Corticosteroid Modulation of Hippocampal Neural Activity

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LOYOLA UNIVERSITY CHICAGO

CORTICOSTEROID MODULATION OF HIPPOCAMPAL NEURAL ACTIVITY

A DISSERTATION SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

BY

DAYNE Y. OKUHARA

CHICAGO, ILLINOIS

JANUARY, 1997
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Dedicated to the my wife Susie and
the memory of Reid K. Shimabukuro
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My sincerest thanks and gratitude to Dr. Sheryl G. Beck for her mentorship, patience, and guidance. I am indebted to Drs. Nancy Muma and Israel Hanin for their guidance throughout my graduate career and Dr. Nancy Muma for including me in her laboratory. I thank my committee members Drs. Rodrigo Andrade, Nancy Muma, Mary Manteuffel, and Russell Pieper for their time and contributions toward the development of this dissertation. My appreciation to the faculty, secretaries, and students of the Department of Pharmacology for their support and friendship. I am particularly appreciative to the Arthur J. Schmitt Foundation for their dissertation fellowship award.

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ABSTRACT

The neurons in hippocampal subfield CA3 contain corticosteroid mineralocorticoid (MR) and glucocorticoid (GR) receptors. The neurons in subfield CA3 have a critical role in hippocampal neural signal processing, yet very little is known about the effects of corticosteroids on neural activity in this subfield. Therefore, the actions of chronic corticosteroid treatment on neuron membrane electrical properties and the 5-HT$_{1A}$ receptor system (receptor-G protein-potassium channel) were examined in rat hippocampal subfield CA3 pyramidal cells. The treatment groups used in these investigations were: adrenalectomy, MR activation with aldosterone or low levels of corticosterone, MR and GR activation with high levels of corticosterone, and sham.

Based on intracellular recording experiments, corticosteroid treatment altered the percentage of nonburst and burst firing neurons. There was a higher percentage of nonbursting cells from adrenalectomized animals compared to aldosterone and sham treated animals. The action potential duration was also longer in cells from adrenalectomized compared to cells from high corticosterone and aldosterone treated animals.

Corticosteroids altered the 5-HT concentration-response curve characteristics for the 5-HT$_{1A}$ receptor. The EC$_{50}$ value was smaller in cells from adrenalectomized animals compared to the other treatment groups. The Emax value was smaller in cells
from animals treated with high corticosterone levels compared to sham and adrenalectomized animals. G protein function was also altered by corticosterone treatment. G protein activation elicited a smaller current in cells from high corticosterone treated animals compared to the other treatment groups and in cells from sham compared to adrenalectomized animals.

Western blot and immunohistochemistry techniques were used to determine the effects of corticosterone on G protein levels and distribution in the hippocampus. Treatment with high concentrations of corticosterone increased the levels of G_s, G_{11} and G_o α-subunits when compared to sham. Adrenalectomy had no effect on G_s, G_{11} and G_o α-subunits levels. No change in the intracellular location of G protein α-subunits was detected using immunohistochemistry.

This dissertation work has demonstrated that corticosteroids alter neuron electrical membrane properties and the 5-HT_{1A} receptor signal transduction system in hippocampal subfield CA3 neurons.
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<td>Description</td>
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<tr>
<td>ADX</td>
<td>adrenalectomized treatment group</td>
</tr>
<tr>
<td>ACSF</td>
<td>artificial cerebrospinal fluid</td>
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<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
</tr>
<tr>
<td>ALD</td>
<td>aldosterone treatment group</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
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<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CRF</td>
<td>corticotropin releasing factor</td>
</tr>
<tr>
<td>CT</td>
<td>12.5 mg corticosterone treatment group</td>
</tr>
<tr>
<td>DLG</td>
<td>dorsal lateral geniculate nuclei</td>
</tr>
<tr>
<td>EPSP</td>
<td>excitatory post synaptic potentials</td>
</tr>
<tr>
<td>fAHP</td>
<td>fast afterhyperpolarization</td>
</tr>
<tr>
<td>GABA</td>
<td>( \gamma )-aminobutyric acid</td>
</tr>
<tr>
<td>GR</td>
<td>glucocorticoid receptor</td>
</tr>
<tr>
<td>GRE</td>
<td>glucocorticoid response elements</td>
</tr>
<tr>
<td>HCT</td>
<td>200-300 mg corticosterone treatment group</td>
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<tr>
<td>HPA</td>
<td>hypothalamus-pituitary-adrenal</td>
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<tr>
<td>IOD</td>
<td>integrated optical density</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
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<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>LP</td>
<td>lateral thalamic posterior nuclei</td>
</tr>
<tr>
<td>mg</td>
<td>miligram</td>
</tr>
<tr>
<td>µs</td>
<td>microseconds</td>
</tr>
<tr>
<td>ml</td>
<td>mililiter</td>
</tr>
<tr>
<td>MΩ</td>
<td>megaohms</td>
</tr>
<tr>
<td>MR</td>
<td>mineralocorticoid receptor</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>ms</td>
<td>milliseconds</td>
</tr>
<tr>
<td>mV</td>
<td>milivolts</td>
</tr>
<tr>
<td>NA</td>
<td>noradrenaline</td>
</tr>
<tr>
<td>pA</td>
<td>picoamperes</td>
</tr>
<tr>
<td>PTX</td>
<td>pertussis toxin</td>
</tr>
<tr>
<td>PVN</td>
<td>paraventricular nucleus</td>
</tr>
<tr>
<td>sAHP</td>
<td>slow afterhyperpolarization</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
</tr>
<tr>
<td>VLG</td>
<td>ventral lateral geniculate nuclei</td>
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<tr>
<td>VNAB</td>
<td>ventral noradrenergic ascending bundle</td>
</tr>
<tr>
<td>VPT</td>
<td>ventral posterior thalamic nuclei</td>
</tr>
<tr>
<td>5-HT</td>
<td>5-hydroxytryptamine or serotonin</td>
</tr>
<tr>
<td>6-OHDA</td>
<td>6-hydroxy-dopamine</td>
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CHAPTER I
INTRODUCTION

Corticosteroids are steroid hormones that maintain energy, ion, and endocrine homeostasis (de Kloet, 1991; Henkin, 1970; Munck et al., 1984). There are two classes of corticosteroids: glucocorticoids and mineralocorticoids. Glucocorticoids regulate glucose metabolism while mineralocorticoids maintain ion homeostasis (reviewed by Dallman et al., 1987, 1994; de Kloet, 1991; Tempel and Leibowitz, 1994).

Corticosterone is the principal glucocorticoid in rats while in man the principal glucocorticoid is cortisol. Aldosterone is the principal mineralocorticoid in both man and rats.

Corticosteroids regulate gene expression through the interaction with two receptor subtypes: mineralocorticoid receptor (MR) and glucocorticoid receptor (GR). MR has a high affinity for corticosterone, \( K_D = 0.5 - 0.7 \) nM, and aldosterone (Krozowski and Funder, 1983; Reul and de Kloet, 1985). GR has a lower affinity for corticosterone, \( K_D = 2.5 - 5 \) nM and aldosterone (Krozowski and Funder; 1983; Reul and de Kloet, 1985).

The synthesis and release of corticosteroids are regulated by the hypothalamic-pituitary-adrenal axis (HPA) (reviewed by Feldman and Weidenfeld, 1995). In response to various stimuli, paraventricular (PVN) neurons in the hypothalamus release
corticotropin releasing factor (CRF) into the portal circulation. CRF then stimulates the synthesis and release of adrenocorticotropic hormone (ACTH) from the corticotropes in the anterior pituitary. ACTH enters the circulatory system and drives the synthesis and release of corticosteroids from the adrenal cortex.

There are two physiological processes that drive HPA axis activity: circadian rhythm and stress (reviewed by Dallman et al., 1987, 1994). Corticosteroid plasma levels are lower (< 10 µg/dl) during hours when the animals is resting (i.e., during day light for rats) and higher (> 15 µg/dl) during hours when the animal is active (i.e., at night for rats) or stressed. The relative occupation of MR and GR changes with physiological fluctuations in the plasma corticosteroid levels. MR is 70-80% occupied at basal physiological corticosterone plasma levels (Reul and de Kloet, 1985; Reul et al., 1987). GR occupancy changes from 15% at basal to 90% at peak and stress circulating corticosterone levels (Reul and de Kloet, 1985; Reul et al., 1987a,b). HPA axis activity is regulated by higher cortical and brainstem inputs that converge at the hypothalamus and influence CRF secretion (reviewed by Feldman and Weidenfeld, 1995). However, corticosteroids also modulate HPA axis activity through feedback inhibition via the activation of MR and GR in the PVN, pituitary, and hippocampus (reviewed by Dallman et al., 1987, 1994).

The relationship between the serotonin (5-HT) system and HPA axis has been examined in different biological states including depression (reviewed by Lesch and
Lerer, 1991), development (reviewed by Meaney et al., 1994), and in animal strains characterized by altered HPA activity (Burnet et al., 1994). For example, it was demonstrated that postnatal handling of rat pups reduces the HPA response to stress (reviewed by Meaney et al., 1994). Meaney et al., (1994) hypothesize that the decreased HPA response to stress is due to a more sensitive corticosteroid feedback inhibition. Furthermore, the increased feedback inhibition may be related to an increase in 5-HT turnover (Smythe et al., 1994) and GR receptor expression in the hippocampus (Meaney and Aitken, 1985; O'Donnell et al., 1994).

The hippocampus contains the highest concentration of corticosteroid receptors in the central nervous system (McEwen et al., 1968; Coirini et al., 1983, 1985; Reul and de Kloet 1985, 1986; Magarinos et al., 1989). Corticosteroid receptors have at least two physiological roles in the hippocampus: influencing HPA axis activity via a negative feedback mechanism and modulating hippocampus related behaviors (reviewed by Jacobson and Sapolsky, 1991; de Kloet et al., 1993). It is still not clear how corticosteroids influence neural signal processing in the hippocampus. Several important questions remained answered. For example, exactly where in the hippocampus are corticosteroids exerting their modulatory effect?

The hippocampus is divided into three regions; hippocampus proper, dentate gyrus, and the subiculum (reviewed by Knowles, 1992; Bernard and Wheal, 1994). The hippocampus proper is further divided into four subfields, CA1-4. The cells in the
different hippocampal regions and subfields have unique roles in hippocampal signal processing based on their different positions within the trisynaptic circuit. The cells in subfield CA3 receive their inputs from the dentate gyrus, perforant pathway axons, neighboring CA3 cells. The cells in subfield CA1 receive their inputs almost entirely from subfield CA3 (reviewed by Knowles, 1992; Bernard and Wheal, 1994).

Corticosteroids modulate pyramidal cell membrane properties and the response elicited by the activation of 5-HT1A receptors on subfield CA1 cells (Joëls and de Kloet, 1994; Beck et al., 1994, 1996). Activation of the 5-HT1A receptor in the hippocampus hyperpolarizes the cell membrane potential (Andrade and Nicoll, 1987; Colino and Haliwell, 1987; Beck and Choi, 1991; Beck et al., 1992). The 5-HT1A receptor signal transduction system mediating the hyperpolarization is composed of a receptor, heterotrimeric guanine nucleotide binding protein (G protein), and potassium channel (Andrade et al., 1986; Okuhara and Beck, 1994).

The actions of corticosteroids on pyramidal cell membrane electrical properties and the 5-HT1A receptor signal transduction system in subfield CA3 are not known, yet this information is crucial towards elucidating the mechanism by which corticosteroids modulate signal processing in the hippocampus. In addition to their differences in neuronal organization and synaptic inputs, the pyramidal neurons in subfields CA1 and CA3 have different cell membrane electrical properties (Nunez et al., 1990; Beck et al., 1992; Spruston and Johnston, 1992), 5-HT1A receptor concentration-response curve
characteristics (Beck et al., 1992, Okuhara and Beck, 1994), and when these regions are stimulated their effects on HPA axis activity are not the same (Kawakami et al., 1968; Dunn and Orr, 1984; Daniels et al., 1994). Therefore information on the actions of corticosteroids on pyramidal cell membrane electrical properties and the 5-HT$_{1A}$ receptor signal transduction system in subfield CA3 will contribute to a basic understanding of how corticosteroids alter cell excitability, responses elicited by the activation of G protein-linked neurotransmitter receptors, and help to elucidate the role of corticosteroid receptors in information processing in the hippocampus.

The **goal of this dissertation** is 1) to determine the effects of chronic corticosteroid treatment on neuron membrane electrical properties and/or 2) the 5-HT$_{1A}$ receptor-mediated response on pyramidal cells in hippocampal subfield CA3 and 3) determine which component(s) of the 5-HT$_{1A}$ receptor signal transduction system is modulated by corticosteroids. The **underlying hypothesis** of this dissertation is that chronic corticosteroid treatment modulates pyramidal cell excitability and/or the 5-HT$_{1A}$ receptor signal transduction system in hippocampal subfield CA3. This hypothesis was tested with experiments designed to address four specific aims.

Four treatment groups were used in this dissertation. Adult rats were surgically treated to achieve 1) no corticosteroid receptor occupancy, 2) MR occupancy only, 3) occupancy of both MR and GR, and 4) sham. All treatments were for 13 -15 days.
Specific Aim 1: Determine if corticosteroids alter pyramidal cell electrophysiological properties in hippocampal subfield CA3.

Intracellular recording techniques were used in hippocampal slices maintained in vivo to measure passive and active membrane electrical characteristics.

Specific Aim 2: Determine if corticosteroids alter the 5-HT concentration-response curve characteristics for the 5-HT<sub>1A</sub> receptor in hippocampal CA3 pyramidal cells.

Intracellular recording techniques were used in hippocampal slices maintained in vivo to examine the 5-HT concentration-response curve characteristic for the 5-HT<sub>1A</sub> receptor.

Specific Aim 3: Determine the effects of corticosteroids on the 5-HT<sub>1A</sub> signal transduction components: (1) The current evoked by direct activation of G proteins and (2) the current-voltage relationship for the potassium current linked to the 5-HT<sub>1A</sub> receptor.

Corticosteroids may modulate one or more of the 5-HT<sub>1A</sub> signal transduction components (i.e., receptor, G protein, or ion channel). Therefore, intracellular recording techniques were used in hippocampal slices maintained in vivo to examine the action of corticosteroids on 1) the outward current evoked by the activation of G proteins with GTPγS, and 2) the current-voltage (I-V) relationship of the potassium channels linked to the 5-HT<sub>1A</sub> receptor.
Specific Aim 4: Determine the effects of corticosteroids on the 5-HT₁A signal transduction components: G protein expression in the hippocampus.

Western blot and immunohistochemistry techniques were used to determine the effects of corticosteroids on G protein levels and distribution in the hippocampus.
CHAPTER II

REVIEW OF RELATED LITERATURE

Introduction

Adrenal corticosteroids are divided into two classes, glucocorticoids and mineralocorticoids (Figure 1). Corticosteroids maintain homeostasis, influence mood, sensory detection, and perception (reviewed by Henkin 1970; Munck et al., 1984; de Kloet, 1991). T. Addison first published his results on the death of patients with adrenal destruction in 1855. Later, H. Cushing (1932) and Harris (1948) established that the hypothalamus-pituitary-adrenal (HPA) axis regulates corticosteroid synthesis and secretion. The actions of corticosteroids are mediated mainly through two receptor subtypes: glucocorticoid and mineralocorticoid receptors.

The first section of this literature review covers the description, pharmacology, function, and regulation of corticosteroid receptors in mammals. Section two describes the components, function, and regulation of the HPA axis. The regulation of HPA axis activity is complex, involving several feedback loops and extrahypothalamic pathways. One system which has an important modulatory influence on HPA axis activity is the limbic system, in particular, hippocampal inputs into the HPA axis. Section three reviews the role of the hippocampus in regulating HPA activity. In addition to their
physiological effects in the periphery, corticosteroids also influence neural activity. Section four reviews the available information on corticosteroid-induced changes in neuron electrophysiological properties and the responses elicited by the activation of the 5-HT$_{1A}$ receptor in the hippocampus. The final section summarizes the known and proposed roles of corticosteroids in pathological conditions. The scientific contribution of this dissertation is also addressed in this section.

**Corticosteroids Receptors**

Glucocorticoids have a wide variety of effects on many cell functions (reviewed by Kraus-Friedmann, 1984; Pilkis and Granner, 1992). Glucocorticoids stimulate gluconeogenesis, increase amino acid uptake by the liver and kidney, increase amino acid release through muscle catabolism, and stimulate lipolysis. The primary role of glucocorticoids is to provide glucose for energy demanding tissues such as the brain and heart, especially under conditions of stress. Glucocorticoids also have immuno-suppressive, anti-inflammatory, and anti-asthmatic properties (Falaschi et al., 1994; Derijk and Sternberg, 1994; Barnes, 1996; Laitinen and Laitinen, 1996; Schwiebert et al., 1996) while supraphysiological levels decrease muscle mass, leading to muscle weakness (Caldwell et., 1978; Goldberg et al., 1980; Florini, 1987; Smith et al., 1990).

Glucocorticoids exert their effects directly by regulating the synthesis of key enzymes and proteins, or permissively, by influencing the sensitivity of cells to other hormones or extracellular signals. For example, glucocorticoids elevate blood glucose
Figure 1. Major corticosteroids in rat and humans. The two classes of corticosteroids are glucocorticoids and mineralocorticoids. Corticosterone is the major glucocorticoid in rat, while cortisol is the major glucocorticoid in humans.
levels directly by increasing the synthesis of hepatic phosphoenolpyruvate carboxykinase (Beale et al., 1982; Lamers et al., 1982) and branched-chain α-ketoacid dehydrogenase (Block et al., 1970; Block and Buse, 1990), rate limiting enzymes for gluconeogenesis and skeletal muscle catabolism respectively. Glucocorticoids act permissively by increasing the sensitivity of adipose cells to catecholamines (reviewed by Munck and Guyre, 1986; Malbon et al., 1988).

Mineralocorticoids maintain fluid and electrolyte homeostasis. Aldosterone primarily acts at the kidney to increase Na\(^+\) reabsorption and excretion of K\(^+\) and H\(^+\) (O'Neil, 1990; Funder, 1993; Horisberger and Rossier, 1995; Eaton et al., 1995). Aldosterone affects electrolyte transport at the distal tubules and collecting ducts by increasing the synthesis of Na\(^+\) (Chu and Edelman, 1972; Rousseau and Crabbe, 1972; Lahav et al., 1973; Rossier et al., 1978; Garty, 1986) and K\(^+\) channels (Kopppen et al., 1983; Stokes, 1985; Sansom and O'Neil, 1986; Sansom et al., 1989; Illek et al., 1990; Schafer et al., 1990) and the Na\(^+\),K\(^+\)-ATPase pump (Geering et al., 1982; Ewart and Klip, 1990; Verry, 1990; Shahedi et al., 1993).

Glucocorticoids regulate gene expression through the interaction with at least two receptor subtypes; type I or MR and the type II or GR. Both types of corticosteroid receptors are intracellular receptors which bind to DNA and influence gene transcription (reviewed by Dahlman-Wright et al., 1992). The two receptors are distinguished pharmacologically by their relative affinity for corticosterone and other selective ligands.
Structure and Mechanism of Action

Corticosteroid receptors are intracellular receptors that regulate the expression of specific genes. Both MR and GR have been cloned and their sequences are highly homologous (Arriza et al., 1987; Patel et al., 1989). GR has been the focus for the majority of the biochemical and functional studies, therefore, the following section reviews the information available for GR. However, since both MR and GR belong to the same steroid receptor family, they have many functional similarities (Pearce, 1994).

The unoccupied receptor usually resides in the cytoplasm, translocating into the nucleus after binding to the steroid (Govindan, 1980; Papmichail et al., 1980; Antakly et al., 1984; Fuxe et al., 1985; Wikstrom, et al., 1987; Picard, 1987; LaFond et al., 1988; Gasc et al., 1989). The unoccupied receptor is maintained in an inactive state by a multi-protein complex. This complex contains GR (Okret, 1985) associated with heat shock proteins hsp56 (Sanchez, 1990), hsp70 (Sanchez, 1990; Sanchez et al., 1990), and hsp90 (Catelli et al., 1985; Sanchez et al., 1985; Sanchez et al., 1990). Steroid binding dissociates hsp90 from GR (Mendel et al., 1986; Sanchez et al., 1987a), forming an 'activated' receptor with increased affinity for DNA (Sanchez et al., 1987b; Howard and Distelhorst, 1988a,b). Activated GRs dimerize and bind to specific DNA sequences (Tsai et al., 1988; Wrange et al., 1989; Chalepakis et al., 1990), known as glucocorticoid response elements (GRE) and influence gene transcription (Yamamoto, 1985).
A great deal of information is known about the structure of MR and GR. Purified GR is 90-100 kDa when resolved by SDS-gel electrophoresis (Govindan and Manz, 1980; Bernard and Joh, 1984; Govindan and Gronemeyer, 1984; Grandics et al., 1984; Idziorek et al., 1985; Lustenberger et al., 1985; Webb et al., 1985). Based on nucleotide sequences of MR and GR, the two receptors are comprised of approximately 780 amino acids and are highly conserved across different species (Hollenberg et al., 1985; Danielsen et al., 1986; Miesfeld et al., 1986; Arriza et al., 1987; Patel et al., 1989). Corticosteroid hormone receptors are single chain polypeptides containing three distinct functional domains: ligand binding, DNA binding, and N-terminal domains (Figure 2) (reviewed by Gustafsson et al., 1987; Dahlman-Wright et al., 1992; Wright et al., 1993). The different domains were first identified through limited proteolysis of the purified protein (Carlstedt-Duke et al., 1977, 1982, 1987; Wrange and Gustafsson, 1978; Westphal et al., 1984; Wrange et al., 1984). Point mutation analysis of the cDNA clones allowed researchers to identify the amino acid residues critical for the proper function of each domain (reviewed by Dahlman-Wright et al., 1992; Gehring, 1993).

The ligand binding domain is in the carboxy-terminal end of the peptide, covering approximately 250 amino acids (Carlstedt-Duke et al., 1987; Hollenberg et al., 1987; Rusconi and Yamamoto, 1987). The hormone binding domain in MR and GR share a 57 % homology in humans (Arriza et al., 1987) and 59 % in rats (Patel et al., 1989). Removal of this domain produces a constitutively active receptor (Danielson et al., 1987;
Figure 2. Schematic representation of the MR and GR protein and its major domains. The domains are labeled at the top of the diagram while the approximate amino acid size of the domain is located at the bottom.

Godowski et al., 1987; Hollenberg et al., 1987; Meisfeld et al., 1987). Biochemical analysis of this domain reveals that it folds into a complex secondary and tertiary structure, important for proper steroid binding (reviewed by Dahlman-Wright, et al., 1992; Gehring 1993) and translocation into the nucleus (Picard and Yamamoto, 1987).

The DNA binding domain is in the middle of the polypeptide. The MR and GR DNA binding domains are 94% homologous in humans (Arriza et al., 1987) and 76% in rats (Patel et al., 1989). This domain contains 70 amino acids which are highly conserved with other steroid receptors (Giguere et al., 1986; Carlstedt-Duke et al., 1987; Danielson et al., 1987; Hollenberg et al., 1987; Meisfeld et al., 1987; Rusconi and Yamamoto, 1987; Evans, 1988; Hard et al., 1990). The prominent feature of this domain is the coordination of cysteine residues and zinc ions to form the DNA binding structure (Freedman et al., 1988; Severne et al., 1988; Hard et al., 1990).
The N-terminal domain sequence is highly variable with other intracellular steroid receptors (reviewed by Evans, 1988; Beato, 1989). The N-terminal domain in MR and GR share only a 15% homology in humans (Arriza et al., 1987) and 40% in rats (Patel et al., 1989). Very little is known about the function of this domain and how the different amino acids contribute to the activity of the receptors. This domain may be important for the transactivation of GR with other transcription factors (Hollenberg et al., 1987; Danielson et al., 1987; Gehring and Segnitz, 1988; Hollenberg and Evans, 1988).

Pharmacology and Distribution

Several experimental techniques, including in situ hybridization, autoradiography, and immunohistochemistry were used to map the distribution of MR and GR in the central nervous system (CNS). Convincing pharmacological evidence for the existence of two corticosteroid receptor subtypes came with the development of drugs that were selective for MR and GR and allowed researchers to distinguish the two receptors and their differential distribution in the CNS.

[^3H]corticosterone binding macromolecules in the brain were initially identified in the early 1970's (McEwen and Plapinger, 1970; Grosser et al., 1971; Gerlach and McEwen, 1972; McEwen et al., 1972; Grosser et al., 1973). These early investigations demonstrated that corticosterone binding was not uniformly distributed. Corticosterone binding was much higher in limbic areas, especially the hippocampus, compared to other regions of the brain (McEwen et al., 1969, 1970, 1972; McEwen and Plapinger, 1970;
Grosser et al., 1971; Stevens et al., 1971; Gerlach and McEwen, 1972; Warembourg, 1975; Stumpf and Sar, 1979; Sapolsky et al., 1983b). However, binding experiments with the native mineralocorticoid aldosterone (Anderson and Fanestil, 1976; Moguilevski and Raynaud, 1980; De Nicola et al., 1981; Veldhuis et al., 1982; Beaumont and Fanestil, 1983; Krozowski and Funder, 1983; Wrange and Yu, 1983) and synthetic glucocorticoid dexamethasone (de Kloet ER et al., 1975; Rees et al., 1975; Rhees et al., 1975; Warembourg, 1975) produced two different corticosteroid receptor distributions. The accumulated data lead investigators to suggest that two corticosteroid receptor populations existed in the brain.

The development of selective MR antagonist RU 26752 and RU 26813 (Coirini et al., 1985), GR agonist RU 26988 and RU 28362 (Moguilewsky and Raynaud, 1980; Coirini et al., 1985), and GR antagonist RU 38486 (Gagne et al., 1985) allowed researchers to pharmacologically characterize and map the distribution of MR and GR in the CNS. The two receptor subtypes are distinguished by their relative affinities for the endogenous corticosteroids corticosterone and aldosterone. MR has a high affinity for corticosterone (K_D = 0.5 nM) and aldosterone. In contrast, GR has a low affinity for corticosterone (K_D = 2 - 5 nM) and aldosterone (Veldhuis et al., 1982; Beaumont and Fanestil, 1983; Krozowski and Funder, 1983; Coirini et al., 1985; Reul and de Kloet, 1985; Sheppard and Funder, 1987). Dexamethasone is a synthetic glucocorticoid with a
higher affinity for GR (Veldhuis et al., 1982; Krokowski and Funder, 1983; Coirini et al., 1985).

The hippocampus contains the highest concentration of MR and GR in the CNS. MR binding is almost exclusively located in the hippocampus while GR binding is also found in other CNS areas including the cortex, ventromedial thalamic nuclei, arcuate, and lateral septum (Coirini et al., 1983, 1985; Reul and de Kloet 1985, 1986; Magarinos et al., 1989).

Regulation

Corticosteroid receptor levels in the brain are dynamically regulated by neuropeptides, neurotransmitters, and corticosteroids (via an autoregulatory mechanism) (reviewed by de Kloet et al., 1986a; Herman, 1993; Budziszewska and Larson, 1994). The regulation of MR and GR depends upon the brain area (i.e., hippocampus versus hypothalamus) and which physiological factor is evoking the change in MR and GR binding (i.e., neurotransmitters versus autoregulation). Furthermore, the regulation of corticosteroid receptors by neuropeptides, neurotransmitters, or corticosteroids may represent three independent physiological mechanisms underlying control of HPA axis activity (see Hypothalamic-Pituitary-Adrenal Axis). A large number of investigations have focused on the regulation of corticosteroid receptors in the hippocampus, where corticosteroid receptors have an important role in HPA axis feedback and behavior (Jacobson and Sapolsky, 1991).
Regulation by Neuropeptides

HPA axis activity is regulated by two principal neuropeptides, CRF and ACTH (see Hypothalamic-Pituitary-Adrenal Axis). CRF and the ACTH analog ORG 2766 both modulate corticosteroid receptor levels. CRF decreases GR binding and mRNA levels in the anterior pituitary and in AT20 anterior pituitary corticotrope cell culture (Sheppard et al., 1990, 1991), but increases the GR mRNA levels in the hippocampus (Sheppard et al., 1990). ORG 2766 is an ACTH analog that has no effect on corticosteroid secretion from the adrenal gland (Greven and de Weid, 1973; de Weid and Jolles, 1982). However, ORG 2766 administration increases corticosteroid receptor binding in the hippocampus (Rigter et al., 1984; Reul et al., 1988). Selective MR and GR ligands were used to demonstrate that ORG 2766 selectively increases MR binding in the hippocampus (Reul et al., 1988). The effects of ORG 2766 in the pituitary and hypothalamus are not known.

Another neuropeptide which is also released from the hypothalamus is vasopressin (Kiss et al., 1984; Plotsky et al., 1985; Davis et al., 1986; Holmes et al., 1986; Fink et al., 1988; Canny et al., 1989). Vasopressin is released by hypothalamic PVN neurons in response to stress (Plotsky et al., 1985; Canny et al., 1989) and influences water-intake, learning and memory (de Wied, 1971; Bohus et al., 1975; de Weid et al., 1975; de Kloet and de Wied, 1980). Vasopressin increases corticosteroid receptor binding capacity in
the hippocampus and GR receptors in the anterior pituitary (Veldhuis and de Kloet, 1982).

The mechanisms underlying the neuropeptide-induced changes in corticosteroid receptor binding are largely unknown. However, the neuropeptide actions are independent of corticosteroid hormones (Veldhuis and de Kloet, 1982; Reul et al., 1988; Shepherd et al., 1990). While the normal physiological roles of CRF-, ACTH-, and vasopressin-induced changes in corticosteroid receptor levels are not known, these peptides generally display trophic effects in certain diseased and aged brains, restoring deficient corticosteroid receptors and HPA responsiveness (de Kloet et al., 1986a; Reul et al., 1988).

Regulation by Neurotransmitters

One brain area in which neurotransmitters appear to have an important role in regulating corticosteroid receptor expression is the hippocampus (Siegel et al., 1983; Weidenfeld et al., 1983). Modulation of corticosteroid receptors in the hippocampus by neurotransmitters may be important in affecting mood, behavior, and neuroendocrine responses (Azmitia et al., 1984; Budziszewska and Larson, 1994).

The 5-HT neurotransmitter system regulates corticosteroid receptor binding. Lesioning 5-HT neurons in the raphe nucleus or depleting neurons of 5-HT decreases corticosteroid receptor binding in the mediobasal hypothalamus and hippocampus (Siegel et al., 1983; Weidenfeld et al., 1983; Weidenfeld and Feldman, 1991a; Novotney and
Lesioning 5-HT neurons in the raphe nucleus also decreases GR and MR mRNA levels in the hippocampus (Seckl et al., 1990; Seckl and Fink, 1991). In cultured hippocampal neurons, 5-HT increases GR binding capacity without altering the binding capacity of MR (Mitchell et al., 1990). The 5-HT receptor mediating the upregulation of GR binding capacity in cultured hippocampal neurons is the 5-HT\textsubscript{2} receptor (Mitchell et al., 1992). In contrast to cultured neurons, 5-HT\textsubscript{1A} agonists increase MR binding capacity in the hippocampus in vivo (Budziszewska et al., 1995).

Other neurotransmitter systems which also modulate corticosteroid receptor binding capacity or mRNA levels in the brain are the noradrenergic (Maccari et al., 1990; 1992a,b; Yau and Seckl, 1992; Kabbaj et al., 1995), cholinergic (Nyakas et al., 1979; Yau et al., 1992), and dopaminergic systems (Casolini et al., 1993). Interestingly, the different neurotransmitter systems have different effects on corticosteroid receptor binding capacity and mRNA levels. Noradrenaline (NA) alters corticosteroid receptor binding capacity and mRNA levels (Maccari et al., 1992a,b; Yau and Seckl, 1992; Kabbaj et al., 1995). Cholinergic neurotransmitters do not modulate MR and GR binding capacity but alter their mRNA levels (Nyakas et al., 1979; Yau et al., 1992). Dopamine does not alter MR and GR binding capacity but alters the affinity of GR for corticosterone (Casolini et al., 1993).

The mechanisms underlying the neurotransmitter-induced changes in corticosteroid receptor binding capacity are not known. Changes in corticosteroid receptor mRNA
levels do not always parallel the changes in receptor binding capacity. Furthermore, in certain cases (i.e., 5-HT$_2$ modulation of GR) (Mitchell et al., 1992), changes in corticosteroid receptor binding capacity are dependent on the production of second-messengers by the activated neurotransmitter receptor. Indeed, protein kinase A dependent mechanisms (Kendal and Robinson, 1990) have been shown to phosphorylate corticosteroid receptors (Dalman et al., 1988; Smith et al., 1989) and convert them to their inactive form (reviewed by Sanchez et al., 1987; Dahlman-Wright et al., 1992), increasing their binding capacity in the cytosol.

Modulation of corticosteroid receptors by neurotransmitters may be the initial phase leading to an eventual shift in HPA axis activity (Kabbaj et al., 1995). For example, activation of 5-HT or NA neurons increases HPA axis activity (Feldman and Weidenfeld, 1995). However, prolonged activation of these receptors have opposite effects on hippocampal corticosteroid receptor binding capacity. NA decreases (Maccari et al., 1992a; Kabbaj et al., 1995) while 5-HT increases (Seckl and Fink, 1991; Budziszewska et al., 1995) corticosteroid receptor levels. Clearly, the selective physiological activation of different neurotransmitter pathways will have different effects on corticosteroid receptor function and may underlie modulatory and pathophysiological processes.

**Autoregulation**

Receptor autoregulation is a mechanism which occurs for a large number of receptor systems, including corticosteroid receptors (reviewed by Schmidt and Meyer,
A common feature of receptor autoregulation in all systems is a correlation between ligand availability and receptor binding capacity. What is different between receptor systems is how ligand availability and receptor binding correlate (directly or inversely) and their mechanism of autoregulation. An adrenalectomy-induced increase in hippocampal corticosteroid binding capacity was first demonstrated by McEwen et al., (1970). Later, it was reported that corticosteroid receptor binding capacity in the hippocampus was reduced by high levels of corticosterone or stress (Tornello et al., 1982; Sapolsky et al., 1984a). Investigators then sought to determine the individual roles of hippocampal MR and GR in receptor autoregulation and their function in HPA axis feedback.

The binding capacities of MR and GR are differentially regulated by changes in steroid levels. Hippocampal MR binding capacity may either increase (Chao et al., 1989) or show no change (Reul et al., 1987b; Reul et al., 1989; Lowy, 1991; Spencer et al., 1991) with adrenalectomy. MR mRNA levels increase with adrenalectomy (Herman et al., 1989a, 1993). Activation of either MR or GR has different effects on MR binding capacity. The activation of MR does not alter MR binding capacity (Reul et al., 1987b; Spencer et al., 1991) while GR activation alone increases MR binding capacity and mRNA levels (Reul et al., 1987b; Reul et al., 1989). Coactivation of MR and GR decreases MR binding capacity and mRNA levels (Lowy, 1991; Spencer et al., 1991). MR binding capacity and mRNA levels also display circadian variation. However, there
are different reports supporting either a peak- (Reul et al., 1987b) or a nadir-associated (Chao et al., 1989) increase in MR binding capacity during the circadian cycle. MR mRNA levels are higher during the nadir (Herman et al., 1993; Holmes et al., 1995a).

Unlike MR, hippocampal GR binding capacity shows a reproducible increase with adrenalectomy that returns to control levels with corticosterone or dexamethasone treatment (Reul et al., 1987; Chao et al., 1989; Lowy, 1991; Spencer et al., 1991). The adrenalectomy-induced increase in GR binding capacity is also paralleled by an increase in GR mRNA levels (Kalinyak et al., 1987; Herman et al., 1989a, 1993; Reul et al., 1989; Sheppard et al., 1990; Peiffer et al., 1991). GR binding capacity in the hippocampus does not change with diurnal variation (Reul et al., 1987b) but GR mRNA levels are higher during the nadir of the circadian cycle (Herman et al., 1993; Holmes et al., 1995a). In contrast to MR, activating both or each corticosteroid receptor selectively, decreases GR binding capacity in hippocampal sections and tissue culture (Sapolsky et al., 1984a; Sapolsky and McEwen, 1985; Luttge et al., 1989; Vedder et al., 1993; O’Donnell and Meaney, 1994).

Corticosteroid receptor autoregulation does not occur in all brain areas. Adrenalectomy increases MR and GR mRNA levels in the hippocampus and amygdala but not in the hypothalamus, neocortex, dorsomedial thalamus, hypothalamic PVN, and arcuate nucleus (Pfeifer et al., 1991; Herman et al., 1993). Corticosterone decreases corticosteroid receptor binding capacity in the rat hippocampus but not in the
hypothalamus, amygdala, or pituitary (Tornello et al., 1982; Sapolsky and McEwen, 1985). Interestingly, dexamethasone decreases GR binding capacity in the pituitary and amygdala but not in the hippocampus (Sapolsky and McEwen, 1985).

The autoregulation of corticosteroid receptors is an important feedback mechanism (Schmidt and Meyer, 1994). The mechanism underlying corticosteroid receptor autoregulation is complex. Influencing the transcription of their own gene (Burstein et al., 1991), mRNA stability, alternative splicing, and receptor protein turnover rate may all be involved in the autoregulation mechanism (Schmidt and Meyer, 1994; Herman and Watson, 1995). Finally, the pronounced autoregulation of corticosteroid receptors in the hippocampus as compared to other brain areas, supports the important role that the hippocampus has in influencing HPA axis activity and corticosteroid-induced behaviors (Jacobson and Sapolsky, 1991).

Function

It has been proposed by de Kloet (1991) that homeostasis is maintained by MR and GR via a 'corticosteroids receptor balance hypothesis'. This hypothesis simply states that the physiological functions of MR and GR depend on the differential occupation of the two receptors and is based on two important corticosteroid receptor properties. First, MR and GR have different affinities for corticosterone. Second, the fractional occupancy of MR and GR changes with physiological fluctuations in corticosterone plasma concentrations. MR has a high affinity for corticosterone (K_D = 0.5 - 0.7 nM)
and is 70-80% occupied at basal physiological corticosterone plasma levels (Krozowski and Funder, 1983; Reul and de Kloet, 1985; Reul et al., 1987). GR has a lower affinity for corticosterone, $K_D = 2.5 - 5 \text{ nM}$, and its occupancy changes from 15% at basal to 90% at peak and stress circulating corticosterone levels (Reul and de Kloet, 1985; Reul et al., 1987a,b). One important point should be emphasized about the physiological function of MR and GR. GR occupation without concomitant MR occupation has not been demonstrated to occur physiologically.

Brain MR and GR have distinct physiological functions that are often complementary and not antagonistic. For example, MR and GR are important in maintaining homeostasis. However MR maintains ion homeostasis while GR maintains glucose availability (Dallman et al., 1987, 1994; Tempel and Leibowitz, 1994). MR and GR influence different aspects of behavior. MR permits the proper acquisition of behavior reactivity in rats while GR facilitates memory storage (Micco et al., 1980; Jefferys et al., 1983; Veldhuis et al, 1985; McEwen and Brinton 1987; Oitzl and de Kloet, 1992, 1994; de Kloet et al., 1993). One example of the different roles MR and GR have on behavior is the performance of rats in the Morris water maze. MR activation is important in developing the strategy used by the animal to find the platform in the water while GR activation is important for acquiring and storing spatial information (Oitzl and de Kloet, 1992; Oitzl et al., 1994).
Concluding Remarks

MR and GR are intracellular receptors that bind to DNA and regulate the expression of specific genes. MR and GR have different physiological functions. MR and GR also have different affinities for corticosterone. Therefore, homeostasis is maintained by MR and GR via changes in their fractional occupancy with fluctuating corticosteroid plasma levels. MR and GR are differentially expressed in the brain. The hippocampus contains the highest density of MR and GR binding sites and mRNA levels.

Corticosteroid receptor levels are autoregulated and influenced by neuropeptides and neurotransmitters which may represent three different physiological mechanisms underlying the modulation of HPA axis activity. Neuropeptides provide trophic effects which may be important in maintaining normal corticosteroid receptor levels (reviewed by de Kloet et al., 1986a). Neurotransmitters provide a rapid mechanism for modulating corticosteroid receptor levels via the activation of selective neurotransmitter pathways. Finally, corticosteroids autoregulate their own receptor levels, providing a HPA axis feedback mechanism (reviewed by Schmidt and Meyer, 1994). Corticosteroid receptor regulation occurs in several brain areas; however, the hippocampus shows the greatest sensitivity.

Hypothalamic-Pituitary-Adrenal Axis

The synthesis and release of corticosteroids are regulated by the hypothalamic-pituitary-adrenal axis (HPA) (Figure 3) (reviewed by Feldman and Weidenfeld, 1995).
Figure 3. Schematic model of the HPA axis. The major components, peptides, and hormones are pictured in this diagram. The solid arrows depict released constituents, while the broken arrow depicts feedback.
In response to various stimuli, PVN neurons in the hypothalamus release CRF into the portal circulation. CRF then stimulates the synthesis and release of ACTH from the corticotropes in the anterior pituitary. ACTH enters the circulatory system and drives the synthesis and release of corticosteroids from the adrenal cortex.

Regulation of The HPA axis

HPA axis activity is driven by two physiological mechanisms, circadian rhythm and stress. Circulating glucocorticoid levels in nocturnal rats are lowest during 'lights-on', peak during 'lights-off' (Guillemin et al., 1959; Scheving and Pauly, 1966; Zimmermann and Critchlow, 1967; Ader et al., 1968; Retiene, 1970; Dunn et al., 1972), and increase in response to stress (Ganong, 1963; Mangili et al., 1966; Yates, 1967; Ader et al., 1968; Gibbs, 1970; Dunn et al., 1972). It is well established that extrahypothalamic structures regulate circadian rhythm and stress-induced HPA axis activity (Slusher, 1964; Davidson and Feldman, 1967; Halász et al., 1967; Palka et al., 1969; Wilson and Critchlow, 1975; Phelps et al., 1978). Extradypothalamic structures affect HPA axis activity through the release of vasopressin, oxytocin, epinephrine or 5-HT at the hypothalamus (Weiner and Ganong, 1978; Fuller, 1981; Swanson et al., 1983; Plotsky 1985, 1987; Antoni, 1986; Plotsky et al., 1987, 1989; Feldman et al., 1990, Feldman and Weidenfeld, 1991).
Neurotransmitter Systems Influencing HPA Axis Activity

5-HT neurons arising from raphe nuclei innervate the parvocellular region of the PVN in the hypothalamus (Palkovits et al., 1977; Sawchenko et al., 1983). 5-HT neurons innervate CRF synthesizing neurons in the PVN (Lipositis et al., 1987) and stimulate the synthesis and release of CRF \textit{in vivo} (Saphier and Feldman, 1989; Swanson and Simmons, 1989; Feldman et al., 1991) and from isolated rat hypothalamic cells (Calogero et al., 1989).

5-HT neurons facilitate circadian- and stress-induced release of CRF or corticosterone (Szafarczyk et al., 1980; Fuller, 1981; Feldman et al., 1995). The 5-HT receptors mediating the release of CRF, ACTH or corticosterone are the 5-HT$_{1A}$ and 5-HT$_{2}$ receptors (Koenig et al., 1987, 1988; Lorens and Van de Kar, 1987; Gilbert et al., 1988; King et al., 1989; Fuller and Snoddy, 1990).

NA fibers located in the ventral noradrenergic ascending bundle (VNAB) have an important role in regulating HPA axis activity (Feldman et al., 1984; Plotsky et al., 1989; Feldman and Weidenfeld, 1995, Feldman et al., 1995). VNAB fibers originate in the brainstem, mainly from the locus coeruleus. A subset of these fibers innervate the hypothalamic PVN, via the medial forebrain bundle (Cunningham and Sawchenko, 1988; Cunningham et al., 1990; Saphier and Feldman, 1991), and stimulate CRF release (Guillaume et al., 1987; Plotsky, 1987; Weidenfeld and Feldman, 1991b).
Lesioning the VNAB or medial forebrain bundle with the catecholamine neurotoxin 6-hydroxy-dopamine (6-OHDA) prevents the circadian release of ACTH and corticosterone (Szafarczyk et al., 1985) and the stress-induced release of CRF (Feldman et al., 1984; Szafarczyk et al., 1985, 1987; Plotsky, 1987; Sawchenko, 1988). The NA receptors mediating the release of CRF are the $\alpha_1$- and $\alpha_2$-receptors (Saphier and Feldman, 1991; Daniels et al., 1993).

The cholinergic system also influences HPA axis activity. Cholinergic agonists stimulate CRF, ACTH, and corticosterone release (Makara and Stark, 1976; Plotsky et al., 1987; Weidenfeld et al., 1989a). Cholinergic antagonists attenuated both basal- and stress-induced release of ACTH (Hedge and Smelik, 1968; Kaplanski and Smelik, 1973; Makara and Stark, 1976; Guillaume et al., 1987). The cholinergic receptors mediating the release of corticosterone are nicotinic receptors (Brenner et al., 1986; Weidenfeld et al., 1989a).

Feedback Regulation by Corticosteroids

While extrahypothalamic pathways are important for the initial stimulation (or inhibition) of HPA axis activity, more direct control of the HPA axis is mediated through feedback loops within the axis (Figure 3) (reviewed by Feldman and Weidenfeld, 1995). Corticosteroids act at both the hypothalamus and pituitary to directly inhibit their own synthesis and secretion. Some controversy still exists surrounding the mechanism by which corticosteroids directly act at the hypothalamus to decrease ACTH secretion.
Corticosteroid receptors and CRF are both present within the same cells in the PVN (Ceccatelli et al., 1989). Adrenalectomy or stress (initiated via photic, acoustic, or sciatic nerve stimulation) increases hypothalamic CRF peptide and mRNA levels (Paull and Gibbs; 1983; Westlund et al., 1985; Plotsky and Sawchenko, 1987; Sawchenko, 1987; Beyer et al., 1988; Fink et al., 1988; Weidenfeld et al., 1989b; Feldman et al., 1992; Kwak et al., 1993), resulting in an increase in ACTH secretion from the pituitary. However, corticosteroid implants in the hypothalamus attenuated only the neural (photic) stimulated CRF mRNA levels and peptide secretion (Feldman et al., 1992) and did not reliably decrease the adrenalectomy stimulated ACTH secretion in animals (Dallman et al., 1985; Kovacs et al., 1986; Sawchenko, 1987; Kovacs and Makara, 1988; Levin et al., 1988). Therefore, HPA axis feedback inhibition may occur at other brain sites in addition to the hypothalamus (Feldman et al., 1992).

Corticosteroids also act in the pituitary to inhibit ACTH secretion (reviewed by Dallman et al., 1987). GR and ACTH are both localized within the same cells in the pituitary (Cintra and Bortolotti, 1992). Corticosteroids decrease CRF- (Koch et al., 1979; Dallman et al., 1985), adrenergic- (Giguere et al., 1982), and stress- (Walker et al., 1986) stimulated ACTH release from the pituitary. The inhibitory effect of corticosteroids on ACTH secretion occurs within 10-30 min (Brattin and Portanova, 1977; Buckingham and Hodges, 1977; Mulder and Smelik, 1977; Gilles and Lowry, 1977; Dallman et al., 1987; Plotsky and Sawchenko, 1987; Sawchenko, 1987; Beyer et al., 1988; Fink et al., 1988; Weidenfeld et al., 1989b; Feldman et al., 1992; Kwak et al., 1993). Therefore, HPA axis feedback inhibition may occur at other brain sites in addition to the hypothalamus (Feldman et al., 1992).
1978; Widmaier and Dallman, 1984; Abou-Samra et al., 1986; Dayanithi and Antoni, 1989) and requires protein synthesis (Dayanithi and Antoni, 1989). The requirement for protein synthesis suggests that corticosteroids decrease CRF and/or ACTH synthesis by increasing the synthesis of inhibitory transcription factors (Dayanithi and Antoni, 1989).

MR and GR have different roles in HPA axis feedback (Dallman et al., 1987, 1994; de Kloet and Reul, 1987; Dayanithi and Antoni, 1989; Ratka et al., 1989; Sapolsky et al., 1990; Bradbury et al., 1991, 1994). MRs mediate HPA axis feedback inhibition during the circadian trough, when MR is 70-80% occupied at basal physiological corticosterone plasma levels (Krozowski and Funder, 1983; Reul and de Kloet, 1985; Reul et al., 1987a,b). GRs mediate HPA axis negative feedback regulation during the diurnal peak or stress, when its occupancy changes from 15% at basal to 90% at peak and stress circulating corticosterone levels (Reul and de Kloet, 1985; Reul et al., 1987a,b).

Interestingly, the hypothalamus-pituitary brain areas are important in feedback during the circadian trough while other brain areas are important during the peak (Akana et al., 1986; Suemaru et al., 1995). Stress-induced increase in HPA axis activity is mediated through several different neural pathways (Feldman, 1985; Canny et al., 1989) and feedback is provided by both the HPA axis and other cortical areas (Kaneko and Hiroshige, 1978a,b; Keller-Wood and Dallman 1984; Dallman et al., 1987; Canny et al., 1989).
The Hippocampus and HPA Axis Feedback Regulation

The hippocampus has a significant role in regulating HPA axis activity. While it is well established that the hippocampus is a site of negative feedback for the HPA axis, it is not known how the hippocampus exerts its influence on the axis (reviewed by Jacobson and Sapolsky, 1991). The neural circuit in the hippocampus is complex. The hippocampal structure is divided into different regions: hippocampus proper, dentate gyrus, and subiculum. These regions are interconnected to form the hippocampal circuitry. The neurons within these regions have different cell properties, neurotransmitter receptor-mediated responses, and when these regions are stimulated their effects on HPA axis activity are not the same.

Hippocampal Anatomy

The hippocampus is divided into three regions; hippocampus proper, dentate gyrus, and the subiculum (reviewed by Knowles, 1992; Bernard and Wheal, 1994). The hippocampus proper is further divided into several subfields, CA1-4. Pyramidal cells are the primary projection neurons in the hippocampal subfields (Knowles, 1992; Bernard and Wheal, 1994) and share the same general shape as the pyramidal cells in the cortex (Peters and Jones, 1984). They have triangular or ovoid shaped cell bodies with one to three long apical dendrites. There are also a wide variety of interneurons in the hippocampus. The interneurons mainly release γ-aminobutyric acid (GABA) as their neurotransmitter and are classified by their shape, neurophysiology, and location
The hippocampal circuitry is established through a hierarchy of connections that produce unidirectional signal processing within the structure (Andersen et al., 1971b; Andersen, 1975; Bernard and Wheal, 1994). Figure 4 illustrates a diagrammatic model of the trisynaptic circuit. The major hippocampal inputs are received at the dentate gyrus granule cells from the entorhinal cortex through the perforant pathway. Mossy fibers from the dentate gyrus project to subfield CA3 pyramidal cells, which in turn, send Schaffer collaterals to subfield CA1 pyramidal cells. Subfield CA1 cells then provide the major output for the hippocampus through the fornix and subiculum.

There are several important differences in the cellular organization and physiology between the various subfields. The pyramidal cells in subfield CA3 are larger and more densely packed compared to the cells in subfield CA1 (Knowles, 1992). The pyramidal cells in subfields CA1 and CA3 are interconnected differently. The pyramidal cells in subfield CA3 are highly interconnected with nearby pyramidal cells and interneurons through an extensive local network of axon collaterals (Miles and Wong, 1986; Ishizuka et al., 1990). In contrast, the pyramidal neurons in subfield CA1 have few excitatory collaterals between neighboring pyramidal cells (Knowles and Schwartzkroin, 1981a,b). The extensive interconnection of cells in subfield CA3 are hypothesized to be important in the synchronized burst firing of these cells (Miles and Wong, 1986; Ishizuka et al., 1990).
Figure 4. Schematic representation of the hippocampal trisynaptic circuit. Only the major cell types, projections, and synapses are depicted in this diagram.
Hippocampal pyramidal cell excitability is determined by at least two factors: membrane electrical properties and neurotransmitter responses and both of these factors are different among the various subfields. For example, in subfields CA1 and CA3, the pyramidal cell’s passive and active membrane properties are different (Nunez et al., 1990; Beck et al., 1992; Spruston and Johnston, 1992). Therefore, the cells in these two subfields may integrate synaptic potentials and encode their outputs differently. The responses elicited by the activation of at least two neurotransmitter receptors are different between subfield CA1 and CA3. The 5-HT or baclofen concentration-response curve characteristics for the 5-HT\textsubscript{1A} and \(\gamma\)-aminobutyric acid\textsubscript{B} (GABA\textsubscript{B}) receptors, respectively, are different between subfields CA1 and CA3 (Beck et al., 1992, 1995).

Corticosteroid Receptor Distribution and Regulation in the Hippocampus

There is some uncertainty concerning the relative distribution of corticosteroid receptors in the individual hippocampal subfields. Based on radioligand binding assays, MR binding density is greatest in subfield CA1 and least in subfield CA3. This is also supported by \textit{in situ} hybridization and immunohistochemistry investigations with probes for the MR mRNA and protein (Arriza \textit{et al.}, 1988; Van Eekelen \textit{et al.}, 1988; Herman \textit{et al.}, 1989a; Ahima \textit{et al.}, 1991). GR binding density is higher in subfield CA3 compared to CA1. However, the results from \textit{in situ} hybridization and immunohistochemistry experiments indicate that subfield CA3 has very little GR mRNA and immunoreactivity
(Fuxe et al., 1985; Van Eekelen et al., 1988; Herman et al., 1989a). In a recent immunohistochemistry investigation, Ahima and Harlan (1990) reported that GR labeling is equally intense throughout subfields CA1-CA3. There has been no attempts to resolve the differences in GR binding, immunoreactivity, and mRNA distribution in the hippocampus. Some of the possible explanations for the disparate results are differences in GR mRNA translation, stability, or post-translation modifications between subfields CA1 and CA3 (Herman et al., 1989a).

As reviewed above, the hippocampus is highly sensitive to corticosteroid receptor autoregulation compared to other brain areas. Interestingly, corticosteroid receptor autoregulation in the hippocampus is different between subfields CA1 and CA3. Corticosterone decreases corticosteroid receptor binding sites in hippocampal subfields CA1 and CA2, not in CA3 (Sapolsky and McEwen, 1985). Adrenalectomy increases GR and MR mRNA levels in subfield CA1 but not in subfield CA3 (Herman et al., 1989a).

**Hippocampus Influence on HPA Axis Activity**

Based on several lines of evidence, the hippocampus is an important site for HPA axis negative feedback regulation. First, corticosterone is capable of decreasing its own release by acting through the hippocampus. Corticosterone or dexamethasone implants into the dorsal hippocampus decreases adrenalectomy-induced ACTH secretion (Davidson and Feldman, 1967; Kovacs and Makara, 1988). Second, the hippocampus
exerts a negative influence on HPA axis activity. Dorsal hippocampectomy decreases
the diurnal variation in circulating corticosterone levels by increasing basal (nadir) ACTH
and corticosterone plasma levels (Fendler et al., 1961; Moberg et al., 1971; Fischette et
al., 1980; Wilson et al., 1980; Margarinos et al., 1987; Sapolsky et al., 1989;).
Hippocampectomy also increases CRF and vasopressin mRNA levels in the PVN
(Herman et al., 1989b, 1992).

Hippocampal lesions alter stress-induced HPA axis activity (Sapolsky et al., 1984b). Corticosterone levels increase during stress and return to pre-stress levels approximately
one hour after the stressor is terminated (Sapolsky et al., 1984b). Rats with hippocampal
lesions have higher circulating plasma corticosterone levels in response to stress and the
high level is maintained for at least four hours after the stressor is terminated (Kant et
al., 1984; Sapolsky et al., 1984b). A common test for corticosterone feedback inhibition
is the use of dexamethasone to suppress the stress-induced corticosterone secretion in
rats. When the hippocampus or fornix are lesioned, the efficacy of dexamethasone to
suppress stress-induced corticosterone secretion is significantly diminished (Feldman and
Conforti, 1976; Margarinos et al., 1987).

Finally, hippocampal regulation of the HPA axis is also supported by investigations
using electrical stimulation of the hippocampus. Electrical stimulation of subfield CA2-
CA3 or the dentate gyrus decreases while stimulating subfield CA1 increases plasma
corticosterone levels in rats (Kawakami et al., 1968; Dunn and Orr, 1984; Daniels et al., 1994).

Concluding Remarks

Several lines of evidence suggest that corticosteroids have an important role in modulating hippocampal neural activity. The hippocampus has the highest density of corticosteroid receptors in the brain. The distribution of MR and GR may be different between subfields CA1 and CA3; therefore, the two receptors may selectively modulate the hippocampal circuitry at specific sites. The hippocampus is a site of HPA axis negative feedback, influencing both circadian and stress driven corticosteroid secretion. However, it is not known how the feedback signal is processed in the hippocampus. Neuronal signal processing in the hippocampus is complex and corticosteroid modification of hippocampal neural activity is not completely understood.

Corticosteroid Modulation of Hippocampal Neuron Properties

Neuron Membrane Electrical Properties

One way that corticosteroids may influence signal processing in the hippocampus is by modulating the electrical properties of hippocampal neurons. Indeed, it has been demonstrated that corticosteroids alter neuron membrane electrical properties in hippocampal CA1 pyramidal cells. Chronic (two weeks) MR activation with basal levels of corticosterone increases the membrane input resistance and time constant of pyramidal
cells in subfield CA1 (Beck et al., 1994). The membrane input resistance and time constant determine the decay of synaptic potentials in the dendrites and soma, which in turn, influences the integration of postsynaptic potentials in the cell (Rall, 1977). Therefore, corticosteroids may alter the integration of postsynaptic potentials in subfield CA1 cells.

Chronic MR activation with basal levels of corticosterone also increases the action potential peak amplitude and duration (Beck et al., 1994). Altering the shape of the action potential may be physiologically important. Wheeler et al., (1996) recently reported that increasing the action potential duration leads to an increase in Ca\(^{2+}\) influx at the presynaptic terminal and enhances synaptic strength. Consequently, MR activation in subfield CA1 neurons may increase the release of neurotransmitters at the nerve terminal.

Corticosteroids alter two characteristics that rely on a Ca\(^{2+}\)-dependent K\(^+\) current: spike accommodation and slow afterhyperpolarization (sAHP) (Madison and Nicoll, 1984; Lancaster and Nicoll, 1987). When a prolonged (300 - 500 ms) depolarizing current pulse is injected into hippocampal pyramidal cells, the cell fires a short train of action potentials at the beginning of the pulse. The frequency of action potentials then decreases over the duration of the pulse. Accommodation is a term used to describe the neuron’s ability to adapt and prevent the firing of action potentials during the depolarizing pulse. Once the depolarizing pulse is terminated, a sAHP is apparent. Accommodation is determined by the magnitude and duration of the sAHP.
In subfield CA1, the sAHP is smaller in cells from adrenalectomized animals compared to cells from adrenally-intact animals (Joëls and de Kloet, 1989; Kerr et al., 1989). Short term (20 min to 4 hrs) and chronic MR activation decreases spike accommodation and the sAHP (Joëls and de Kloet, 1989, 1990; Kerr et al., 1989; Beck et al., 1994). MR and GR activation or GR activation alone increases accommodation and the sAHP (Joëls and de Kloet, 1989, 1990; Kerr et al., 1989).

The actions of corticosteroids on several ionic conductances in the hippocampus have also been examined. The L- and T- type Ca\(^{2+}\) currents are larger in cells from adrenalectomized as compared to intact animals (Karst and Joëls, 1994). Short-term MR activation returns the magnitude of the L- and T- type Ca\(^{2+}\) currents to control values (Karst and Joëls, 1994). Due to conflicting reports, the effects of GR activation on Ca\(^{2+}\) currents in the hippocampus is still unclear. There are reports that GR activation induces either larger (Kerr et al., 1992) or smaller (Karst and Joëls, 1994) Ca\(^{2+}\) currents in subfield CA1 pyramidal cells compared to cells from adrenalectomized animals. \(I_Q\) is also modulated by MR and GR activation. The \(I_Q\) current is small when either MR or GR are occupied alone but increases when both MR and GR are occupied (Karst et al., 1993).
Hippocampus $5\text{-HT}_{1A}$ Signal Transduction System in Subfields CA1 and CA3

In addition to altering cell membrane electrical properties, corticosteroids may also influence neural activity by modulating the responses elicited by the activation of neurotransmitter receptors. Corticosteroids have been shown to modulate neurotransmitter receptor systems in the brain including NA, muscarinic, glutamate, and 5-HT systems (reviewed by Joëls and de Kloet, 1994; McEwen, 1996). The action of corticosteroids on the $5\text{-HT}_{1A}$ receptor system has been reported by several different groups (Joëls and de Kloet, 1994; Chaouloff, 1995; Beck et al., 1996). However, the mechanism by which corticosteroids alter the responses elicited by the activation of the $5\text{-HT}_{1A}$ receptor is still not known. One reason the mechanism remains elusive is the effects of corticosteroids on the different components of the $5\text{-HT}_{1A}$ receptor signal transduction pathway are not known.

The hippocampus receives an extensive 5-HT innervation from the dorsal and median raphe (Azmitia and Segal 1978; Molliver, 1987). Stimulation of ascending 5-HT containing axons from the raphe decreases the cell firing rate of hippocampal subfield CA3 pyramidal cells (Segal, 1975). Locally applied 5-HT also decreases pyramidal cell firing rate (De Montigny and Aghajanian, 1978; Otmakhov and Bragin, 1982). Activation of the $5\text{-HT}_{1A}$ receptor in the hippocampus hyperpolarizes the cell membrane potential (Andrade and Nicoll, 1987; Colino and Haliwell, 1987; Beck and Choi, 1991; Beck et al., 1992). The $5\text{-HT}_{1A}$ receptor signal transduction system mediating the
hyperpolarization is composed of a receptor, heterotrimeric guanine nucleotide binding protein (G protein), and potassium channel (Andrade et al., 1986; Okuhara and Beck, 1994).

5-HT1A Receptor Structure and Distribution

The 5-HT1A receptor belongs to the family of G protein-coupled receptors (reviewed by Iismaa and Shrine, 1992). G protein-coupled receptors are single chain polypeptides that contain seven putative membrane-spanning domains. The human 5-HT1A receptor was first cloned in the late 1980's (Kobilka et al., 1987; Fargin et al., 1988). The rat 5-HT1A receptor was later cloned by two separate groups (Albert et al., 1990; Fujiwara et al., 1990) and encoded a protein approximately 420 amino acids in length.

There are three important features of G protein-coupled receptors which is also shared by the 5-HT1A receptor. First, the three dimensional arrangement of the seven transmembrane domains are proposed to form a ligand binding pocket (Hartig, 1989; Sylte et al., 1993) (Figure 5). The three dimensional arrangement places specific amino acid residues, located within the transmembrane domains, in areas critical for the interaction with receptor agonists and antagonists (Guan et al., 1992; Ho et al., 1992; Chanda et al., 1993). Second, the third cytoplasmic loop is the proposed site where G protein coupling occurs. The amino acids in the third cytoplasmic loop are highly divergent as compared with other G protein-coupled receptors (El Mestikawy et al.,
Figure 5. Diagrammatic representation of the 5-HT$_{1A}$ receptor. The seven transmembrane regions (cylinders labeled in roman numerals) are shown in a hypothetical arrangement. The amino terminal (N) is located outside the cell while the carboxy terminal (C) is in the cytoplasm. The third cytoplasmic loop is also displayed.
The amino acids bordering each end of the third cytoplasmic loop are thought to confer G protein selectivity (Strader et al., 1987; Kobilka et al., 1988; O'Dowd et al., 1988). Finally, 5-HT$_{1A}$ receptor binding is modulated by phosphorylation. In a cell line where the 5-HT$_{1A}$ receptor was stably transfected, protein kinase C activation phosphorylated and desensitized the receptor (Raymond, 1991).

The 5-HT$_{1A}$ receptor is expressed throughout the periphery and brain. The distribution of 5-HT$_{1A}$ receptors has been examined in several mammalian species (i.e., rat, pig, cat, mouse, and human) with similar results. The highest density of 5-HT$_{1A}$ binding sites and mRNA are located in limbic system structures: hippocampus, amygdala, septum, and cortical limbic areas (reviewed by Zifa and Fillion, 1992; Boess and Martin, 1994). Within the hippocampus, the number of 5-HT$_{1A}$ receptor binding sites is different between subfields CA1 and CA3. Subfield CA1 has approximately a two times higher density of receptor binding sites compared to CA3 (Vergé et al., 1986; Radja et al., 1991).

G protein Structure and Function

G proteins are heterotrimeric proteins that bind guanine nucleotides (reviewed by Birnbaumer and Birnbaumer, 1995). G proteins that are involved in receptor signal transduction systems function as transducers. These G proteins couple, or link, the activation of specific plasma membrane receptors to other cellular proteins (commonly referred to as effector proteins) located within the membrane.
The G protein complex is composed of three subunits: $\alpha$, $\beta$, and $\gamma$. The $\alpha$-subunit binds to guanosine triphosphate (GTP) and hydrolyzes it to guanosine diphosphate (GDP). The $\alpha$-GTP complex is capable of dissociating from the $\beta\gamma$ subunits (Fung, 1983; Northup et al., 1983a,b). The $\beta\gamma$ subunits anchor the G protein complex to the plasma membrane and facilitates the interaction between G proteins and receptors (Kanaho et al., 1984; Florio and Sternweis, 1985, 1989; Watkins et al., 1985; Navon and Fung, 1987). All three subunits have been reported to interact with effector molecules (reviewed by Birnbaumer, 1992; Birnbaumer and Birnbaumer, 1995). There are at least three major G protein families; $G_s$, $G_i$, and $G_q$. The G protein families, and their respective subtypes, were originally classified by their ability to either stimulate ($G_s$) or inhibit ($G_i$) adenylyl cyclase, or other effector molecules. Presently, they are classified by their $\alpha$-subunit sequences (reviewed by Birnbaumer et al., 1990).

Activation of receptors coupled to G proteins initiates a cycle which is the hallmark of G protein-coupled receptor signal transduction systems (reviewed by Birnbaumer and Birnbaumer, 1995). The signal transduction cycle is described in Figure 6. The activated receptor couples with a G protein. The G protein $\alpha$-subunit binds a molecule of GTP and begins to hydrolyze it to GDP. The $\alpha$-GTP complex dissociates from the $\beta\gamma$ subunits. The $\alpha$-GTP and/or $\beta\gamma$ subunits interact with effector molecules. The hydrolysis of GTP to GDP terminates the interaction of the $\alpha$-subunit with the effector molecule and the $\alpha\beta\gamma$ complex is reformed.
Figure 6. Cartoon illustration of the G protein cycle. 1. The activated receptor couples with the inactive G protein (GDP bound to the α-subunit). 2. The G protein α-subunit binds a molecule of GTP and begins to hydrolyze it to GDP. 3. The α-GTP complex dissociates from the βγ subunits. The α-GTP and/or βγ subunits interact with their effector proteins (E1 and E2). 4. The hydrolysis of GTP to GDP terminates the interaction of the α-subunit with the effector molecule. The mechanism (?) underlying the dissociation of the βγ subunits from their effector molecule is not known. 5. The G protein subunits dissociate from their effector proteins and reform the αβγ complex.
A tool that is commonly used to determine which G protein subtypes are involved in a receptor signal transduction system is the *Bordetella* pertussis toxin (PTX). PTX covalently modifies (process known as ADP-ribosylation) particular G protein α-subunits (i.e., Gi and Go, not Gs) and inhibits the coupling between the receptor and G protein (Gilman, 1984; Watkins *et al.*, 1985). Using PTX, it was demonstrated that the 5-HT1A receptor couples to a PTX-sensitive G protein in hippocampal subfields CA1 and CA3 (Andrade *et al.*, 1986; Okuhara and Beck, 1994).

**Potassium Channel Linked to the 5-HT1A receptor**

Activation of the 5-HT1A receptor on hippocampal subfield CA1 pyramidal cells evokes an inward rectifying potassium current that hyperpolarizes the cell membrane potential (Andrade and Nicoll, 1987; Colino and Haliwell, 1987; Beck *et al.*, 1992). Activation of the 5-HT1A receptor on subfield CA3 pyramidal cells also produces a hyperpolarization via an increase in an inward rectifying potassium current (Beck and Choi, 1991; Beck *et al.*, 1992; Okuhara and Beck, 1994).

Very little is known about the potassium channel linked to the 5-HT1A receptor signal transduction system. The channel is probably a member of the recently cloned G protein inward rectifying potassium (GIRK) channel family (Lesage *et al.*, 1994, 1995; Kobayashi *et al.*, 1995; Ponce *et al.*, 1996; Spausches *et al.*, 1996). The potassium channels linked to the 5-HT1A receptor in subfield CA1 appear to be activated by
G protein $\beta\gamma$ subunits, and the channel gating kinetics modified by ATP (Oh et al., 1995).

While the 5-HT$_{1A}$ receptor signal transduction system is composed of similar components in subfields CA1 and CA3, the pharmacological properties of the receptor is different between the two subfields. For example, 5-HT is less potent and elicits a greater hyperpolarization response in subfield CA3 cells compared to cells from subfield CA1 (Beck et al., 1992). 5-HT$_{1A}$ receptor antagonist that are competitive in subfield CA1 are insurmountable in subfield CA3 (Beck et al., 1992). The different pharmacological properties of the 5-HT$_{1A}$ receptor signal transduction system in subfield CA1 and CA3 may be due to differences in signal transduction components (i.e., G protein or potassium channel subtype) or coupling affinity between components.

Corticosteroid Modulation of the 5-HT$_{1A}$ Receptor System in Subfield CA1

Corticosteroids modulate the 5-HT$_{1A}$ receptor system in the hippocampus. Short-term (20 min to 4 hrs) activation of MR with basal levels of corticosterone or aldosterone decreases the 5-HT$_{1A}$ receptor-mediated hyperpolarization compared to cells from adrenalectomy or sham animals (Joëls et al., 1991, Joëls and de Kloet, 1992; Beck et al., 1996). Short-term GR activation does not affect the magnitude of the 5-HT$_{1A}$ receptor-mediated hyperpolarization (Joëls and de Kloet, 1992; Beck et al., 1996) but increases the EC$_{50}$ value of the 5-HT concentration-response curve (Beck et al., 1996).
Due to conflicting results, the effects of corticosteroids on the number of 5-HT$_{1A}$ binding sites is not clear. Adrenalectomy either increases (Chalmers et al., 1993; Tejani-Butt and Labow, 1994) or has no effect on the number of 5-HT$_{1A}$ binding sites in subfield CA1 (de Kloet et al., 1986b; Mendelson and McEwen, 1992a,b; Meijer and de Kloet, 1994; Kuroda et al., 1994; Holmes et al., 1995b). In the reports where adrenalectomy increased the number of 5-HT$_{1A}$ binding sites, corticosterone treatment reversed the adrenalectomy-induced effect.

The corticosteroid-induced alterations in the 5-HT$_{1A}$ receptor-mediated responses cannot always be explained by changes in the number of 5-HT$_{1A}$ receptor binding sites. For example, an increase in the number of 5-HT$_{1A}$ receptor binding sites (Chalmers et al., 1993; Tejani-Butt and Labow, 1994) in adrenalectomized animals would predict a greater or more sensitive 5-HT$_{1A}$ receptor-mediated response. However, there is no difference in the 5-HT$_{1A}$ receptor-mediated hyperpolarization between adrenalectomized and adrenally-intact animals (Joëls et al., 1991, Joëls and de Kloet, 1992; Beck et al., 1996). Therefore, corticosteroids may be altering the 5-HT$_{1A}$ receptor signal transduction system at sites downstream of the receptor.

In addition to changes in the number of 5-HT$_{1A}$ receptor binding sites, corticosteroids may alter G protein expression or activity. The regulation of G protein levels by glucocorticoids has been examined in several tissue types including liver (Kawai et al., 93), aorta (Haigh et al., 1990) vascular smooth muscle (Schelling et al., 1994),
fetal forebrain tissue (Slotkin et al., 1994), cortex (Saito et al., 1989), and hippocampus (Wolfgang et al., 1994; Gannon et al., 1995).

The action of glucocorticoids on G protein expression is different in the cortex and hippocampus. In the cerebral cortex, adrenalectomy decreases $G_i$, increases $G_s$, and has no effect on $G_o \alpha$-subunit levels when compared to sham (Saito et al., 1989). Corticosterone replacement reversed the adrenalectomy-induced effects on G protein levels in the cortex. In contrast to the cortex, adrenalectomy has no effect on $G_i$ and $G_s$ $\alpha$-subunit levels in the hippocampus (Gannon et al., 1994). Only one study has examined the effects of high corticosteroid exposure on G protein levels in the hippocampus. Chronic stress increases $G_s$ and $G_o$ but does not affect $G_i \alpha$-subunit levels (Wolfgang et al., 1994).

Finally, corticosteroids may also alter effector molecule activity. To date, corticosteroid-induced changes were only examined in effector proteins that regulate second messenger production. For example, glucocorticoids alter adenylyl cyclase activity and mRNA levels in the rat hippocampus (Gannon et al., 1994; Wolfgang et al., 1994). The effects of corticosteroids on the properties of the potassium channel linked to the 5-HT$_{1A}$ receptor signal transduction system in the hippocampus are not known.
Concluding Remarks

Corticosteroids modulate hippocampal neural activity at two known levels. First corticosteroids alter the electrical membrane properties of pyramidal cells. Second they modulate the responses elicited by the activation of neurotransmitter receptors. Current research has focused on the action of corticosteroids on the electrical membrane properties and responses elicited by the activation of the 5-HT$_{1A}$ receptor on hippocampal pyramidal cells in subfields CA1. Nothing is known about effects of corticosteroids on the electrical membrane properties and 5-HT$_{1A}$ receptor-mediated responses in subfield CA3. This deficiency of information makes it difficult to elucidate the role of corticosteroids in hippocampal information processing. As reviewed in the previous section (The Hippocampus and HPA Axis Feedback Regulation) signals in the hippocampus are processed along a trisynaptic pathway; the neurons and the local network in each subfield have distinct properties. Therefore, it is important to determine the effects of corticosteroids in each subfield to understand how neural signaling in the hippocampus is influenced by the steroids.

Clinical Importance

Corticosteroids maintain homeostasis by regulating sugar metabolism, ion balance, immune responses and the responses to stress. Prolonged altered corticosteroid secretion induces pathological conditions that often become neurotoxic (Uno et al., 1994). There are several conditions that are associated with altered corticosterone secretion including
prolonged or chronic stress, senescence, and depression. The following section reviews the current information on stress, senescence, and depression and their relationship to corticosteroids.

**Stress**

One of the key physiological responses to stress is an increase in corticosteroid secretion, thereby increasing energy sources and limiting non-vital processes (Selye, 1936, 1946; Munck et al., 1984). Normally, corticosterone levels are brought back to basal levels through feedback regulation. Prolonged stress is known to produce brain damage and psychiatric disorders (reviewed by Sapolsky, 1992). Cerebral cortical atrophy and hippocampal cell loss are commonly found in the brains of torture or abuse victims (Jensen et al., 1982; Uno et al., 1989). Cognitive functions are also disrupted with prolonged stress (reviewed by McEwen and Sapolsky, 1995). For example, certain types of learning and memory performances are impaired in rats exposed to repeated stress (Luine et al., 1994a,b).

Hippocampal neurons are particularly sensitive to prolonged stress and high concentrations of glucocorticoids. Stress or high glucocorticoid levels induces neuronal cell loss, (Sapolsky et al., 1985a; Uno et al., 1989; Stein-Behrens et al., 1995) atrophy, and reduced branching of subfield CA3 pyramidal cell apical dendrites (Woolley et al., 1990; Watanabe et al., 1992; Magarinos and McEwen, 1995a,b). Furthermore, stress or
high glucocorticoid levels exacerbate hippocampal cell damage induced by a number of different insults, i.e., ischemia (Sapolsky, 1985).

The exact mechanism underlying the toxic effects of glucocorticoids in hippocampal subfield CA3 are not known. There is some indication that part of the deleterious effects of glucocorticoids is due to the disruption of energy availability (Sapolsky, 1986; Kadekaro, et al., 1988; Freo et al., 1992). Another important factor in glucocorticoid-induced toxicity in the hippocampus is the involvement of excitatory amino acid neurotransmitters and calcium. Briefly, glucocorticoids increase excitatory amino acid neurotransmission and they impair the ability of hippocampal neurons to maintain intracellular calcium concentrations (reviewed by Sapolsky, 1994).

Senescence

Senescence is associated with elevated HPA axis activity in rats (reviewed by Sapolsky et al., 1986; Sapolsky, 1992) and humans (Raskind et al., 1994). Aged rats display higher ACTH and/or corticosterone plasma levels, and prolonged corticosterone plasma levels following the removal of the stressor (Sapolsky et al., 1984b; 1985b; Landfield, 1987).

The mechanism underlying the senescence-induced changes in HPA axis activity involves the hippocampus (reviewed by Landfield and Eldridge, 1994). The hippocampus in aged rats display several physiological changes that may influence HPA axis activity. For example, hippocampal corticosteroid receptor binding is decreased in aged
rats (Sapolsky et al., 1983a; Rigter et al., 1984; Reul et al., 1988) and their regulation is altered (Eldridge et al., 1989a,b). The changes in HPA axis activity in senescent rats resemble the changes that occur with hippocampectomy (Fendler et al., 1961; Feldman and Conforti 1976, 1980; Kant et al., 1984; Sapolsky et al., 1984b, 1989; Margarinos et al., 1987). An age related loss in hippocampal neurons has also been reported (Coleman et al., 1987; Sapolsky, 1992).

Based on the association between high glucocorticoid levels and hippocampal neuron damage with stress and aging, Sapolsky and colleagues (1986) put forth a glucocorticoid cascade hypothesis of neuronal damage. Briefly, a prolonged exposure to elevated glucocorticoid levels, due to either stress or prolonged excitatory neural inputs into the HPA axis, decreases corticosteroid receptor levels in the hippocampus. The reduced corticosteroid receptor levels attenuate HPA axis feedback, further prolonging the elevated glucocorticoid exposure. Eventually, the glucocorticoids produce toxic effects (discussed above), inducing hippocampal cell loss.

Depression

Altered HPA axis activity is often associated with depression. The abnormalities of the HPA system in depression are manifested as an: 1) increased level of cortisol in the urine (free cortisol), plasma, and cerebral spinal fluid; 2) alterations in cortisol levels during the diurnal rhythm; 3) an attenuation of the CRF-induced release of ACTH; and 4) decreased ability of dexamethasone to suppress cortisol secretion (reviewed by
Murphy 1991a; Checkley et al., 1992). Similar abnormalities are also observed in aged rats (Issa et al., 1990).

It is not known why the HPA axis activity is elevated in depression, or how corticosteroids and depression are linked. However, several lines of evidence implicate corticosteroids in the etiology and treatment of depression (Barden et al., 1995). For example, suppressing hypercortisol secretion has been reported to abate depression in several independent studies (Jeffcote et al., 1979; Kelly et al., 1980; 1983; Nieman et al., 1985; Murphy 1991b; Murphy et al., 1991a,b). Also chronic treatment with antidepressant agents increases MR and GR binding capacity (Reul et al., 1993) and mRNA levels in the rat hippocampus (Brady et al., 1991; Seckl and Fink, 1992; Yau et al., 1995).

Cushing Syndrome and Hypercorticosteroid Secretion

Drugs targeting the HPA axis may be a viable resource for treating pathological conditions associated with hypercorticosteroid secretion. It is well documented that hypersecretion of corticosteroids is associated with prolonged stress, aging, and depression. However, it has been difficult for investigators to show directly that hypercorticosteroid secretion can precipitate these conditions. The best evidence available supporting a direct link between hypercorticosteroid secretion with stress and depression is derived from Cushing’s syndrome patients.
One of the clinical features of Cushing's syndrome is a persistent elevation in plasma corticosteroid levels (Cushing, 1932). These patients also display many of the same features characteristic of prolonged stress. For example, cerebral cortical atrophy and hippocampal cell loss are commonly found in the brains of patients with Cushing's syndrome (Jensen et al., 1982; Uno et al., 1989; Starkman et al., 1992). Depression is a clinical symptom in approximately half of the Cushing's syndrome cases (Jeffcoate et al., 1979; Cohen, 1980) and suppressing the hypercorticosteroid secretion in these patients dramatically reduces their depression (Jeffcote et al., 1979; Kelly et al., 1980, 1983; Nieman et al., 1985).

Concluding Remarks

The hippocampus contains the highest density of corticosteroid receptors in the brain, which leads to the suggestion that corticosteroids may have a profound influence on hippocampal signal processing. Previous investigations have demonstrated that corticosteroids modulate several cell membrane electrophysiological characteristics and responses elicited by the activation of several different neurotransmitter receptors on pyramidal cells in the hippocampus (Joëls et al., 1991, Joëls and de Kloet, 1992; Beck et al., 1994; Birnstiel and Beck, 1995; Beck et al., 1996). The mechanism by which corticosteroids modulate signal processing in the hippocampus is not completely resolved. One piece of information that is missing is the effects of corticosteroids on neural activity in all of the different hippocampal regions and subfields. The effects of
corticosteroids on pyramidal cell membrane electrophysiological properties and 5-HT$_{1A}$ receptor signal transduction system are not known in subfield CA3. This is an important point since the neurons in subfield CA3 have a critical role in processing synaptic information based on their unique synaptic organization (Knowles, 1992; Bernard and Wheal, 1994), electrophysiological characteristics (Nunez et al., 1990; Beck et al., 1992; Spruston and Johnston, 1992) and responses elicited by the activation of neurotransmitter receptors (Beck et al., 1992, 1995). Information on the actions of corticosteroids on pyramidal cell membrane electrophysiological properties and the 5-HT$_{1A}$ receptor signal transduction system will contribute to a basic understanding of how corticosteroids alter cell excitability, alter responses elicited by the activation of G protein-linked neurotransmitter receptors, and will help to elucidate the role of corticosteroid receptors in information processing in the hippocampus.
CHAPTER III

CORTICOSTEROIDS ALTER PYRAMIDAL CELL ELECTROPHYSIOLOGICAL CHARACTERISTICS IN HIPPOCAMPAL SUBFIELD CA3

Abstract

The hippocampus contains the highest density of MR and GR in the central nervous system. The modulation of neuron excitability by corticosteroids in hippocampal subfield CA1 is well documented. However, it is not known if corticosteroids produce different effects across the various hippocampal subfields. Therefore, we used intracellular recording techniques to examine the actions of chronic corticosteroid treatment (2 weeks) on the electrophysiological properties of rat hippocampal subfield CA3 pyramidal cells. The treatment groups used in this investigation were, adrenalectomy (ADX), selective MR activation with aldosterone (ALD), MR and GR activation with high levels of corticosterone (HCT), and SHAM.

Corticosteroid treatment altered the percentage of nonburst and burst firing neurons. The largest differences were observed with ADX animals where the percentage of nonbursting cells was 74% compared to 42% and 41% in ALD and SHAM animals. Corticosteroids did not alter the membrane input resistance,
resting potential, or time constant. Corticosteroids also had no effect on the slow- or fast-afterhyperpolarizing potential amplitude or duration. The action potential duration was longer in cells from ADX animals compared to cells from HCT and ALD animals. Based on our results, we conclude that corticosteroids altered the firing mode and decreased the action potential duration in subfield CA3 pyramidal cells. Both of these findings are novel results which are distinct from those previously reported for subfield CA1, suggesting that corticosteroids have different effects across hippocampal subfields.

Introduction

Corticosteroids (glucocorticoids and mineralocorticoids) are steroid hormones that maintain homeostasis by influencing energy production and electrolyte balance (Henkin, 1970; Munck et al., 1984, de Kloet, 1991). Corticosteroids are synthesized in the adrenal cortex and their release regulated by the hypothalamic-pituitary-adrenal (HPA) axis. Corticosteroids regulate gene expression through two receptor subtypes: MR (or type I) and GR (or type II) (Joëls et al., 1994b). MR has a high affinity for corticosterone ($K_D = 0.5 - 0.7$ nM) and is 70-80% occupied at basal physiological corticosterone plasma levels (Krozowski and Funder, 1983; Reul and de Kloet, 1985; Reul et al., 1987a,b). GR has a lower affinity for corticosterone, $K_D = 2.5 - 5$ nM, and its occupancy changes from 15% at basal to 90% at peak and stress circulating corticosterone levels (Reul and de Kloet, 1985; Reul et al., 1987a,b).
The hippocampus contains the highest concentration of corticosteroid receptors in the CNS (McEwen et al., 1968; Warembourg, 1975; Sapolsky et al., 1983b; Sarrieau et al., 1984; Reul and de Kloet, 1985, 1986; Magarinos et al., 1989). Corticosteroids have a profound influence on cells in the hippocampus (de Kloet et al., 1993; McEwen et al., 1993). The effects of MR and GR activation on pyramidal cell excitability and ionic currents are well documented for subfield CA1. Generally, MR activation increases CA1 pyramidal cell excitability while GR activation decreases cell excitability (Beck et al., 1994; Joëls and de Kloet, 1994).

It is not known if corticosteroids have a different effect on neuron electrophysiological properties in subfield CA3. This is an important issue since the hippocampus processes information along a trisynaptic lamellar pathway (Andersen et al., 1971b; Andersen, 1975) with the pyramidal cells in subfield CA1 receiving their inputs via Schaffer collaterals from the pyramidal cells in subfield CA3. It is important to determine how corticosteroids modulate synaptic transmission along this pathway. Neurons in each subfield have a distinct role in processing synaptic transmission based on their different local interneuron circuits (Knowles, 1992), electrophysiological characteristics (Brown et al., 1981; Nunez et al., 1990; Beck et al., 1992; Spruston and Johnston, 1992) and neurotransmitter-mediated responses (Beck et al., 1992, 1995). Furthermore, the MR/GR ratio is greater in subfield CA1 (6.5:1) compared to CA3 (1:1) as determined by a receptor binding investigation (Reul and de Kloet, 1985).
We used intracellular recording techniques to examine the effects of chronic corticosteroid treatment on the electrophysiological properties of pyramidal cells in hippocampal subfield CA3. Our hypothesis is that corticosteroids will produce a different modulatory action in subfield CA3 neurons as compared to those previously reported for subfield CA1. These results will contribute to a basic understanding of how corticosteroids alter cell excitability and will help to elucidate the role of corticosteroid receptors in information processing in the hippocampus.

Materials and Methods

Materials

Corticosterone pellets were purchased from Innovative Research of America (Sarasota, FL). Aldosterone was purchased from Steraloids Inc. (Wilton, NH). Osmotic minipumps were purchased from Alzet Cooperation (Palo Alto, CA). All other chemicals were obtained from standard commercial sources.

Animals

Four different treatment groups were used for the experiments in this investigation (Table 1). The adrenalectomy was performed as described previously (Beck et al., 1994, 1996; Birnstiel and Beck, 1995). Male Sprague-Dawley rats (Harlan) 75-100g were used for all treatment groups. Bilateral adrenalectomies were performed on all treatment groups, except the SHAM group, to remove circulating corticosteroids.
Table 1.—Treatment groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surgery</th>
<th>Implant</th>
<th>ACSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>Intact</td>
<td>Nothing</td>
<td>1 nM corticosterone</td>
</tr>
<tr>
<td>ADX</td>
<td>Adrenalectomy</td>
<td>Nothing</td>
<td>no steroid</td>
</tr>
<tr>
<td>ALD</td>
<td>Adrenalectomy</td>
<td>Aldosterone (10 µg/h minipump)</td>
<td>3 nM aldosterone</td>
</tr>
<tr>
<td>HCT</td>
<td>Adrenalectomy</td>
<td>200 to 300 mg corticosterone</td>
<td>100 nM corticosterone</td>
</tr>
</tbody>
</table>

Animals were anesthetized with ether. A small incision was made into the abdominal cavity, just below the rib cage, and the adrenal glands carefully removed. The muscle wall was then sutured and the skin closed using wound clips. The ADX group received no further treatment. The ALD group had an osmotic minipump containing aldosterone (Alzet model 2002) implanted subcutaneously at the time of adrenalectomy, to selectively activate MR. The minipump delivered 10 µg/h aldosterone dissolved in propylene glycol. MR and GR were activated in another group of animals (HCT) by subcutaneously implanting 200-300 mg corticosterone pellets, 2 to 3 week release, in the back of the neck at the time of adrenalectomy. The SHAM group of animals was produced by visualizing the adrenal glands but leaving them intact. After surgery the animals were maintained on a standard twelve hour light/dark cycle and rat chow, ad libitum. SHAM animals were given standard drinking water while the ADX, ALD, and HCT animals were given 0.9 % NaCl drinking water.
ad libitum. At the end of 13 - 15 days, the animals were sacrificed in the morning and hippocampal slices immediately prepared for electrophysiological recording. At the time of sacrifice, trunk blood was collected to determine the plasma corticosterone levels by radioimmune assay (Burgess and Handa, 1992).

Hippocampal Slice Preparation

Hippocampal slices were prepared for electrophysiological recording as previously described (Okuhara and Beck, 1994). Rats were sacrificed by decapitation and the brain rapidly removed and placed in ice cold artificial cerebrospinal fluid (ACSF) containing in mM: NaCl 125, KCl 3, NaH₂PO₄ 1.25, MgSO₄ 2, CaCl₂ 2.5, dextrose10, and NaHCO₃ 28. The ACSF was also supplemented with steroids, as outlined in Table 1, to maintain the treatment paradigm. We previously reported that steroids must be present in the ACSF to preserve the corticosteroid-induced effects on neuron cell properties (Beck et al., 1994). The hippocampus was dissected free and the dorsal section cut in 500 - 550 µm sections on a vibratome. The hippocampal slices were then placed in a holding vial containing ACSF bubbled with 95% O₂-5% CO₂ at room temperature. The slices remained in the holding vial for at least one hour after dissection before being transferred to the recording chamber. In the recording chamber, the slice was stabilized between two nylon nets and continuously perfused with ACSF bubbled with 95% O₂-5% CO₂ at a rate of 2-3 ml/min, at 31-32°C.
Intracellular Recording

Intracellular recordings were made as previously described (Okuhara and Beck, 1994). Electrodes were pulled from borosilicate capillary tubing on a Brown and Flaming electrode puller (Sutter Instruments) to a resistance of 30-35 MΩ (2 M KCl). Pyramidal cells were impaled with brief ejections of positive current through the electrode. The impaled cells were sealed by applying 1 nA hyperpolarizing current. Electrical signals were collected and amplified using an Axoclamp 2A and Cyberamp 320 amplifier (Axon Instruments, Foster City, CA) and recorded on a Gould Series 3200 chart recorder (Gould Electronics, Valley View, OH). Data were collected online with pCLAMP software (Axon Instruments).

Statistical Analysis

Statistical comparisons were performed using analysis of variance (ANOVA). The Student-Newman-Keuls method was used for post-hoc tests. A chi-square test was used to determine if the percentage of bursting and nonbursting cells was different with corticosterone treatment. All values are reported as mean ± S.E.M. A p < 0.05 was considered significant.

Results

Corticosterone Plasma Levels

SHAM corticosterone plasma levels ranged from 0 - 4.13 µg/dl (1.23 ± 0.24 µg/dl,
n = 22). ADX corticosterone plasma levels ranged from 0 - 0.5 µg/dl (0.21 ± 0.05 µg/dl, n = 20). ALD corticosterone plasma levels ranged from 0 - 0.6 µg/dl (0.12 ± 0.05 µg/dl, n = 18). The lower and upper limits of the corticosterone radioimmune assay were 0.05 and 50 µg/dl respectively. Adrenalectomies that produced corticosterone concentrations ≤ 0.6 µg/dl were considered successful. Two SHAM, 12 ADX, and 13 ALD animals had corticosterone levels less than 0.05 µg/dl. The concentration of aldosterone used in our experiments was based on an investigation by Kuroda et al., (1994). The ALD treatment decreased MR binding by 61% (n = 5 animals) in the cytoplasm (data not shown) as determined using a homogenate binding assay. HCT plasma concentrations greater than 20 µg/dl were considered successful. HCT corticosterone plasma levels ranged from 25 to > 50 µg/dl (39.96 ± 2.90 µg/dl, n = 11).

Passive Membrane Properties

The analysis of cell characteristics was as described previously (Beck et al., 1994). Two different firing modes have been described for neurons in subfield CA3 (Wong and Prince, 1978, 1981; Masukawa et al., 1982; Bilkey and Schwartzkroin, 1990). One type of firing mode is a regular spiking pattern of action potentials in response to a long depolarizing pulse (nonbursting cells) while the other mode is a cluster of spikes which reside on a slow depolarizing potential (bursting cells). We also observed burst
and nonburst firing in the present investigation (Figure 7). A chi-square analysis indicated that the percentage of bursting and nonbursting cell types was dependent on the type of corticosteroid treatment (Table 2). Since it is not known if corticosteroids have different effects on nonbursting and bursting cell types, the pyramidal cell characteristics data were divided into nonbursting and bursting cell types. The data were first analyzed using a two-way ANOVA with treatment as one variable and cell type as the other variable. If there were no significant effects between cell type, the data were then analyzed using one-way ANOVA, collapsing across cell type and grouping according to treatment only.

The membrane potential was read directly from the amplifier display. Corrections for the input offset were done after the electrode was withdrawn from the cell. The membrane input resistance and membrane time constant were calculated from current-voltage plots. The current-voltage plot data were generated by injecting 300 ms pulses of -900 to 0 pA in 100 pA increments into the cell while recording the cell's membrane potential. The membrane input resistance was calculated from the slope of the linear portion of the current-voltage plots. The membrane time constant corresponded to the length of time required for the membrane potential to reach 63% of its final value during a 300 ms pulse of -100 pA. There were no differences in resting membrane potential, input resistance, or membrane time constant between either cell type or corticosteroid treatment (Table 3).
Figure 7. Membrane potential changes in a nonbursting and burst firing cell. 
A: Nonbursting cell. B: Bursting cell. The cells received an injection of -700 and 
400 pA current pulses, 300 ms in duration. Plots were generated from digitized data, 
therefore, action potentials appear truncated. Notice the slow depolarizing envelope in 
the bursting cell (arrow).
Table 2.—Number of nonbursting and bursting cells recorded from the different corticosterone treatment groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SHAM</th>
<th>ADX</th>
<th>ALD</th>
<th>HCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>nonbursting</td>
<td>13</td>
<td>29</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>bursting</td>
<td>19</td>
<td>10</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>% nonbursting</td>
<td>41</td>
<td>74</td>
<td>42</td>
<td>62</td>
</tr>
</tbody>
</table>

Proportion of nonbursting and bursting cells are different between treatment groups.

$$\chi^2 = 9.592, \text{ d.f.} = 3, P = 0.022.$$ 

Table 3.—The effects of corticosteroids on passive membrane electrophysiological properties

<table>
<thead>
<tr>
<th>Treatment &amp; cell type</th>
<th>RMP (mV)</th>
<th>Resistance (MΩ)</th>
<th>Tau (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM NB</td>
<td>68.4 ± 1.4 (13)</td>
<td>50.1 ± 2.8 (13)</td>
<td>49.3 ± 4.2 (13)</td>
</tr>
<tr>
<td>B</td>
<td>72.0 ± 1.2 (19)</td>
<td>51.0 ± 1.9 (19)</td>
<td>49.3 ± 3.6 (19)</td>
</tr>
<tr>
<td>ADX NB</td>
<td>68.8 ± 0.9 (29)</td>
<td>51.6 ± 2.3 (29)</td>
<td>42.8 ± 2.4 (29)</td>
</tr>
<tr>
<td>B</td>
<td>69.3 ± 1.4 (10)</td>
<td>48.6 ± 3.0 (10)</td>
<td>53.4 ± 5.7 (10)</td>
</tr>
<tr>
<td>ALD NB</td>
<td>68.4 ± 1.8 (11)</td>
<td>45.2 ± 2.3 (11)</td>
<td>43.8 ± 5.4 (11)</td>
</tr>
<tr>
<td>B</td>
<td>71.7 ± 1.2 (15)</td>
<td>49.8 ± 2.9 (15)</td>
<td>48.1 ± 6.0 (15)</td>
</tr>
<tr>
<td>HCT NB</td>
<td>68.6 ± 1.5 (16)</td>
<td>54.7 ± 1.7 (16)</td>
<td>44.5 ± 4.3 (15)</td>
</tr>
<tr>
<td>B</td>
<td>68.6 ± 2.1 (10)</td>
<td>47.7 ± 4.8 (9)</td>
<td>55.1 ± 6.1 (9)</td>
</tr>
</tbody>
</table>

All values are reported as mean ± SEM (n)

Cell type NB, nonbursting; B, bursting
Table 4 summarizes the effects of corticosteroids on action potential and fAHP properties. Action potentials were generated with short 2-4 ms depolarizing pulses. The action potential threshold was the membrane potential value just before the action potential spike began to rise. The action potential threshold was used as the baseline value to calculate its height and baseline duration. The action potential height was measured from baseline to the peak of the spike. The action potential half-width was the width of the spike at 50% of its peak amplitude. The action potential baseline duration was the spike width along its baseline. fAHP’s were generated with 20 ms pulses of enough current (300-600 pA) to generate a single action potential. The fAHP amplitude was measured from threshold to the peak hyperpolarizing amplitude which occurred 1-4 ms after the end of the spike. The fAHP t_{1/2} corresponds to the amount of time for the fAHP to decrease from its peak amplitude to one-half of its value.

Based on a two-way ANOVA examination, neither corticosteroid treatment nor cell type had an effect on the action potential threshold. The action potential peak amplitude was smaller in bursting cells (80.3 ± 0.7 mV, n = 52.) compared to nonbursting cells (82.7 ± 0.6 mV, n = 69) (F = 6.252; d.f. = 1, 113; P = 0.014). However, corticosteroid treatment had no effect on the action potential peak amplitude (F = 2.021; d.f. = 3, 113; P = 0.115). Corticosteroid treatment did alter the action potential duration. The action potential half- and baseline widths were wider in cells
from ADX animals compared to cells from ALD and HCT animals (Figure 8). The action potential half-width was also wider in cells from SHAM animals compared to ALD.

sAHP and Accommodation

The effects of corticosteroids on sAHP and accommodation properties are summarized in Table 5. sAHP’s were generated by injecting 300-500 ms pulses of 800-1200 pA to elicit a train of sodium action potentials. A train of sodium action potentials usually generate two types of afterhyperpolarizing potentials, a medium-afterhyperpolarizing potential followed by a sAHP (Madison and Nicoll, 1984). Therefore, the sAHP amplitude was measured after the medium-afterhyperpolarizing potential ended (approximately 100 to 250 ms after the current pulse terminated). The sAHP t_{1/2} corresponds to the amount of time for the sAHP to decrease from its peak amplitude to one-half of its baseline value. Spike accommodation corresponds to the number of action potentials that occurred during a 300 ms depolarizing pulse.

Two-way ANOVA analysis of the data indicated that the sAHP amplitude was smaller in nonbursting cells (10.8 ± 0.5 mV, n = 65) compared to bursting cells (12.4 ± 0.5 mV, n = 38) (F = 5.822; d.f. = 1, 111; P = 0.017). Consistent with the larger sAHP in bursting cells, accommodation was also greater in bursting cells (4.5 ± 0.47 spikes, n = 53) compared to nonbursting cells (5.7 ± 0.3, n = 64) (F = 5.743; d.f. = 1, 110;
Table 4.—The effect of corticosteroids on action potential and fAHP properties.

<table>
<thead>
<tr>
<th>Treatment &amp; cell type</th>
<th>Action Potential</th>
<th>fAHP</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Threshold (mV)</td>
<td>Amplitude (mV)</td>
<td>Half-widtha (µs)</td>
<td>Baseline widthb (ms)</td>
<td>Amplitude (mV)</td>
<td>t1/2 (ms)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHAM NB</td>
<td>50.9 ± 0.6 (13)</td>
<td>82.2 ± 1.4 (13)</td>
<td>963 ± 40 (13)</td>
<td>1.67 ± 0.06 (13)</td>
<td>7.8 ± 0.4 (13)</td>
<td>3.3 ± 0.3 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>50.8 ± 0.4 (19)</td>
<td>79.7 ± 0.8 (19)</td>
<td>926 ± 20 (19)</td>
<td>1.60 ± 0.04 (19)</td>
<td>7.7 ± 0.4 (17)</td>
<td>3.0 ± 0.2 (17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADX NB</td>
<td>51.4 ± 0.5 (29)</td>
<td>84.0 ± 1.1 (29)</td>
<td>975 ± 20 (28)</td>
<td>1.68 ± 0.04 (28)</td>
<td>7.8 ± 0.6 (26)</td>
<td>2.9 ± 0.2 (26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>51.7 ± 0.7 (10)</td>
<td>82.5 ± 0.5 (10)</td>
<td>940 ± 30 (10)</td>
<td>1.64 ± 0.07 (10)</td>
<td>6.7 ± 0.5 (9)</td>
<td>2.4 ± 0.2 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALD NB</td>
<td>51.9 ± 0.7 (11)</td>
<td>83.5 ± 1.3 (11)</td>
<td>877 ± 20 (11)</td>
<td>1.52 ± 0.04 (11)</td>
<td>7.2 ± 0.8 (11)</td>
<td>2.7 ± 0.2 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>50.1 ± 0.5 (14)</td>
<td>79.9 ± 1.4 (14)</td>
<td>862 ± 20 (14)</td>
<td>1.49 ± 0.05 (14)</td>
<td>7.6 ± 0.5 (15)</td>
<td>2.9 ± 0.3 (15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCT NB</td>
<td>52.0 ± 0.7 (16)</td>
<td>81.0 ± 1.3 (16)</td>
<td>896 ± 20 (16)</td>
<td>1.53 ± 0.04 (16)</td>
<td>9.0 ± 0.6 (14)</td>
<td>2.8 ± 0.2 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>51.7 ± 0.6 (9)</td>
<td>79.2 ± 1.4 (9)</td>
<td>896 ± 30 (9)</td>
<td>1.58 ± 0.06 (9)</td>
<td>8.4 ± 0.7 (10)</td>
<td>3.1 ± 0.4 (10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values are reported as mean ± SEM (n)

Cell types NB, nonbursting; B, bursting

* ADX (966 ± 20 µs, n =38) different from ALD (869 ± 10 µs, n = 25) and HCT (896 ± 20 µs, n = 25)
  SHAM (941 ± 20 µs, n =32) different from ALD. One-way ANOVA F = 4.419; d.f. = 3,113; P = 0.006.
  Student-Newman-Keuls p < 0.05

b ADX (1.67 ± 0.04 ms, n = 38) different from ALD (1.51 ± 0.03 ms, n = 25) and HCT (1.55 ± 0.03 ms, n = 15)
Corticosteroid treatment had no effect on the sAHP amplitude ($F = 2.096$, d.f. = 3, 111; $P = 0.105$) or accommodation properties ($F = 0.04$; d.f. = 3, 110; $P = 0.989$).

Figure 8. Superimposed action potentials in a cell from an ADX and HCT animal. Each trace represents an average of five action potentials in a cell from either an ADX (solid line) or HCT (broken line) treated animal.
Table 5.—The effect of corticosteroids on sAHP and accommodation properties

<table>
<thead>
<tr>
<th>Treatment &amp; cell type</th>
<th>sAHP Amplitude (mV)</th>
<th>sAHP t₁/₂ (ms)</th>
<th>Accommodation (no. of spikes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>NB 11.8 ± 0.9 (13)</td>
<td>1001 ± 86 (6)</td>
<td>5.2 ± 0.6 (13)</td>
</tr>
<tr>
<td></td>
<td>B 13.7 ± 0.8 (19)</td>
<td>945 ± 55 (11)</td>
<td>5.1 ± 0.4 (19)</td>
</tr>
<tr>
<td>ADX</td>
<td>NB 11.7 ± 0.6 (28)</td>
<td>1126 ± 84 (19)</td>
<td>6.4 ± 0.7 (26)</td>
</tr>
<tr>
<td></td>
<td>B 11.3 ± 1.1 (10)</td>
<td>961 ± 65 (9)</td>
<td>4.0 ± 0.7 (9)</td>
</tr>
<tr>
<td>ALD</td>
<td>NB 9.9 ± 1.2 (11)</td>
<td>1186 ± 238 (10)</td>
<td>4.9 ± 0.5 (11)</td>
</tr>
<tr>
<td></td>
<td>B 13.8 ± 1.1 (15)</td>
<td>1022 ± 55 (12)</td>
<td>4.1 ± 0.5 (15)</td>
</tr>
<tr>
<td>HCT</td>
<td>NB 9.7 ± 1.0 (13)</td>
<td>853 ± 106 (11)</td>
<td>6.3 ± 0.9 (14)</td>
</tr>
<tr>
<td></td>
<td>B 10.9 ± 1.1 (9)</td>
<td>1052 ± 58 (6)</td>
<td>3.9 ± 0.5 (10)</td>
</tr>
</tbody>
</table>

Discussion

We examined the effects of corticosteroids on pyramidal cell electrical properties in subfield CA3. The primary finding was that the percentage of nonbursting and bursting cell types was dependent upon corticosterone treatment. Therefore, we analyzed the cell characteristics according to treatment and cell type. There were several cell characteristics that were different between nonbursting and bursting cell types. Nonbursting cells had a larger action potential peak amplitude, less accommodation, and a smaller sAHP amplitude compared to bursting cells. However, corticosteroid treatment had no effect on the these cell characteristics. Corticosteroids also had no effect on the passive membrane properties (membrane potential, input resistance, and time constant), fAHP amplitude and duration. Corticosteroids did
increase the action potential duration in cells from ADX animals compared to ALD and HCT. The action potential duration was also longer in cells from SHAM animals compared to ALD.

Percentage of Nonbursting and Bursting Cells

The pyramidal cells in subfield CA3 are divided into bursting and nonbursting cell types, depending on their firing pattern. Bursting cells produce a burst of 3-5 action potentials on top of a slow depolarizing envelope (Wong and Prince, 1978, 1981; Masukawa et al., 1982; Bilkey and Schwartzkroin, 1990). The percentage of nonbursting cells we observed in subfield CA3 in SHAM and ALD animals (41% - 42%) is similar to the values reported for naive animals (40% - 41%) (Masukawa et al., 1982; Bilkey and Schwartzkroin, 1990). ADX produced the largest shift in the percentage of nonbursting cells, increasing the amount of nonbursting cells to 74%.

The effect of corticosteroids on the ratio of burst and nonbursting cells do not appear to be secondary to changes in cell viability. The cell resting membrane potential, resistance, and time constant were not altered by corticosterone treatment, and were comparable to those reported in naive animals (Beck et al., 1992).

Adrenalectomy has been reported to induce granule cell degeneration in the dentate gyrus. While the most severe cell loss occurs between 4 or more months after adrenalectomy (Sloviter et al., 1989; 1993; Roy et al., 1990; McNeil et al., 1991; Sapolsky et al., 1991; Jaarsma et al., 1992), granule cell degeneration was also
reported to occur between 1 to 3 weeks after adrenalectomy (Gould et al., 1990; Sloviter et al., 1993). Granule cells form synapses with subfield CA3 pyramidal cells and the loss of granule cells induced by adrenalectomy may account for the large shift in the percentage of nonbursting cells. However, there is no evidence to support that the degenerating granule neurons are altering the physiological or biochemical properties of CA3 pyramidal cells.

Based on several lines of evidence it is unlikely that the large shift in percentage on nonbursting cells observed with ADX treatment is due to the loss of dentate granule cells. First, Sloviter et al. (1993, 1995) report a large variation in granule cell degeneration at any given time point and the percentage of degenerating granule cells following 14 days of adrenalectomy was not reported. The large variation in degenerating granule cells may have a significant effect on the results if only a small number of animals were used. However, we examined the effects of ADX in twenty rats, a sample size comparable if not greater then that used by Sloviter et al., (1993). Second, it is unlikely that the massive loss of granule cells only influenced the firing pattern and action potential duration of CA3 neurons. Such a huge loss in synaptic inputs should attenuate numerous electrical membrane properties. Finally, it was reported that within one week of adrenalectomy both cell loss and mitosis was induced (Cameron and Gould, 1996), therefore, the overall synaptic integrity between the dentate and subfield CA3 may not altered with adrenalectomy. While the loss of
granule cells following 14 days of adrenalectomy is important and may have a significant effect on neuronal signaling between the dentate and subfield CA3, it does not readily explain or diminish the significance of the large difference in the percentage of nonbursting cells observed in ADX treated animals.

A possible explanation for the increased number of nonbursting cells is that corticosteroids modulate the ionic current that influences the bursting firing mode. The exact nature of burst firing is not known. Early investigations reported that burst firing is due to a slow Ca\(^{2+}\) current (Wong and Prince, 1978, 1981), however, there is also evidence supporting the role of Na\(^+\) current in burst firing, possibly a persistent Na\(^+\) current (Konnerth et al., 1986; French et al., 1990; Azouz et al., 1996; Jensen et al., 1996). The ionic conductances underlying pyramidal cell burst firing may be different across hippocampal subfields (Azouz et al., 1996; Jensen et al., 1996). Burst firing in subfield CA3 may depend on both Na\(^+\) and Ca\(^{2+}\) currents (Wong and Prince, 1978, 1981; Konnerth et al., 1986), whereas, burst firing in subfield CA1 cells depend upon Na\(^+\) currents only (Azouz et al., 1996; Jensen et al., 1996). Since corticosterone treatment influences burst firing in subfield CA3 neurons, it may be possible to determine the exact ion currents and channels underlying burst firing in subfield CA3 cells by comparing the isolated ion currents in cells from adrenalectomized and corticosterone treated animals.
The effects of corticosteroids on isolated Ca\textsuperscript{2+} and Na\textsuperscript{+} currents in subfield CA3 are not known. The action of corticosteroids on Ca\textsuperscript{2+} currents in subfield CA1 have been determined but the results are not straightforward. Adrenalectomy decreased the Ca\textsuperscript{2+} action potential area (Kerr et al., 1989; 1992) but increased Ca\textsuperscript{2+} currents (Karst and Joëls, 1994). Corticosteroids also modulate Ca\textsuperscript{2+} homeostasis (Elliott and Sapolsky, 1993), which in turn, may influence the Ca\textsuperscript{2+} driving potential and/or ion channels. Other factors contribute to burst and nonburst firing and include the relative densities and distribution of Ca\textsuperscript{2+}-independent K\textsuperscript{+} channels along a neuron (Migliore et al., 1995) and neuron morphology (Bilkey and Schwartzkroin, 1990).

Passive Membrane and Action Potential Properties

The membrane input resistance and time constant are determined by passive and voltage-dependent ion channels that normally function near the cell's resting membrane potential (Rall, 1977). The membrane input resistance and time constant determine the decay of synaptic potentials in the dendrites and soma, therefore, influencing the integration of postsynaptic potentials (Rall, 1977). Corticosteroids did not alter the resting membrane potential, input resistance, or time constant in subfield CA3 pyramidal neurons. In contrast, the membrane input resistance and time constant in subfield CA1 pyramidal cells are altered by corticosteroids. The changes in CA1 cells may be due to alterations in I\textsubscript{O} (Karst et al., 1993; Beck et al., 1994), which is less
prominent in CA3 cells (Spruston and Johnston 1992). Therefore, corticosteroids may alter the integration of postsynaptic potentials in CA1 but not in CA3 cells.

The decrease in action potential half- and baseline width in cells from ALD and HCT animals suggest that corticosteroids may play a role in the shaping of action potentials. Furthermore, corticosteroids appear to have different effects on action potential characteristics in cells from subfields CA1 and CA3. Chronic low (basal) levels of corticosterone increased action potential height and duration in pyramidal cells from subfield CA1 and decreased the fAHP amplitude (Beck et al., 1994) when compared to cells from adrenalectomized animals. In the present study we observed a decrease in the action potential duration and no change in the action potential height and fAHP amplitude in cells from ALD animals when compared to ADX.

The broader action potential duration in cells from SHAM and ADX animals may be physiologically important. Wheeler et al., (1996) reported that increasing the action potential duration leads to an increase in Ca$^{2+}$ influx at the presynaptic terminal and enhances synaptic strength. Therefore, MR activation could conceivably have different effects on Ca$^{2+}$ influx at the axon terminals arising from subfields CA1 and CA3. The narrowing of action potentials may result from changes in any of the K$^+$ and Ca$^{2+}$ ion currents which shape the action potential (Rutecki, 1992). Further experiments are required to determine which current(s) are altered by corticosteroids.
sAHP and Accommodation

The sAHP and accommodation characteristics in cells from subfield CA1 were the first electrophysiological membrane characteristics identified that were altered by corticosteroids (Joëls and de Kloet, 1989; Kerr et al., 1989; Joëls and de Kloet, 1990). Both of these properties rely on a Ca$^{2+}$-dependent K$^+$ current (Madison and Nicoll, 1984; Lancaster and Nicoll, 1987). In subfield CA1, short term and chronic MR activation decreased spike accommodation and sAHP amplitude (Kerr et al., 1989; Joëls and de Kloet, 1990; Beck et al., 1994).

Unlike the cells in subfield CA1, corticosteroids did not alter the sAHP or accommodation in subfield CA3 neurons. This is an interesting result because both the sAHP and burst firing are triggered by Ca$^{2+}$ currents (Wong and Prince, 1978, 1981; Schwartzkroin and Stafstrom, 1980; Konnerth et al., 1986; Sah and Isaacson, 1995), yet corticosteroids only appear to alter the cell’s firing mode. The relationship between burst firing and sAHPs is not well known. There are several lines of evidence suggesting that burst firing and sAHP may be independent properties. The channels underlying burst firing and sAHP’s may be located in different areas of the neurons (soma versus dendrites) (Wong et al., 1979; Bernardo et al., 1982; Wong and Stewart et al., 1992; Sah and Bekkers, 1996). In subfield CA1, the ionic currents mediating the sAHP and burst firing appear to be separate (Azouz et al., 1996). Finally, in subfield CA3, both Na$^+$ and Ca$^{2+}$ currents influence burst firing (Wong and Prince, 1978, 1981;
Konnerth *et al.*, 1986). Under our experimental conditions, it was not possible to determine the nature of the current(s) that shifted the fraction of nonbursting and bursting cells.

In conclusion, corticosteroids have numerous effects on neurons located in the hippocampus. Corticosteroids modulate neuron electrophysiological properties (*Beck et al.*, 1994; *Joëls and de Kloet*, 1994; *Joëls et al.*, 1994a, 1994b), morphology (*Woolley et al.*, 1990; *Gould et al.*, 1991; *Watanabe et al.*, 1992; *Magarinos and McEwen*, 1995a, 1995b) and neurotransmitter receptor-mediated responses in the hippocampus (*Joëls and de Kloet*, 1994; *Birnstiel and Beck*, 1995; *Beck et al.*, 1996). Corticosteroids may alter signal processing in the hippocampus through a complex mechanism, producing different effects in each area of the trisynaptic circuitry (dentate-CA3-CA1). For example, corticosteroids selectively alter dendritic morphology within subfield CA3 (*Woolley et al.*, 1990; *Watanabe et al.*, 1992; *Magarinos and McEwen*, 1995a,b). Corticosteroids also have different effects on the neuron’s electrophysiological properties in subfields CA1 and CA3. This distinct role of corticosteroid modulatory actions among the different hippocampal subfields may be very important in signal processing in the hippocampus.
CHAPTER IV
CORTICOSTEROIDS ALTER 5-HT_{1A} SIGNAL TRANSDUCTION SYSTEM IN HIPPOCAMPAL SUBFIELD CA3 PYRAMIDAL CELLS

Abstract

Corticosteroids influence neuron activity in the hippocampus through the activation of MR and GR receptors. For example, corticosteroids modulate the responses elicited by the activation of several different neurotransmitter receptors on hippocampal pyramidal cells. However, the effects of corticosteroids on the 5-HT_{1A} receptor signal transduction system (receptor-G protein-potassium channel) in subfield CA3 are not completely known. Therefore, we used single-electrode voltage clamp techniques to examine the actions of chronic corticosteroid treatment on the 5-HT_{1A} signal transduction system in rat hippocampal subfield CA3 pyramidal cells. The treatment groups used in this investigation were: ADX, ALD, HCT, and SHAM.

Corticosteroids altered the 5-HT concentration-response curve characteristics for the 5-HT_{1A} receptor. The EC_{50} value was smaller in cells from ADX compared to the other treatment groups. The Emax value was smaller in cells from HCT compared to SHAM and ADX animals. G protein function was also altered by corticosterone
treatment. Less current was elicited by GTPγS in cells from HCT compared to the other treatment groups and in cells from SHAM compared to ADX animals. Corticosteroid treatment did not alter the current-voltage relationship or the reversal potential of the potassium current linked to the 5-HT₁A receptor. Based on these new findings we conclude that corticosteroids alter the 5-HT₁A receptor mediated-response in hippocampal subfield CA3 neurons. Furthermore, corticosteroids alter the 5-HT₁A signal transduction system at site(s) downstream of the receptor.

**Introduction**

Activation of corticosteroid receptors in the hippocampus influences hippocampus-related behaviors (de Kloet et al., 1993) and modulates HPA axis activity (Jacobson and Sapolsky, 1991). Corticosteroid hormones (glucocorticoids and mineralocorticoids) regulate gene expression through the interaction with two receptor subtypes: MR and GR (Joëls et al., 1994b). MR and GR have a 5-10 fold difference in their affinity for the rat corticosteroid hormone corticosterone. MR has a high affinity for corticosterone and is 70-80% occupied at basal corticosterone plasma levels (Krozowski and Funder, 1983; Reul and de Kloet, 1985; Reul et al., 1987a,b). GR has a lower affinity for corticosterone and its occupancy changes from 15% at basal to 90% at peak circadian or stress-induced circulating corticosterone levels (Reul and de Kloet, 1985; Reul et al., 1987a,b).
One way corticosteroids alter neural activity in the hippocampus is by modulating the responses elicited by the activation of neurotransmitter receptors (Joëls and de Kloet, 1994; McEwen, 1996), including the 5-HT$_{1A}$ receptor (Joëls and de Kloet, 1994; Beck et al., 1996). The 5-HT$_{1A}$ receptor is a G protein-coupled receptor. Activation of the 5-HT$_{1A}$ receptor in the hippocampus increases an inward rectifying potassium current linked to a PTX sensitive G protein (Andrade et al., 1986; Andrade and Nicoll, 1987; Colino and Halliwell, 1987; Beck 1989; Beck and Choi, 1991; Okuhara and Beck, 1994). Corticosteroids alter the 5-HT$_{1A}$ receptor-mediated responses in hippocampal subfield CA1 (Joëls et al., 1991, Joëls and de Kloet, 1992; Beck et al., 1996). However, the effects of corticosteroids on the 5-HT$_{1A}$ receptor-mediated responses in subfield CA3 are not known.

Previous studies have examined the effects of corticosteroids on the number of 5-HT$_{1A}$ binding sites and mRNA levels (Chaouloff, 1995). Corticosteroids appear to alter 5-HT$_{1A}$ receptor binding sites in subfield CA3, but not in subfield CA1 (Mendelson and McEwen, 1992a,b; Kuroda et al., 1994). Recently, we reported that chronic exposure to high levels of corticosteroids increased the protein levels of $G_s$, $G_{i1}$ and 2, and $G_\alpha$ $\alpha$-subunits in the hippocampus (Okuhara et al., 1996a). However, the actions of corticosteroids on G protein function in the individual hippocampal subfields and on G protein-evoked currents are not known.
In the present study, we examined the effects of chronic corticosteroid treatment on different components of the 5-HT1A signal transduction system in hippocampal subfield CA3 pyramidal cells. Single-electrode voltage clamp techniques were used in the brain slice preparation. We hypothesized that chronic corticosteroid treatment would alter the 5-HT1A receptor signal transduction system in subfield CA3. The results from these experiments will contribute to a basic understanding of how corticosteroids alter responses elicited by the activation of G protein-linked neurotransmitter receptors and help to elucidate the role of corticosteroids in hippocampus-related behaviors and HPA axis feedback.

Materials and Methods

Materials

Corticosterone pellets were purchased from Innovative Research of America (Sarasota, FL). Aldosterone was purchased from Steraloids Inc. (Wilton, NH). Osmotic minipumps were purchased from Alza Cooperation (Palo Alto, CA). Tetrodotoxin and 5-HT hydrochloride were purchased from Sigma Chemicals (St. Louis, MO). GTPγS was purchased from Boehringer Mannheim (Indianapolis, IN). All other chemicals were obtained from standard commercial sources.
Animals

Four different treatment groups were used for the experiments in this investigation (Table 1, pg. 64). The adrenalectomy was performed as described previously (Beck et al. 1994, 1996; Birnstiel and Beck, 1995). Male Sprague-Dawley rats (Harlan) 75-100g were used for all treatment groups. Bilateral adrenalectomies were performed on all treatment groups, except the SHAM group, to remove circulating corticosteroids. The animal was anesthetized with ether, a small incision was made into the abdominal cavity, just below the rib cage, and the adrenal glands carefully removed. The muscle wall was then sutured and the skin closed using wound clips. The ADX group received no further treatment. The ALD group had an osmotic minipump containing aldosterone (Alzet model 2002), implanted subcutaneously at the time of adrenalectomy, to selectively activate MR. The minipump delivered 10 µg/h aldosterone dissolved in propylene glycol. MR and GR were activated in another group of animals (HCT) by subcutaneously implanting 200-300 mg corticosterone pellets, 2 to 3 week release, in the back of the neck at the time of adrenalectomy. The SHAM group of animals was produced by visualizing the adrenal glands but leaving them intact. After surgery the animals were maintained on a standard twelve hour light/dark cycle and rat chow, ad libitum. SHAM animals were given standard drinking water while the ADX, ALD, and HCT animals were given 0.9 % NaCl drinking water ad libitum. At the end of 13 - 15 days, the animals were sacrificed in the morning and
hippocampal slices immediately prepared for electrophysiological recording. At the
time of sacrifice, trunk blood was collected to determine the plasma corticosterone
levels by radioimmune assay (Burgess and Handa, 1992).

Hippocampal Slice Preparation

Hippocampal slices were prepared for electrophysiological recording as previously
described (Okuhara and Beck, 1994). Rats were sacrificed by decapitation and the
brain rapidly removed and placed in ice cold artificial cerebrospinal fluid (ACSF)
containing in mM: NaCl 125, KCl 3, NaH₂PO₄ 1.25, MgSO₄ 2, CaCl₂ 2.5, dextrose10,
and NaHCO₃ 28. The ACSF was also supplemented with steroids, as outlined in
Table 1 (pg. 64), to maintain the treatment paradigm. We previously reported that
steroids must be present in the ACSF to preserve the corticosteroid-induced effects on
neuron cell properties (Beck et al., 1994). The hippocampus was dissected free and the
dorsal section cut in 500-550 μm sections on a vibratome. The hippocampal slices
were then placed in a holding vial containing ACSF bubbled with 95% O₂-5% CO₂ at
room temperature. The slices remained in the holding vial for at least one hour after
dissection before being transferred to the recording chamber. In the recording
chamber, the slice was stabilized between two nylon nets and continuously perfused
with ACSF bubbled with 95% O₂-5% CO₂ at a rate of 2-3 ml/min, at 31-32°C.
Intracellular Recording

Intracellular recordings were made as previously described (Okuhara and Beck, 1994). Electrodes were pulled from borosilicate capillary tubing on a Brown and Flaming electrode puller (Sutter Instruments) to a resistance of 30-35 MΩ (2 M KCl). Pyramidal cells were impaled with brief ejections of positive current through the electrode. The impaled cells were sealed by applying 1 nA hyperpolarizing current. Electrical signals were collected and amplified using an Axoclamp 2A and Cyberamp 320 amplifier (Axon Instruments, Foster City, CA) and recorded on a Gould Series 3200 chart recorder (Gould Electronics, Valley View, OH). Data were collected online with pCLAMP software (Axon Instruments).

Discontinuous single-electrode voltage clamp (dSEVC) technique was used under the following conditions. Briefly, the slice was perfused with 1 µM tetrodotoxin and the capacitance compensation was set and switching frequency adjusted to 4 kHz at the beginning of the experiment. The cells membrane potential was clamped at a gain of 8 nA/mV and headstage continuously monitored.

5-HT Concentration-Response

Cells were voltage clamped at -65 mV and data for the 5-HT concentration response curves were collected by perfusing the slice with increasing 5-HT concentrations (3, 10, 30, and 100 or 110 µM) and the maximum current response
evoked during the drug application was recorded. During drug perfusion, if the voltage clamp deviated by more than 1 mV, the data was discarded.

The magnitude of the current response was fitted to a hyperbolic function (Okuhara and Beck, 1994; Beck et al., 1996):

\[ E = \frac{E_{\text{max}}}{1 + (EC_{50} / [5-HT])^N} \]

where \( E \) is the current produced at the 5-HT concentration \([5-HT]\), \( E_{\text{max}} \) is the maximal current response, \( EC_{50} \) is the 5-HT concentration which produces half the \( E_{\text{max}} \) response, and \( N \) is the slope index. The fitted \( E_{\text{max}} \), \( EC_{50} \) and \( N \) values within each experimental group were used for statistical comparisons.

Experiments with GTP\(_{\gamma}\)S

The effect of corticosteroids on G protein activity was determined by measuring the magnitude of outward current evoked by 15 mM GTP\(_{\gamma}\)S which was included in the recording electrode. After the cell's membrane potential stabilized, the cell was voltage clamped at its resting membrane potential (i.e., the potential where no current was required to maintain the voltage clamp). The voltage clamp was then moved to -60 mV and the amount of current required to maintain the clamp was recorded.

I-V Relationship

The effect of corticosteroids on the potassium current linked to the 5-HT\(_{1A}\) receptor was determined by plotting the I-V curve for the outward current evoked by
the activation of the 5-HT$_{1A}$ receptor. After the cell's membrane potential stabilized, the cell was voltage clamped at its resting membrane potential (the potential where no current was required to maintain the voltage clamp). Data for IV plots were obtained by running voltage ramps in the presence and absence of 100 µM 5-HT. Voltage ramps were run from approximately -125 mV to -30 mV at a rate of 1 mV/s. The I-V plot for the potassium current evoked by the activation of the 5-HT$_{1A}$ receptor was constructed by subtracting the control ramp values (no 5-HT) from the ramp values in the presence of 100 µM 5-HT. The reversal potential and potential where inward rectification occurred were analyzed for each curve.

Statistical Analysis

Statistical comparisons were performed using analysis of variance (ANOVA). The Student-Newman-Keuls method was used for post-hoc tests. All values are reported as mean ± SEM. A p < 0.05 was considered significant.

Results

Corticosterone Plasma Levels

SHAM corticosterone plasma levels ranged from 0 - 4.13 µg/dl (1.35 ± 0.32 µg/dl, n = 15). ADX corticosterone plasma levels ranged from 0 - 0.22 µg/dl (0.06 ± 0.03 µg/dl, n =12). ALD corticosterone plasma levels ranged from 0 - 0.56 µg/dl (0.07 ±
0.05 \mu g/dl, n = 16). The lower and upper limits of the corticosterone radioimmune assay were 0.05 and 50 \mu g/dl respectively. Adrenalectomies that produced corticosterone concentrations \leq 0.6 \mu g/dl were considered successful. Two SHAM, 8 ADX, and 14 ALD animals had corticosterone levels less than 0.05 \mu g/dl. The concentration of aldosterone used in our experiments was based on an investigation by Kuroda et al., (1994). The ALD treatment decreased MR binding by 61% (n=5 animals) in the cytoplasm (data not shown) as determined using a homogenate binding assay. HCT plasma concentrations greater than 20 \mu g/dl were considered successful. HCT corticosterone plasma levels ranged from 25 to > 50 \mu g/dl (34 \pm 3 \mu g/dl, n = 16).

5-HT Concentration Response Curve Characteristics for the 5-HT_{1A} Receptor

Activation of the 5-HT_{1A} receptor evoked an outward current in a concentration-dependent manner (Figure 9A). The 5-HT concentration-response curve characteristics recorded from cells from corticosterone treated animals are summarized in Table 6. The EC_{50} obtained in cells from ADX treated rats was smaller compared to cells from either SHAM, ALD, or HCT treated animals (Figure 9B). The Emax value was also smaller in cells from HCT when compared to cells from SHAM or ADX treated animals (figure 9B). Corticosterone treatment did not alter the slope index.

Perfusion of hippocampal slices with 5-HT may activate a number of different 5-HT receptor subtypes. Two 5-HT receptors subtypes which may be activated by 5-HT
and thereby modulate the 5-HT_{1A} current are the 5-HT_{4} and 5-HT_{7} receptors. Both of these receptors are located in the hippocampus (Lovenberg et al., 1993; Meyerhor et al., 1993; Ruat et al., 1993; Jakeman et al., 1994; Roychowdhury et al., 1994; Waeber et al., 1994) and are positively coupled to adenylyl cyclase (Lovenberg et al., 1993; Ruat et al., 1993; Shen et al., 1993; Tsou et al., 1994). Activation of the 5-HT_{4} receptor produces a slow depolarizing response in subfield CA1 pyramidal cells (Andrade and Chaput, 1991). Since the 5-HT_{4} and 5-HT_{7} receptors have not been characterized in subfield CA3, we determined if activation of these receptors altered the 5-HT induced outward current recorded in subfield CA3. The magnitude of the outward current elicited by 30 µM 5-HT was not altered by the administration of 1 µM GR113808 and ritanserin (n = 6) (Figure 10), 5-HT_{4} and 5-HT_{7} receptor antagonists, respectively (Boess and Martin, 1994; Torres et al., 1994). Perfusion of the slice with 30 µM 5-HT while blocking the 5-HT_{1A} receptor with either 1 µM WAY 100635 or 3 µM spiperone did not produce either a depolarizing or inward current response (n=3) (data not shown).

Activation of G proteins with GTPγS

The injection of GTPγS into neurons produced a hyperpolarization of the resting membrane potential. There was no difference in the resting membrane potential between treatment groups. The resting membrane potentials were SHAM -76 ± 1 mV, ADX -79 ± 2 mV, ALD -74 ± 1 mV, and HCT -76 ± 1 mV. The relative amount of
Figure 9. Concentration-dependent response of subfield CA3 pyramidal cells to 5-HT perfusion. A: Chart recording of the concentration-dependent current response of a CA3 cell to 5-HT perfusion. The drug was applied for the duration depicted by the line above the chart record. B: Concentration-response curves from data collected from all of the cells from SHAM (open circles), ADX (closed circles) and HCT (closed squares) treated animals. The data were fit to a hyperbolic function as described in MATERIALS AND METHODS in this chapter.
Table 6.—The effect of corticosteroids on the 5-HT concentration-response curve characteristics for the 5-HT₁A receptor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>EC₅₀ (-log)</th>
<th>Emax (pA)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>4.96 ± 0.06 (15)</td>
<td>551 ± 46 (15)</td>
<td>2.0 ± 0.1 (15)</td>
</tr>
<tr>
<td>ADX</td>
<td>5.12 ± 0.04* (18)</td>
<td>540 ± 47 (16)</td>
<td>2.3 ± 0.1 (18)</td>
</tr>
<tr>
<td>ALD</td>
<td>4.96 ± 0.03 (16)</td>
<td>469 ± 39 (15)</td>
<td>2.4 ± 0.2 (16)</td>
</tr>
<tr>
<td>HCT</td>
<td>4.85 ± 0.04 (15)</td>
<td>360 ± 30 (15)b</td>
<td>2.3 ± 0.2 (15)</td>
</tr>
</tbody>
</table>

All values are reported as mean ± SEM (n)

* ANOVA F = 11.631; d.f. = 3, 60; P < 0.001. Student-Newman-Keuls, ADX different from SHAM, ALD, and HCT, p < 0.05.

b ANOVA F = 4.302; d.f. = 3, 60; P < 0.008. Student-Newman-Keuls, HCT different from SHAM and ADX, p < 0.05.
Figure 10. The outward current elicited by the perfusion of 30 µM 5-HT in the presence and absence of 5-HT$_4$ and 5-HT$_7$ receptor antagonists. The outward current elicited by the perfusion of 30 µM 5-HT in the presence and absence of 1 µM of the 5-HT$_4$ and 5-HT$_7$ receptor antagonists GR113808 and ritanserin. The drugs were applied for the duration depicted by the line above the chart record. The magnitude of the outward current evoked by 5-HT was not different ($p = 0.141$, $n = 6$, paired t-test) in the presence or absence of GR113808 and ritanserin.

current evoked by the activation of G proteins with GTP$\gamma$S was determined by voltage clamping the cell’s membrane at its resting potential (when zero current is required to maintain the voltage clamp). The voltage clamp was then moved to -60 mV for several minutes (Figure 11A). The amount of current required to maintain the voltage clamp at -60 mV was used as a measure of the amount of current evoked by the activation of G proteins with GTP$\gamma$S. Figure 11B summarizes the results of these experiments.
Significantly less current was required in cells from HCT when compared to cells from SHAM, ADX, and ALD treated animals. The amount of current measured in cells from SHAM was also smaller compared to cells from ADX treated animals.

**I-V relationship**

I-V plots for the 5-HT$_{1A}$ evoked potassium current were analyzed in 2 to 3 cells from ADX, SHAM and HCT treated animals. Representative I-V plots in Figure 12A-C demonstrate that corticosterone does not appear to alter the reversal potential nor the inward rectification properties for the potassium current linked to the 5-HT$_{1A}$ receptor. The reversal potential was between -97 to -104 mV and rectification started at approximately -50 mV.

**Discussion**

The 5-HT$_{1A}$ receptor is a member of the G protein-linked receptor family. In the present study, we report the effects of chronic corticosteroid treatment on different components of the 5-HT$_{1A}$ signal transduction system in hippocampal subfield CA3 pyramidal cells using electrophysiological techniques. The EC$_{50}$ value for the 5-HT concentration-response curve was shifted to the left in cells from ADX treated animals compared to SHAM, ALD, and HCT. The Emax value was smaller in cells from HCT animals compared to ADX and SHAM. The shift in the 5-HT$_{1A}$ response may be due to alterations at sites downstream of the 5-HT$_{1A}$ receptor. Therefore, we compared the
Figure 11. The outward current evoked by GTPγS. **A:** Chart recording of a cell injected with GTPγS. The cell’s membrane potential was first voltage clamped at its resting potential (-74 mV, I = 0 pA). The clamp was then moved to -60 mV and the current recorded (I = 340 pA). Perfusion of 100 µM 5-HT (duration depicted by the line above the chart record) did not elicit a current response, indicating all the G proteins linked to 5-HT receptors were activated by GTPγS. **B:** Summary graph depicting the amount of current evoked by GTPγS in cells from corticosterone treated animals. Treatment group and number of cells are labeled at the bottom axis.

ANOVA F = 12.691; d.f. = 3, 61; P < 0.001.

*HCT different from SHAM, ADX, and ALD. Student-Newman-Keuls, p < 0.05,

#ADX different from SHAM. Student-Newman-Keuls, p < 0.05.
A

100 µM 5-HT

100 pA

2 min

B

Current (pA)

SHAM (15)  |  ADX (18)  |  ALD (17)  |  HCT (15)

#  |  *  |  *
Figure 12. I-V plots of steady-state 5-HT mediated current in corticosteroid treated animals. I-V plot of steady-state 5-HT mediated current in a cell from either a A: SHAM, B: ADX, or C: HCT treated animal.
magnitude of the outward current evoked by the activation of G proteins with GTP\(\gamma\)S. Less current was evoked by GTP\(\gamma\)S in cells from HCT animals compared to ADX, SHAM, and ALD. Furthermore, less current was evoked by GTP\(\gamma\)S in cells from SHAM compared to ADX animals. Finally, we determined the effects of corticosteroids on the I-V relationship of the potassium current elicited by the activation of the 5-HT\(_{1A}\) receptor. Corticosteroid treatment did not alter the reversal potential or inward rectification properties of the potassium current linked to the 5-HT\(_{1A}\) receptor. Based on our results and those previously reported by other investigators, we conclude that corticosteroids alter the response elicited by the activation of 5-HT\(_{1A}\) receptors by modulating the receptor’s signal transduction system at different sites.

Alterations in the number of 5-HT\(_{1A}\) binding sites may account for some of the changes we observed in the 5-HT concentration response curve characteristics. Previous investigators reported that adrenalectomy increases the number of 5-HT\(_{1A}\) receptor binding sites in subfield CA3 when compared to either SHAM, aldosterone, low or high corticosterone treated animals (Mendelson and McEwen, 1992a,b; Chalmers et al., 1993; Kuroda et al., 1994; Tejani-Butt and Labow, 1994). The increased number of 5-HT\(_{1A}\) receptor binding sites may account for the shift in EC\(_{50}\), with no change in Emax, for the 5-HT concentration-response curve that we observed
in cells from ADX compared to SHAM and ALD treated animals. A shift in EC$_{50}$, with no change in Emax, may occur with spare receptors (Stephenson, 1956; Yocca et al., 1992).

It is not clear if the smaller Emax in cells from HCT compared to SHAM treated animals is due to a decrease in the number of 5-HT$_{1A}$ binding sites. It has been reported that high levels of corticosterone (plasma concentrations $> 40$ µg/dl) do not change the number of 5-HT$_{1A}$ binding sites in subfield CA3 compared to sham treated animals (Mendelson and McEwen, 1992a). However, social and restraint stress does decrease the number of 5-HT$_{1A}$ binding sites in the hippocampus (Watanabe et al., 1993; McKittrick et al., 1995). One possible explanation for the discrepancy is the treatment length. Mendelson and McEwen (1992b) treated their animals with a corticosterone pellet implant for 7 days. The investigations that examined the effects of stress treated their animals for 14 days (Watanabe et al., 1993; McKittrick et al., 1995). It is possible that exposure to elevated corticosterone plasma levels must exceed 7 days before the down regulation of 5-HT$_{1A}$ binding sites occurs. Alternatively, the stress response may be activating other physiological processes, in addition to increasing corticosterone plasma levels, that induces the down regulation of 5-HT$_{1A}$ binding sites.

Corticosteroids also alter the 5-HT$_{1A}$ signal transduction system downstream of the receptor. ADX increased while HCT decreased the magnitude of the current
evoked by the activation of G proteins with GTPγS. Corticosteroids could be altering G protein levels, G protein coupling, or the properties of the channels linked to the G proteins. However, the attenuated 5-HT<sub>1A</sub> mediated-response and G protein-evoked current cannot be attributed to a decrease in G protein expression. We observed that HCT treatment increased G<sub>0</sub> and G<sub>i1&2</sub> α-subunit levels in the hippocampus (Okuhara et al., 1996a), the PTX-sensitive G proteins thought to be linked to the 5-HT<sub>1A</sub> receptor (Andrade et al., 1986; Okuhara and Beck 1994). Based on our present results, we propose that the increased G protein levels may be a compensatory response to a decrease in G protein function.

Interestingly, while corticosterone clearly had an effect on the magnitude of the outward current evoked by GTPγS, there was no change in the resting membrane potential of pyramidal cells between treatment groups. A simple explanation for this observation is the non-linear relationship between voltage and resistance (shunting). Shunting accounts for large changes in current with only small changes in membrane potential. Therefore, the activation by GTPγS of ion channels with non-linear I-V relationships (i.e., GIRK) could evoke different amounts of current but produce similar changes in membrane potential in cells from the different treatment groups. Alterations in the 5-HT<sub>1A</sub> receptor-mediated response and G protein-linked current could be attributed to a change in pyramidal cell resting input resistance. However, corticosteroid treatment did not alter neuron resting input resistance in subfield CA3
pyramidal cells (Okuhara et al., 1996b). While we were able to detect corticosteroid-induced changes in the current evoked by the activation of G proteins we do not know if corticosterone is altering G protein function or the coupling between the G protein and potassium channel. We also do not know which G protein-linked current(s) was modulated by corticosterone.

The potassium channel linked to the 5-HT$_{1A}$ receptor signal transduction system is probably a member of the recently cloned G protein inward rectifying potassium (GIRK) channel family (Lesage et al., 1994, 1995; Kobayashi et al., 1995; Ponce et al., 1996; Spausches et al., 1996). ADX, ALD, and HCT treatment did not alter the reversal potential or inward rectification properties of the potassium current linked to the activation of the 5-HT$_{1A}$ receptor. However, alterations in potassium channel kinetic properties cannot be ruled out.

ADX had different effects on the 5-HT concentration response curve characteristics in cells from subfield CA1 and CA3. Previous studies reported that the concentration-response curve characteristics for the 5-HT$_{1A}$ mediated hyperpolarization in subfield CA1 was not altered by adrenalectomy or chronic activation of MR with low levels of corticosterone as compared to sham (Joëls et al., 1991, Joëls and de Kloet, 1992; Beck et al., 1996). In the present investigation, ADX shifted the EC$_{50}$ value for 5-HT$_{1A}$ mediated outward current compared to SHAM and ALD (chronic MR activation). Previously, we reported that the 5-HT$_{1A}$ receptor signal transduction
system is not identical between subfields CA1 and CA3, based on different 5-HT concentration-response curve characteristics for the 5-HT\textsubscript{1A} receptor in the two subfields (Beck \textit{et al.}, 1992; Okuhara and Beck, 1994). Therefore, it is possible that corticosteroids have different effects on the 5-HT\textsubscript{1A} receptor-mediated response in subfields CA1 and CA3.

In conclusion, chronic corticosterone treatment alters the response elicited by the activation of 5-HT\textsubscript{1A} receptors in hippocampal subfield CA3 pyramidal cells. Some of the modulatory actions of corticosterone occur downstream of the receptor, at the G protein level. Furthermore, ADX and high corticosterone treatment have opposite effects on the 5-HT\textsubscript{1A} signal transduction system. Our results provide important information towards understanding how corticosterone modulates neurotransmitter receptor-mediated responses in the hippocampus.
CHAPTER V
DIFFERENTIAL IMMUNOHISTOCHEMICAL LABELING OF Gs, Gi1 and 2 AND G0 α-SUBUNITS IN RAT FOREBRAIN

Abstract

In the previous chapter, it was concluded that corticosterone treatment alters the 5-HT1A receptor signal transduction system at sites downstream of the receptor. The 5-HT1A receptor couples to a PTX-sensitive G protein, therefore, corticosteroids may influence G protein levels and/or their intracellular location. Since a comparative study of the anatomical and morphological distribution of the G proteins Go, Gi1 and 2, and Gs α-subunits in the hippocampus has not been reported, such an investigation was conducted using immunohistochemical techniques with rat forebrain sections prepared from naive animals. Diffuse Go labeling occurred in the neuropil throughout the cortex, superficial layers of the entorhinal cortex, thalamus, several white matter fiber tracts and hippocampus. Gi1 and 2 immunoreactivity was also located in the neuropil but produced a more fibrous pattern. Fibrous labeling of Gi1 and 2 was observed in the cortex, amygdala, hippocampal subfield CA3 and several white matter fiber tracts. Both Go and Gi1 and 2 labeling was present in the pencil fibers within the striatum and lateral geniculate nucleus. Gs labeling, in contrast to Go and Gi1 and 2, was generally cytoplasmic. Cytoplasmic
Gs labeling was observed in the thalamus, habenula, dentate, geniculate nucleus, hypothalamus, and hippocampus. Intense Gs labeling was observed in the striatum parenchyma, choroid plexus and infundibular stem.

Based on our results, we conclude that the G proteins G0, G1 and 2, and Gs are anatomically distributed differently throughout the brain. The diffuse neuropil labeling of G0, fibrous neuropil labeling of G1 and 2 and cytoplasmic labeling of Gs strongly suggests that the G proteins are also differentially distributed morphologically within a neuron. The differential anatomical and cellular location of G proteins in the CNS may contribute to the coupling specificity between neurotransmitter receptors and G proteins.

**Introduction**

Heterotrimeric G proteins transpose extracellular signals into a cell by coupling plasma membrane receptors with effector molecules. G proteins are composed of three subunits: α, β, and γ. The α-subunit binds and hydrolyzes GTP and modulates effector molecule activity (Gilman, 1987). There are several subtypes of G proteins based on the sequence of their α-subunits. Four of these are G<sub>i</sub>, G<sub>o</sub>, G<sub>s</sub> and G<sub>t</sub> (Birnbaumer et al., 1990). The different G protein α-subunits are differentially sensitive to ADP-ribosylation by several toxins. The G proteins G<sub>i</sub> and G<sub>o</sub> are ADP ribosylated by PTX while G<sub>s</sub> is sensitive to cholera toxin (Gilman, 1987). The βγ subunits anchor the
G protein complex to the cytoplasmic membrane and also modulate the activity of the effector molecule (Birnbaumer et al., 1990).

The effector molecules are either ion channels or proteins that synthesize second messenger molecules (i.e., cyclic AMP or inositol triphosphate), which in turn, modulate enzyme activity in the cell (reviewed by Birnbaumer et al., 1990; Clapham, 1994). For example, $G_s$ increases adenylyl cyclase activity and modulates $Ca^{2+}$ activated-potassium channels, $G_i$ decreases adenylyl cyclase activity and activates potassium channels while $G_o$ decreases the current linked to $Ca^{2+}$ channels in the central nervous system.

Current research has focused on elucidating the specificity between receptor and G protein coupling. A cursory examination indicates that a cell may express more types of receptors than G proteins and that these multiple receptor subtypes can share the same G protein (Birnbaumer, 1992). The specificity of receptor-G protein coupling is probably established at several different levels. Factors that contribute to the receptor-G protein specificity are: amino acid sequences in the different G protein subunits (Gilman, 1987; Kleuss et al., 1993), cytoskeletal proteins (Rodbell, 1990; Sarndahl et al., 1991), and compartment localization (i.e., golgi and mitochondria) (Ercolani et al., 1990). The asymmetrical distribution of G proteins within rat enterocytes (Van den Berghe et al., 1991) and in the kidney (Stow et al., 1991) suggests that the cellular location of G proteins may also contribute to G protein-receptor coupling specificity.
In the brain, two other factors also contribute to the coupling specificity between receptors and G proteins: the intracellular (i.e., soma and dendrites) and anatomical location of the G protein. There is information on the distribution of G proteins in only a few brain areas including the cortex (Asano et al., 1987, 1990a,b; Aoki et al., 1992), hippocampus (Asano et al., 1990b; Aoki et al., 1992), basal ganglia (Hervé et al., 1993), cerebellum (Asano et al., 1987, 1990a; Nishida et al., 1991) and olfactory bulb (Asano et al., 1990a; Shinohara et al., 1992). In the previous chapter, it was concluded that corticosterone treatment alters the 5-HT$_{1A}$ receptor signal transduction system at sites downstream of the receptor. The 5-HT$_{1A}$ receptor couples to a G protein, therefore, corticosteroids may influence G protein levels and/or their intracellular location. Since the comparative anatomical and morphological distribution of the G$_o$, G$_{i1}$ and G$_{i2}$, and G$_s$ α-subunits in the hippocampus are not known, in the present study, we examined the anatomical and intracellular distribution of G$_{i1}$ and G$_{i2}$, G$_o$, and G$_s$ α-subunits in the rat forebrain using immunohistochemical techniques with rat forebrain sections prepared from naive animals.

**Materials and Methods**

**Materials**

Primary anti-G protein polyclonal antibodies were purchased from Dupont-New England Nuclear (Boston, MA). Monoclonal rabbit peroxidase-antiperoxidase antibody was purchased from Cappel (Durham, NC). Picric acid was purchased from Aldrich.
(Milwaukee, WI). 3,3'-diaminobenzidine tetrachloride (DAB) was purchased from Sigma (St. Louis, MO). All other chemicals were obtained from commercial sources.

Immunohistochemical Procedure

Four male Sprague-Dawley rats (170-230g) were sacrificed with a lethal injection of phenobarbital and perfused with 200 ml PBS (10 mM NaHPO₄, pH 7.4, and 150 mM NaCl) via the aorta at 650 ml/hr followed by 250 ml PA fixative (4% paraformaldehyde, 1% glutaraldehyde, 0.2% picric acid, 2% sucrose, 0.1 M acetate buffer, pH 6.0) as described previously (Asano et al., 1990b). After fixation, the brain was removed, blocked, and placed in PA for 4-6 hrs. After rinsing and overnight incubation in TBS (10 mM Tris, pH 7.6 and 150 mM NaCl) with 10% sucrose, the tissue was embedded in paraffin, sectioned at 7 µm, and mounted on microscope slides.

Immunohistochemistry was performed following protocols described by Sternberger et al., (1970). Briefly, tissue sections were blocked with TBS/5% bovine serum albumin (BSA)/3% normal goat serum (NGS) for one hour at room temperature. After washing the sections with TBS, three times for 10 minutes each, they were incubated with 1:50 anti-\(G_{sa}\) (RM1), 1:50 anti-\(G_{ic1}\) and 2 (AS7), or 1:100 anti-\(G_{ox}\) (GC2) overnight at room temperature. Several sections were incubated with 2% NGS in place of primary antibody to determine the level of nonspecific labeling. Sections were then washed with TBS, three times for 10 minutes each, and incubated with 1:20 goat anti-rabbit IgG. After one hour, sections were washed with TBS and incubated with rabbit peroxidase-
antiperoxidase for one hour. Subsequently, the sections were washed in TBS and developed with hydrogen peroxide and DAB as the chromagen. All antibody dilutions were made in TBS/5% BSA/3% NGS.

Silver Intensification

Selected DAB developed sections were intensified with silver as described by Kitt et al., (1994). All reagents used for intensification were freshly prepared. Briefly, after developing the sections with DAB and hydrogen peroxide, sections were dehydrated and rehydrated through a graded series of deionized water, alcohol, and xylenes. Sections were then placed in a 2.5% silver nitrate solution for 1 hour at 56°C. After washing in deionized water for 10-15 min, the sections were incubated in 2% gold chloride solution for 10 min at room temperature. Sections were then rinsed in deionized water for 5 min and incubated in a 5% sodium thiosulfate solution for 5 min at room temperature. Sections were washed for 10 min in deionized water and immediately dehydrated through a series of graded alcohols and xylenes.

Western Blot Analysis

Western blots were performed on membrane protein preparations from the hippocampus as described elsewhere (Sternweis and Robinshaw, 1984). Protein isolation procedures were carried out at 4°C. The hippocampus was homogenized with a hand-held dounce homogenizer in TBS, 10% sucrose, and 0.5 mM phenylmethan-
sulfonyl fluoride. The homogenate was then centrifuged at 20,000 X g for 30 min. The supernatant was removed and the membrane pellet resuspended in a second identical wash and centrifuged again at 20,000 X g for 30 min. The supernatant was discarded and the membrane pellet resuspended in TBS, 1 mM EDTA and 1% sodium cholate and incubated for 60 min. with constant mixing. The soluble membrane protein was collected by centrifugation at 35,000 rpm for 60 min. and stored at -80°C.

Western blotting techniques used are described elsewhere (Lesch and Manji, 1992). Membrane proteins were resolved by SDS-PAGE and electrophoretically transferred to nitrocellulose membrane filters. After transfer, the nitrocellulose was blocked with TBS, 0.2% NP-40, and 5% nonfat dry milk at room temperature. After blocking for 60 min. the nitrocellulose was incubated with a 1/10,000 dilution of AS7, RM1, or GC2 overnight at 4°C. The nitrocellulose was then incubated with a 1/10,000 dilution of goat anti-rabbit IgG for 60 min. at room temperature followed by an incubation with 1/10,000 dilution of rabbit PAP. The blots were developed with the ECL detection kit (Amersham, Arlington Heights, IL) and exposed to x-ray film. All antibody dilutions were in TBS, 5% BSA, and 0.2% NP-40. The nitrocellulose filter was washed with TBS/0.2% NP-40 between every antibody incubation and prior to development with the ECL kit.
Results

Western blot analysis

The RM1 antibody consistently labeled a 45 kDa protein band in the Western blots (Figure 13) corresponding to the short form of Gs (Bray et al., 1986; Robinshaw et al., 1986; Kozasa et al., 1988). A 54 kDa band (long form of Gs) was also detected but its detection was not consistent (data not shown). When the band was detected, it was faint and indistinct. The AS7 and GC2 antibodies labeled a single 40 kDa band (Figure 13) corresponding to the appropriate size for Gi1 and 2 and Go, respectively (Goldsmith et al., 1987; Cooper et al., 1990).

Figure 13. Western blot of G proteins in the rat hippocampus.
Lane 1: Gs (RM1 antibody). Lane 2: Gi1and 2 (AS7 antibody). Lane 3: Go (GC2 antibody).
Immunohistochemical Techniques

The immunohistochemical labeling with the primary antibodies described in this investigation is very sensitive to variations in labeling and tissue preparation protocols. For instance, we found that the inclusion of 0.05% NP-40 detergent decreased $G_s$ and $G_{i1}$ and $G_{i2}$ labeling throughout the entire brain and the exclusion of BSA from the blocking solution increased nonspecific $G_s$ labeling. The fibrous neuropil staining pattern observed with $G_{i1}$ and $G_{i2}$ in paraffin embedded tissue was distorted in cryostat cut sections. Tissue prepared with a paraformaldehyde-lysine-periodate (PLP) fixative produced less intense but similar antibody labeling patterns as the picric acid fixed tissue. In the present study, the tissue preparation and immunohistochemical protocols were maximized and identical for all three antibodies. Silver intensification and dark field microscopy were used to increase visualization of the specific staining in selected sections.

In control sections (2% NGS in place of primary antibody), nonspecific labeling was faint under normal light microscopy. Under dark field, a low level of nonspecific labeling was detected in some white matter fiber tracts, cortical cell bodies and hippocampal pyramidal cell bodies (data not shown). Serial dilutions of the primary antibody decreased the intensity of labeling. The differential labeling by the three antibodies used in this study also demonstrates the specificity of antibody binding.

The technical data sheets supplied by Du Pont NEN also provided information on the specificity of the three antibodies. The $G_{i1}$ and $G_{i2}$ antibody, AS7, is specific for only
The \( G_{\text{ac}} \) antibody, RM1, cross reacts with \( G_{\text{olf}} \). The \( G_0 \) antibody, GC2, is specific for \( G_{\text{oc}} \).

**Cortex**

We observed \( G_0 \) and \( G_{i1} \) and 2 immunoreactivity in the neuropil of the rat cortex but with different labeling patterns. \( G_0 \) immunoreactivity was very intense and diffuse. \( G_{i1} \) and 2 immunoreactivity was very fibrous and less intense than \( G_0 \). Diffuse \( G_0 \) labeling was observed in the neuropil throughout the entire cortex while neuronal cell bodies were not labeled. The immunoreactivity in the molecular layer (layer I) of the cortex was more intense than layers II-VI. The pyramidal cell bodies and dendritic processes in layer V were immunonegative and stood out against the positive neuropil (Figure 14A).

\( G_{i1} \) and 2 labeling in the cortex, and throughout the forebrain, was characterized by a fibrous pattern in the neuropil. The antibody also labeled individual fibers extending from the external capsule into the deep cortical layers V and VI (Figure 14B). The \( G_0 \) or \( G_s \) antibody labeled the neuropil in a diffuse pattern and fiber labeling was not observed. In the entorhinal cortex, the superficial layers adjacent to the cortical amygdala and piriform cortex were also strongly immunopositive for \( G_i \) and \( G_0 \), however, we were not able to distinguish the different nuclei in the amygdala.
Figure 14. Photographs of G protein labeling in the cortex.

A: \( G_0 \) labeling in the superficial cortical layers. Immunolabeling was located in the neuropil, while the neuronal cell body (large arrow) and processes (small arrow) were immunonegative. Note the intense labeling of \( G_0 \) in layer I of the cortex (located near the top of photomicrograph). X200.

B: \( G_{i1} \) and \( G_{i2} \) labeling (intensified with silver) in the deep cortical layers adjacent to the external capsule (ec). Fibers arising from the external capsule (arrow) were intensely stained. X200.

C: Cytoplasmic \( G_s \) labeling in a cell (arrow) located in the lateral middle cortical layers. X200. Insert: The same neuron (arrow) viewed at X300.
Gs labeling in the cortex displayed a generally light diffuse neuropil staining. However, in a few sections, cytoplasmic staining was present in cortical cells (Figure 14C). In the entorhinal cortex, the Gs antibody labeled the neuropil in a diffuse pattern. The Gs antibody did not label neuronal cell bodies in the entorhinal cortex.

**Hippocampus**

G protein immunoreactivity in the hippocampus was different for all three antibodies. In subfield CA1, Go and Gi1 and 2 labeling were detected in the neuropil while the pyramidal cell bodies were immunonegative (Figure 15A, C). Go staining was more intense than Gi1 and 2 and dendritic processes were often distinguished against the heavily stained neuropil. Gs immunoreactivity was also detected in subfield CA1 but as a thin cytoplasmic ring near the perimeter of the pyramidal cells (Figure 15E). Go labeling in subfield CA3 was similar to subfield CA1 (Figure 15B). Gi1 and 2 and Gs labeling in subfield CA3 was different from the patterns observed in subfield CA1. Gi1 and 2-like immunoreactivity was detected as fibrous patches surrounding the pyramidal cells in subfield CA3 (Figure 15D). Gs immunoreactivity in subfield CA3 was found throughout the entire neuronal cytoplasm (Figure 15F). Intense Go and Gi1 and 2-like immunoreactivity was also detected in the fimbria.
Figure 15. Photographs of G protein labeling in the hippocampus.
The labeling of G proteins in the hippocampal subfields CA1 (A,C,E) and CA3 (B,D,F). The radiata (Rad), oriens (Or), and pyramidal striata (Py) in subfield CA1 (Panel A) and CA3 (Panel B) are indicated. All photomicrographs are at X200. A: G$_o$ labeling in hippocampal subfield CA1. Location of the alveus (alv) is marked by an arrow. Arrowheads point to immunonegative dendrites. B: G$_o$ labeling in subfield CA3. The diffuse labeling makes it difficult to delineate the different striata. Location of the fimbria (fi) is marked by an arrow. C: G$_{11}$ and G$_{22}$ labeling (intensified with silver) in subfield CA1. alv, alveus. D: G$_{11}$ and G$_{22}$ labeling (intensified with silver) in subfield CA3. A fibrous patch of G$_{11}$ and G$_{22}$ labeling (small arrow) surrounding a pyramidal cell (hallow circle). Location of the fimbria (fi) is marked by the larger arrow. E: G$_s$ labeling in the perimeter (arrowhead) of subfield CA1 pyramidal cells. alv, alveus. F: Cytoplasmic labeling of G$_s$ in a pyramidal cell (arrowhead) located in subfield CA3. fi, fimbria.
Subcortical Nuclei

The three antibodies produced different labeling patterns in several subcortical nuclei. Generally, the $G_0$ antibody labeled the neuropil in a diffuse pattern; $G_{i_{1} and 2}$ immunoreactivity was also detected in the neuropil but produced a fibrous pattern; and $G_s$ staining was located in the cytoplasm.

G protein labeling with all three antibodies in the striatum appears as irregular patches surrounding immunonegative cell bodies. Intense $G_0$ and $G_{i_{1} and 2}$ -like immunoreactivity was detected in the white matter pencil fibers of the striatum and a much lighter label in the parenchyma (Figure 16A). In contrast, $G_s$ staining was absent from the pencil fibers but diffusely present within the striatum parenchyma and not detected in the cell cytoplasm (Figure 16B). In the habenula, $G_0$ and $G_{i_{1} and 2}$ immunoreactivity was located in the neuropil (Figure 16C) while $G_s$ immunoreactivity was localized to the cell cytoplasm (Figure 16D).

G protein labeling in the thalamus also produced different immunoreactive patterns. Intense $G_0$ and $G_{i_{1} and 2}$ -like immunoreactivity was located in the neuropil of the ventral posterior thalamic nuclei (VPT) (Figure 17A). $G_0$ and $G_{i_{1} and 2}$ immunopositive myelinated fibers were also detected in the VPT. These fibers probably arise from the medial lemniscus and internal capsule. Moderate $G_0$ and $G_{i_{1} and 2}$ -like immunoreactivity was detected throughout the lateral thalamic posterior nuclei (LP), and dorsal and ventral lateral geniculate nuclei (DLG, VLG). Moderate $G_s$ labeling was detected in the
Figure 16. Photographs of G protein labeling in the striatum and habenula. A: $G_{i1}$ and $G_{i2}$ labeling (intensified with silver) in the striatal pencil fibers (pf) and parenchyma (stm). X100. B: $G_s$ labeling in the striatum. pf pencil fibers; stm, parenchyma X100. C: $G_o$ labeling in the neuropil of the habenula. Note the immunonegative cell bodies (arrowhead). 3v, third ventricle. X25. D: Cytoplasmic $G_s$ labeling of a cell (arrowhead) in the habenula. The third ventricle is indicated (3v). X25.

cytoplasm of cells in the VPT, LP, DLG, VLG nuclei, central medial thalamic nuclei, mediodorsal thalamic nuclei, and paraventricular thalamic nuclei.

Light to moderate $G_o$ and $G_{i1}$ and $G_{i2}$ -like immunoreactivity was observed in the hypothalamus and median eminence. Moderate to intense cytoplasmic $G_s$ labeling was
detected in the hypothalamic tuberomammillary nucleus, median eminence, and infundibular stem (Figure 17B).

Figure 17. Photographs of G protein labeling in the thalamus, infundibular stem, and white matter fiber tracts. A: $G_{11}$ and $G_{12}$ labeling (intensified with silver) in the ventral posterior thalamus. See figure 18 for orientation. Immunopositive white matter fibers of the internal capsule are indicated by a white arrow. X25. B: $G_{3}$ labeling in the infundibular stem X200. C: $G_{11}$ and $G_{12}$ labeling (in the mammillothalamic tract (mt) and fornix (fx). 3v, third ventricle. X25.
Fiber Tracts and Choroid Plexus

In general, the $G_0$, $G_{i1}$ and $G_{i2}$-like immunoreactivity was very intense in most of the forebrain white matter fiber tracts. The mammillothalamic (Figure 17C), fornix (Figure 17C), optic tract, medial lemniscus, internal capsule (Figure 17A), and lateroventral aspect of the external capsule were heavily labeled by the $G_0$ and $G_{i1}$ and $G_{i2}$ antibodies. The fasciculus retroflexus was the area with the heaviest labeling of $G_0$. $G_s$-like immunoreactivity was not readily found within white matter fiber tracts. In several sections, the fornix and mammillothalamic tract were actually lighter than the surrounding background (data not shown). The choroid plexus was labeled by all three antibodies and was the location of intense $G_s$ staining. Table 7 and Figure 18 highlight the $G$ protein labeling patterns.

Discussion

We compared the anatomical and intracellular distribution of the $G$ proteins $G_s$, $G_0$ and $G_{i1}$ and $G_{i2}$ in the rat forebrain and our results suggest that the $\alpha$-subunits of $G_s$, $G_0$ and $G_{i1}$ and $G_{i2}$ are differentially located both within neurons and among different brain areas. $G_0$ was detected in the neuropil throughout all areas of the brain. Cell bodies and dendritic processes were often immunonegative. $G_{i1}$ and $G_{i2}$ labeling was also located in the neuropil but produced a very fibrous pattern throughout many brain areas. Cell bodies were immunonegative but the dendritic and axonal processes in certain brain areas were
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<td>++</td>
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</tr>
<tr>
<td>infundibular stem (InfS)</td>
<td>++++</td>
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</tr>
<tr>
<td>Striatum parenchyma (stm)</td>
<td>++++d</td>
<td>+d</td>
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</tr>
<tr>
<td>pencil fibers (pf)</td>
<td>-</td>
<td>++++</td>
<td>+++</td>
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<tr>
<td>optic tract (opt)</td>
<td>-</td>
<td>++++</td>
<td>+++</td>
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Continued next page
Table 7.-- Continued

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Gₜ</th>
<th>G₁₁ and₂</th>
<th>Gₐ</th>
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<tbody>
<tr>
<td>Internal capsule (ic)</td>
<td>+</td>
<td>+++</td>
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</tr>
<tr>
<td>External capsule (ec)</td>
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<tr>
<td>mediodorsal</td>
<td>-</td>
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<td>lateroventral</td>
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<tr>
<td>Medial lemniscus (ml)</td>
<td>+</td>
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<td>++</td>
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<tr>
<td>Fornix (fx)</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mammillothalamic tract (mt)</td>
<td>+</td>
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</tr>
</tbody>
</table>
| Choroid plexus (cp)           | +++ | ++ to +++| ++ to +++
| Fasiculus retroflexus (fr)    | -   | +++      | +++ |

Direct comparisons are made within a column. Indirect comparisons may be made between columns.

Key: negative -; light +; moderate, ++; intense +++; highest amount of staining, ++++.

d diffuse neuropil

f fibrous neuropil

c cytoplasm.
Figure 18. Camera lucida of G protein labeling in the subcortex. Cytoplasmic $G_s$ labeling is indicated by dots in upper figure while $G_{11}$ and 2 neuropil and white matter fiber tract labeling is indicated by diagonal lines in the lower figure. A star marks the area of the photomicrograph in figure 17A. Abbreviations: ARC, hypothalamic arcuate nucleus; CM, thalamic central medial nucleus; DLG, thalamic dorsal lateral geniculate nucleus; DM, hypothalamic dorsal medial nucleus; fr, fasiculus retroflexus; fx, fornix; Hb, habenula; ic, internal capsule; InfS, infundibular stem; LP, thalamic lateral posterior nucleus; MD, thalamic mediodorsal nucleus; ml, medial lemniscus; mt, mammillothalamic tract; optic tract; Pv, thalamic paraventricular nucleus; stm, striatum; TMC, hypothalamic tuberal nucleus; VLG, thalamic ventral lateral geniculate nucleus; VPT, thalamic ventral posterior nucleus; 3v, third ventricle.
immunopositive. In contrast to $G_0$ and $G_{i1}$ and $G_2$, $G_s$ was detected primarily in the neuronal cytoplasm.

**Cortex**

In our investigation, the three different antibodies label the cortex with different intensities with $G_0 > G_{i1}$ and $G_2 > G_s$, consistent with quantitative Western blot studies (Saito et al., 1989). The cortex is enriched with neurotransmitter receptors that couple to $G_0$, $G_{i1}$ and $G_2$, or $G_s$ (McCormick, 1992). Our results show that $G_0$ immunoreactivity in the cortex is located in the synaptic rich neuropil and is consistent with those reported by other investigators (Worley et al., 1986; Asano et al., 1987; Terashima et al., 1988; Aoki et al., 1992). Interestingly, we did not observe any $G_0$ labeling within the perikarya as described by Aoki et al., (1992) and Jordon (1990). There are a number of reasons for the discrepancy concerning the morphological location of $G_0$. Different antibody sources and immunohistochemical protocols were used in previous reports. Indeed, Aoki et al., (1992) report that $G_0$ labeling in the perikarya was observed only after higher dilutions of the primary antibody were used to decrease the signal in the neuropil.

Our detection of $G_{i1}$ and $G_2$ in the neuropil agrees with the observations of Asano et al., (1990b) and Aoki et al., (1992). However, we also detected $G_{i1}$ and $G_2$ in the fibers arising from the external capsule. The $G_{i1}$ and $G_2$ immunoreactivity may be located in a subset of cortical fibers since the antibody labeled discrete individual fibers.
There is very little information available from previous reports on the morphological location of $G_s$ in cortical neurons. The low abundance of $G_s$ in the central nervous system and antibody specificity contribute to the difficulty of analyzing the distribution of $G_s$ using immunohistochemical techniques. However, we detected $G_s$ in the cytoplasm of some cortical neurons. It is unlikely that our observations are due to a cross-reactivity with $G_{olf}$ since a previous study reported that the cortex did not contain detectable levels of $G_{olf}$ protein or mRNA (Hervé et al., 1993).

Hippocampus

Our results generally agree with earlier investigations which established the location of $G$ proteins in the hippocampus (Worley, et al., 1986; Terashima et al., 1988, 1993; Asano et al., 1990b; Aoki et al., 1992). In addition, we also report that the different $G$ protein antibodies produced different labeling patterns within the same subfield. The differential labeling patterns may represent different synaptic locations for the $G$ proteins. The patchy $G_{i1}$ and $G_{i2}$ immunoreactivity concentrated near the perimeter of the pyramidal cell body in subfield CA3, suggests that the $G$ proteins are located at terminals near the cell soma. A previous report by Aoki et al. (1992) described $G_o$ and $G_{i1}$ and $G_{i2}$ immunoreactivity within neuronal postsynaptic densities, axons, somata, and dendrites using electron microscopy. The contrasting locations of $G_o$ (diffuse neuropil) and $G_s$ (cytoplasm) is also very interesting. Different locations of the $G$ proteins may reflect different synaptic locations but other factors may also contribute to the different labeling
patterns. Some of these factors include differences in cell function and G protein storage. Our results also extend into subfield CA3 where we observed different immunoreactive patterns when compared to subfield CA1. The different labeling patterns maybe due to differences in synaptic locations or differences in cell size or type.

The hippocampus contains several types of G protein-linked receptors. The 5-HT$_{1A}$, adenosine, and GABA$_B$ receptors all couple to PTX-sensitive G proteins (i.e., G$_o$ and G$_i$) (Andrade et al., 1984; Zgombick et al., 1989; Okuhara and Beck, 1994) but it is not known if these receptors couple to the same PTX-sensitive G protein. Our results suggest that if these receptors are located at different sites on the neuron, they may indeed couple to different PTX-sensitive G proteins.

The different labeling patterns for G$_i$ and G$_o$ in subfields CA1 and CA3 may provide an explanation for the differences in 5-HT concentration-response curves in the two subfields. In the hippocampus, 5-HT$_{1A}$ receptor-activation increases an inward rectifying potassium current in both subfields CA1 (Andrade and Nicoll, 1987; Colino and Halliwell, 1987) and CA3 (Okuhara and Beck, 1994). However, 5-HT has a smaller maximal response and is more potent in subfield CA1 (Beck et al., 1992). One possible explanation for this difference is that the 5-HT$_{1A}$ receptor may couple to different G proteins in the two subfields. Both the 5-HT$_{1A}$ receptor and G protein appear to be at different sites along the neuron in subfields CA1 and CA3. The 5-HT$_{1A}$ receptor is closer to the cell body in subfield CA3 than in subfield CA1 (Pazos and Palacios, 1985).
Therefore, based on our observations, the $5$-$\text{HT}_{1A}$ receptor may couple to $G_i$ in subfield CA3 and couple with either $G_i$ or $G_o$ in subfield CA1.

**Striatum**

The presence of all three $G$ protein types in the striatum is not surprising since these nuclei contain many different $G$ protein-linked receptors (Graybiel, 1990) along with mRNA for $G_s$ and $G_o$ (Brann et al., 1987; Largent et al., 1988). There is very little $G_i$ mRNA in the striatum (Aronin and DiFiglia, 1992), suggesting that the $G_{i1}$ and $2$ immunoreactivity observed in the parenchyma in our study and in the study by Aronin and DiFiglia (1992) is located in the axon terminals. However, based on the detailed light and electron microscopic localization of $G_i$ in the different basal ganglia structures (Aronin and DiFiglia, 1992) cytoplasmic labeling cannot be ruled out. While the immunohistochemical localization of the $G$ proteins in the striatum is not a novel finding, there are several differences between our observations and previous reports. The $G$ protein labeling pattern in our investigation and others (Worley et al., 1986; Aronin and DiFiglia, 1992) does not resemble the patch-matrix distribution often observed in the striatum with antibodies against tyrosine hydroxylase or autoradiographic localization of $\mu$-opiate receptor binding sites (Gerfen et al., 1987). However, while the detection of $G_o$ and $G_{i1and 2}$ in the striatal pencil fibers is the outstanding feature in our investigation, $G_o$ and $G_{i1and 2}$ labeling in the parenchyma is the outstanding feature in previous reports (Worley et al., 1986; Aronin and DiFiglia, 1992).
RM1 labeling in the striatum is difficult to interpret because this antibody cross-reacts with $G_{olf}$. Previous studies with the RM1 antibody (Cooper et al., 1990; Drinnan et al., 1991) report at least two immunoreactive bands on Western blots prepared with tissue from the striatum. One of these bands corresponds to $G_{olf}$ based on immunological and mRNA analysis (Drinnan et al., 1991; Hervé et al., 1993). Our observations in the striatum with RM1 may reflect $G_{olf}$ labeling since our labeling pattern resembles the one previously reported for $G_{olf}$ (Hervé et al., 1993). However, it is clear from our results that neither $G_{s}$ nor $G_{olf}$ is located with the striatal pencil fibers.

**Thalamus**

The homogeneous distribution of $G_{o}$ throughout the different thalamic nuclei is in agreement with a previous study by Worley et al. (1986). However, the fibrous labeling pattern of $G_{\text{il}1\text{and}2}$ in the neuropil is a novel finding. The thalamus contains a variety of neurotransmitter systems, including G protein-linked receptors, enabling it to function as a relay between the cortex and several sensory systems (McCormick, 1992). The moderate to intense presence of both $G_{\text{il}1\text{and}2}$ and $G_{o}$ in the ventral posterior thalamic nuclei, medial lemniscus, and internal capsule suggest that these G proteins have important roles in conveying somatosensory signals. The ventral posterior nuclei relay somatosensory information from the dorsal column-medial lemniscal system to the somatosensory cortex via the posterior limb of the internal capsule. The geniculate nucleus also contains neurotransmitter receptors that link to G proteins (Matute et al.,
1993). The moderate to intense $G_{i2}$ and $G_o$ immunoreactivity in the optic tract and lateral geniculate nuclei suggests that these G proteins may also have important roles in conveying visual signals.

**Hypothalamus**

In our study we demonstrate that the G proteins are differentially localized in cells of the hypothalamus, median eminence, and arcuate nucleus. Our results are consistent with previous mRNA and Western blot analysis detecting G protein expression in the hypothalamus (Largent *et al.*, 1988; Viollet *et al.*, 1994). The detection of PTX-sensitive G proteins $G_i$ and $G_o$ in the median eminence and infundibular stem coincide with the presence of 5-HT$_1$ (Biegon *et al.*, 1982) and melatonin receptors (Vanecek *et al.*, 1987; Lopez-Gonzalez *et al.*, 1991), two receptors which couple to PTX-sensitive G proteins (Andrade *et al.*, 1986; Reppert *et al.*, 1994). The detection of $G_i$ and $G_o$ immunoreactivity in the median eminence and infundibular stem suggests that these G proteins may be important in the secretions of hormones at these sites.

The release of growth hormone-releasing hormone and dopamine by the parvocellular neurons in the arcuate nucleus is regulated by G protein-linked neurotransmitter receptors. For example, growth hormone-releasing hormone and the somatostatin receptor have been shown to colocalize within the same neurons in the arcuate nucleus (Bertherat *et al.*, 1992; McCarthy *et al.*, 1992). The detection of $G_s$,
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$G_{i1}$ and $2$, and $G_o$ in the arcuate nucleus supports the observations that G protein-linked receptors play a role in hormone secretion in the hypothalamus (reviewed by Renaud et al., 1992; Liposits, 1993).

Very little is known about the function of the tuberomamillary nucleus and the types of neurotransmitter receptors located in the nucleus. However, our detection of G proteins in the tuberomamillary nucleus suggests that these neurons may contain neurotransmitter receptors that link to G proteins and may also help to explain the nucleus' role in Alzheimer's disease. The tuberomamillary nucleus projects to the cerebral cortex and is severely affected in Alzheimer's and Parkinson's disease (Braak and Braak, 1992). Furthermore, G protein and neurotransmitter receptor coupling are attenuated in cortical tissue isolated from Alzheimer's disease patients (Warpman et al., 1993; Wang and Friedman, 1994). Therefore, altered coupling between neurotransmitter receptors and G proteins in the tuberomamillary nucleus may contribute to some of the symptoms observed in patients with Alzheimer's disease.

Fiber Tracts and Choroid Plexus

Our novel detection of G proteins within white matter fiber tracts should be interpreted cautiously, especially since previous immunohistochemical studies have not described the labeling of G proteins in white matter fiber tracts. It is unlikely that our detection of $G_o$ and $G_{i1}$ and $2$ is due to nonspecific labeling since the labeling intensity and distribution were different in at least three tracts: the alveus, hippocampal fissure, and the
dorsomedial and lateroventral aspects of the external capsule. Furthermore, we also observed white matter fiber tract labeling using a different immunohistochemical protocol utilizing a substantially lower antibody concentration.

Since the G proteins are located at axon terminals (Aoki et al., 1992; Aronin and DiFiglia, 1992), G protein immunoreactivity within fiber tracts may be due to axonal transport of the proteins (Hendry and Crouch, 1991; Crouch et al., 1994). The presence of G protein immunoreactivity may also be due to the labeling of G proteins in oligodendrocytes. Indeed, neurotransmitter receptors have been detected in glial cells (reviewed by Ransom and Sontheimer, 1992; Chiu and Kriegler, 1994). It is clear from our investigation and previous reports that the G proteins are located and serve functions at sites beyond the synapse.

All three antibodies labeled the choroid plexus and this was the area of intense $G_3$ immunoreactivity. The presence of $G_0$ in the choroid was previously described by Péraldi et al. (1989) where it was detected at the apical pole of choroidal ependymocytes. The distribution of G proteins, Na-K-ATPase (Ernst et al., 1986), neurotransmitter receptors (Yagaloff and Hartig, 1985; Conn et al., 1986), and other ion channels in the choroid plexus suggests that the function of the choroid may be modulated by molecules in the cerebral spinal fluid or neuronal innervation (Péraldi et al., 1989).
Concluding remarks

The differential distribution of G proteins and neurotransmitter receptors within neurons and in different brain areas may contribute to the coupling specificity between the receptors and G proteins. In our investigation, we observed that the G proteins $G_s$, $G_0$ and $G_{i1}$ and $G_{i2}$ are distributed differently within the rat forebrain. We also observed differences in the intraneuronal labeling patterns between the G proteins. $G_s$ labeling was observed in the cytoplasm while $G_0$ and $G_{i1}$ and $G_{i2}$ labeling was located in the neuropil. $G_0$ and $G_{i1}$ and $G_{i2}$ labeling in the neuropil also produced different patterns. $G_0$ labeling was diffuse while $G_{i1}$ and $G_{i2}$ labeling was fibrous. The differences in G protein labeling patterns between brain areas and in neurons suggests that unique synaptic organizations may exist between the different brain nuclei. For example, certain brain areas may contain a high concentration of dendrodendritic synapses while other areas are characterized by dendrosomatic synapses. Unique synaptic organizations may also exist among different cell types. The colocalization of receptor and G proteins may be different between a mossy fiber and pyramidal cell synapse when compared to one between a basket cell and pyramidal cell. In order to determine if unique synaptic organizations do exists among the different brain areas, the brain nuclei in which differential labeling patterns for the G proteins were observed (i.e., hypothalamus, white matter fiber tracts, and habenula) should be analyzed, in detail, at the electron microscopic level.
CHAPTER VI

CORTICOSTERONE ALTERS G PROTEIN α-SUBUNIT LEVELS IN THE RAT HIPPOCAMPUS.

Abstract

The HPA axis regulates the synthesis and secretion of glucocorticoid steroid hormones. The hippocampus, a component of the limbic system, contains the highest concentration of corticosteroid receptors in the brain and may play an important role in regulating hypothalamic-pituitary-adrenal axis activity and mediating physiological responses to stress. In Chapter IV, it was concluded that corticosterone treatment alters the 5-HT₁A receptor signal transduction system at sites downstream of the receptor. The 5-HT₁A receptor couples to a PTX-sensitive G protein, therefore, corticosteroids may influence G protein levels and/or their intracellular location. The glucocorticoid corticosterone alters the response elicited by activation of several different G protein-linked neurotransmitter receptors in the hippocampus. In the present study we used Western blot and immunohistochemical techniques to determine the effects of chronic adrenalectomy (ADX), low (CT) and high (HCT) plasma corticosterone concentrations on Gₛ, G₁₁ and ₂ and Gₒ α-subunit levels and intracellular location in the rat hippocampus.
CT treatment increased \( G_s \alpha \)-subunit levels and HCT treatment increased the levels of \( G_s, G_{i1} \) and \( G_o \alpha \)-subunits when compared to sham as detected on Western blots. No change in the intracellular location of the \( G \) protein \( \alpha \)-subunits was detected using immunohistochemistry. Based on our results, we conclude that corticosterone alters \( G \) protein \( \alpha \)-subunit levels in the rat hippocampus without altering their intracellular location. These results provide an important piece of information towards understanding how glucocorticoids alter \( G \) protein-linked neurotransmitter responses.

**Introduction**

Glucocorticoids directly influence protein expression by binding to intracellular steroid receptors designated as MR and GR. In rats, the two corticosteroid receptors have different affinities for the glucocorticoid corticosterone (Corini et al., 1985; Reul and de Kloet, 1985). MR binds to corticosterone with a \( K_D = 0.5 \) nM. GR has a lower affinity for corticosterone with a \( K_D = 2.5 - 5 \) nM. The corticosteroid receptors in humans and other primates have affinities for corticosterone that are similar to rats (Tsuboi et al., 1979; Yu et al., 1981; Brooke et al., 1994). The hippocampus contains the highest concentration of corticosteroid receptors in the central nervous system (McEwen et al., 1968; Gerlach and McEwen, 1972; Maraginos et al., 1990) consequently, the function of corticosteroid receptors in the hippocampus is under intense investigation (review by de Kloet et al., 1993; Joëls and de Kloet, 1994).
Corticosterone alters the response elicited by the activation of several different G protein-linked neurotransmitter receptors. For example, in the rat hippocampus, corticosterone alters the responses elicited by activation of 5-HT\textsubscript{1A} receptors linked to G\textsubscript{i}/G\textsubscript{o} (Joëls \textit{et al.}, 1991; Joëls and de Kloet, 1992; Beck \textit{et al.}, 1996) and 5-HT\textsubscript{4} (Birnstein and Beck, 1995) and β-adrenergic receptors linked to G\textsubscript{i} (Roberts \textit{et al.}, 1984; Harrelson \textit{et al.}, 1987; Joëls \textit{et al.}, 1989; Gannon and McEwen, 1990). In Chapter IV it was concluded that corticosteroids also alter the response elicited by activation of 5-HT\textsubscript{1A} receptors in hippocampal subfield CA3. The mechanism by which glucocorticoids alter the G protein-linked neurotransmitter receptor responses is not completely known and is currently under investigation. Depending on the receptor system, glucocorticoids may alter the receptor, G protein, and/or effector molecules. For example, in the rat brain glucocorticoids alter dopamine D1 and D2 (Biron \textit{et al.}, 1992) and serotonin 5-HT\textsubscript{2C} (Holmes \textit{et al.}, 1995a,b) receptor mRNA levels, β-adrenergic receptor binding levels (Roberts and Bloom, 1981), and 5-HT\textsubscript{1A} receptor binding and mRNA levels (reviewed by Chaouloff, 1995). Glucocorticoids also modulate effector molecule function and expression. For example, glucocorticoids alter adenylyl cyclase activity and mRNA levels (Gannon \textit{et al.}, 1994; Wolfgang \textit{et al.}, 1995) in the rat hippocampus.

In addition to the changes in neurotransmitter receptor levels and effector molecule activity, glucocorticoids may also alter G protein expression or activity. The regulation of G protein levels by glucocorticoids has been examined in several tissue types including
liver (Kawai and Arinze, 1993), aorta (Haigh et al., 1990), fetal forebrain tissue (Slotkin et al., 1994), and vascular smooth muscle (Schelling et al., 1994). However, there is little information on the effects of corticosterone on G protein expression and function in the hippocampus. In Chapter IV it was concluded that corticosteroids altered the outward current evoked by the activation of G proteins with GTPγS. Changes in G protein function may reflect changes in protein levels and/or intracellular location, altering the interaction between specific receptor-G protein-effector molecule complexes. Therefore, in the present study, we used Western blotting and immunohistochemical techniques to determine the effects of corticosterone on G protein α-subunit levels and distribution in the hippocampus.

**Materials And Methods**

**Materials**

Corticosterone pellets were purchased from Innovative Research of America (Sarasota, FL). G protein α-subunit polyclonal antibodies RM1 (Gα), AS7, (Gα1 and 2), and GC2 (Gα) were purchased from Dupont-New England Nuclear (Wilmington, DE). Monoclonal rabbit peroxidase-antiperoxidase antibody was purchased from Cappel (Durham, NC). Picric acid was purchased from Aldrich (Milwaukee, WI). 3,3'-diaminobenzidine tetrachloride (DAB) was purchased from Sigma (St. Louis, MO). All other chemicals were obtained from commercial sources.
Animals

Male Sprague-Dawley rats (75-100 g; Harlan) were maintained on a twelve hour light-dark cycle (7 a.m. to 7 p.m. light on). Bilateral adrenalectomies (ADX) were performed under ether anesthesia. An incision was made just below the rib cage and the adrenal gland removed. The muscle wall was then sutured and the skin closed using wound clips. To predominately activate MR and mimic basal levels of plasma corticosterone, one group of animals (CT) received a subcutaneous 12.5 mg corticosterone pellet. To activate both MR and GR, another group of animals (HCT) received 300 mg corticosterone pellets subcutaneously. A sham group of animals was produced by visualizing the adrenal glands but leaving them intact. Following surgery, all animals were given standard laboratory chow ad libitum. SHAM animals were given standard drinking water while the ADX, CT, and HCT animals were given 0.9 % NaCl drinking water ad libitum. All animals were sacrificed 2 weeks after surgery. Sacrifices occurred before 12:00 noon and blood samples were collected at that time to determine plasma corticosterone levels (Burgess and Handa, 1992).

Membrane Preparation

Membrane proteins were isolated from hippocampal tissue using a procedure described elsewhere (Sternweis and Robinshaw, 1984; Okuhara et al., 1996c). Protein isolation procedures were carried out at 4°C. Briefly, the animals were decapitated and the hippocampus dissected free. The hippocampus was homogenized with a hand-held
dounce homogenizer in TBS (10 mM Tris, pH 7.6 and 150 mM NaCl), 10% sucrose, and 0.5 mM phenylmethansulfonyl fluoride. The homogenate was then centrifuged at 20,000 X g for 30 min. The supernatant was removed and the membrane pellet was resuspended in a second identical wash and centrifuged again at 20,000 X g for 30 min. The supernatant was removed and the membrane pellet was resuspended in TBS, 1 mM EDTA and 1% sodium cholate and incubated for 60 min with constant mixing. The supernatant containing the soluble membrane protein was separated by centrifugation at 35,000 rpm for 60 min and stored at -80°C.

Western Blot Analysis

Western blotting techniques used in this investigation are described elsewhere (Lesch and Manji, 1992. Briefly, membrane proteins (6 µg) were resolved by SDS-PAGE and electrophoretically transferred to nitrocellulose membrane filters. Equal loading of protein samples was verified by staining a parallel polyacrylamide gel with Coomassie blue. After transfer, the nitrocellulose was incubated in TBS, 0.2% NP-40, and 5% nonfat dry milk at room temperature to block non-specific binding. After 60 min the nitrocellulose was incubated with a 1/10,000 dilution of AS7, RM1, or GC2 overnight at 4°C. The nitrocellulose was then incubated with a 1/10,000 dilution of goat anti-rabbit IgG for 60 min at room temperature followed by an incubation with 1/10,000 dilution of rabbit PAP. The blots were developed with the ECL chemiluminiscent
detection kit (Amersham, Arlington Heights, IL) and exposed to x-ray film. All antibody
dilutions were in TBS, 5% BSA, and 0.2% NP-40. The nitrocellulose filter was washed
with TBS/0.2% NP-40 between every antibody incubation and prior to development
with the ECL kit.

The optical density and area of the G protein bands were obtained using the NIH
Image analysis program. Gray scale density readings were calibrated using a transmission
step wedge standard fitted to the Rodbard equation. An integrated optical density (IOD)
was calculated by summing the optical densities within the area of a band. To determine
the IOD of the G protein bands, the bands were outlined three times and the mean IOD
was calculated. An area adjacent to the G protein band was used to calculate the
background optical density of the film and was subtracted from the integrated optical
density of the G protein band. The linear range of the XAR film was determined by
including a range of hippocampal protein (0.5 to 3 fold) in each Western blot. Five
animals were used for each treatment group. Western blot experiments were repeated
three times for each antibody. Each blot contained tissue samples from SHAM and
either ADX, CT, and HCT treated animals, therefore, the IOD values for the ADX, CT,
and HCT groups were standardized to their respective SHAM values and reported as
% change from SHAM (SHAM = 0%). The results from three Western blot experiments
were averaged and a two-way analysis of variance was performed using ADX, CT, and
HCT treatment as one factor and G protein α-subunit as another factor. The Student-
Newman-Keuls method was used as the post-hoc test. To compare the effects of ADX, CT, or HCT directly to the effects of SHAM treatment, the IOD of the G protein bands on each Western blot were compared using a Student's *t*-test.

**Immunohistochemical Procedure**

The animals were sacrificed with a lethal injection of phenobarbital and perfused with 200 ml PBS (10 mM NaHPO₄, pH 7.4, and 150 mM NaCl) *via* the aorta at 650 ml/h, followed by 250 ml PA fixative (4% paraformaldehyde, 1% glutaraldehyde, 0.2% picric acid, 2% sucrose, 0.1 M acetate buffer, pH 6.0) as described previously (Asano *et al.*, 1990; Okuhara *et al.*, 1996c). After fixation, the brain was removed, blocked, and placed in PA for 4-6 h. After rinsing and overnight incubation in TBS/10% sucrose, the tissue was embedded in paraffin, sectioned at 7 µm, and mounted on microscope slides.

Immunohistochemical labeling of Gₐ and Gₒ α-subunits was performed following protocols described by Sternberger *et al.*, (1970). Briefly, tissue sections were blocked with TBS/5% bovine serum albumin (BSA)/3% normal goat serum (NGS) for 1 hour at room temperature. After washing the sections with TBS, three times for 10 min each, they were incubated with RM1 diluted 1:50 or GC2 diluted 1:100 overnight at room temperature. Several sections were incubated with 2% NGS in place of primary antibody to determine the level of nonspecific labeling. Sections were then washed with TBS, three times for 10 min each, and incubated with goat anti-rabbit IgG diluted 1:20.
After 1 h, sections were washed with TBS and incubated with rabbit peroxidase-antiperoxidase for 1 h. All antibody dilutions were made in TBS/5% BSA/3% NGS. Immunohistochemical labeling of G_{11} and 2 α-subunits was performed with the Vectastain Elite kit (Vector Laboratories, Inc., Burlingame, CA). Briefly, after blocking with NGS, tissue sections were incubated overnight with AS7 diluted 1:400. All sections were washed in TBS and developed with hydrogen peroxide and DAB as the chromagen.

Each immunohistochemistry experiment was repeated once and analyzed independently without knowledge of the treatment. The location of the antibody label within the dorsal hippocampus was recorded for each slide. Three animals were used for each treatment group.

Results

Corticosterone Plasma Levels

The mean plasma corticosterone levels were (mean ± SD, n=3): 3.2 ± 4.2 µg/dl for SHAM, 0 ± 0 for ADX, 3.1 ± 1.5 for CT, and 25.1 ± 6.4 for HCT. These corticosterone levels are comparable to those reported previously by our laboratory (Beck et al., 1994; 1996; Birnstiel and Beck 1995) and other investigators (Reul and de Kloet, 1985; Holmes et al., 1995a).
Western Blots

On Western blots prepared with the membrane fraction from hippocampal tissue, antibodies labeled the appropriate size bands (Okuhara et al., 1996c). The RM1 antibody labeled a 45 kDa and 54 kDa protein (Figure 19A) corresponding to the short and long forms of the G\(_\text{a}\) subunit (Bray et al., 1986; Robinshaw et al., 1986; Kozasa et al., 1988). Detection of the 54 kDa band was inconsistent. When the band was detected, it was a faint and indistinct. The AS7 and GC2 antibodies labeled a 40 kDa band (Figure 19A) corresponding to the appropriate size bands for G\(_{i1}\) and G\(_{i2}\) and G\(_{o}\) \(\alpha\)-subunits respectively (Goldsmith et al., 1987; Cooper et al., 1990).

The coefficient of variation (standard deviation \(\div\) mean) for the SHAM IOD values were calculated for each antibody to determine the variability in the control data. The mean coefficient of variation (mean \(\pm\) S.E.M, \(n = 9\) for all antibodies) was 0.20 \(\pm\) 0.05, 0.15 \(\pm\) 0.01, and 0.14 \(\pm\) 0.02 for the AS7, GC2, and RM1 antibodies respectively.

A two-way analysis of variance of the data indicated that corticosterone treatment produced a significant effect (\(F = 11.3, P < 0.001\)) on G protein \(\alpha\)-subunit levels. The percent change in G protein \(\alpha\)-subunit levels relative to SHAM was greater in tissue from HCT treated animals compared to ADX (\(q = 6.390, p < 0.05\)) and CT (\(q = 5.037, p < 0.05\)) based on Student-Newman-Keuls post hoc tests. In comparison to the SHAM group, ADX treatment did not alter the levels of G\(_{s}\), G\(_{i1}\) and G\(_{i2}\) and G\(_{o}\) \(\alpha\)-subunits. CT treatment only increased G\(_{s}\) \(\alpha\)-subunit levels. HCT treatment increased the levels of G\(_{s}\),
FIGURE 19. Western blots of G protein α-subunits prepared from hippocampal membrane fractions. A: Antibody labeling of G protein α-subunits. Lane 1, anti-Gs (RM1). Lane 2, anti-G_{i1 and 2} (AS7). Lane 3, anti-G_o (GC2). B: Antibody labeling of G protein α-subunits isolated from five SHAM (S) and five HCT (HCT) animals. G protein α-subunit subtype is indicated on the left.
$G_{11}$ and $G_2$ and $G_\alpha$-subunits when compared to SHAM (Figure 19B). Analysis of Coomassie blue stained gels verified that equal amounts of protein were loaded into each lane and that there were no overall changes in total protein levels. However, the Coomassie stained gels revealed that HCT appeared to alter the protein levels for several bands when compared to SHAM; some bands increased while others decreased (Figure 20). Based on the similar migration of the labeled bands, neither ADX, CT, or HCT treatment appeared to alter $G$ protein size. Figure 21 summarizes the results from three different Western blot experiments for each $G$ protein $\alpha$-subunit antibody.

Figure 20. Coomassie blue stained gel of tissue samples used for Western Blots. A SDS-PAGE gel was prepared identically as in figure 19 and stained with Coomassie blue. S, SHAM; H, HCT. Molecular weight markers are in the first lane on the left.
Figure 21. Changes in G protein α-subunit levels with different corticosterone treatments. The graph summarizes the results from three Western blot experiments (mean ± SEM), n = 3. The bars are grouped by G protein α-subunit subtype; G<sub>0</sub> (open), G<sub>s</sub> (light stippled gray), and G<sub>11 and 2</sub> (dark stippled gray). Treatment group and G protein α-subunit type is indicated along the x-axis.

* Significantly different from SHAM, Students t-test, P < 0.05. SHAM is 0%.

Immunohistochemistry

While Western blot analysis can be used to detect a change in G protein levels, changes in the location of G protein α-subunits within specific areas of the hippocampus or in specific locations within neurons cannot be determined. Therefore, we used immunohistochemistry techniques to address these questions. As reported previously, the labeling pattern for the G protein α-subunits varied across subfields and among the
different G proteins examined (Okuhara et al., 1996c). G, α-subunit labeling was located in the cytosol of the soma in hippocampal subfields CA1-CA4 (Figure 22B). In contrast to Gs, the labeling of Go α-subunits was located in the neuropil in subfields CA1-CA4 (Figure 22D) and dentate gyrus (Figure 22F). G11 and 2 α-subunits labeling was equally intense in the soma cytosol and neuropil. In control tissue sections, where the primary antibody was omitted, nonspecific labeling was very faint and distributed evenly throughout the entire tissue section (data not shown). The distribution of the G proteins among the various hippocampal subfields and their location within neurons was not altered in any treatment group.

Discussion

G proteins couple plasma membrane receptors to intracellular effector molecules to transduce extracellular signals into biochemical and physiological changes within a cell. In the present study, we examined the effects of several chronic corticosterone treatments on G protein α-subunit levels and distribution in the hippocampus. Chronic basal concentrations of corticosterone increased Gs α-subunit levels while higher concentrations of corticosterone increased the α-subunit levels of Gs, G11 and 2 and Go as determined by Western blot analysis. None of the corticosterone treatments altered the intracellular location of G proteins based on our immunohistochemistry results.
Figure 22. Immunohistochemical labeling of G protein α-subunits and cresyl violet staining of nissel bodies in the hippocampus. B: Gα labeling in subfield CA3 in sections from a SHAM animal. X200. Or, oriens layer; Py, pyramidal layer; rad, radiatum layer. D: Cytoplasmic labeling of Gα in a pyramidal cell (arrow head). Or, oriens layer; Py, pyramidal layer; rad, radiatum layer. Gα labeling in subfield CA1 in sections from a SHAM animal. X200. F: Gα labeling in the dentate gyrus in sections from an ADX animal. X100. g, granular layer. Panels A, C, and E are photomicrographs of cresyl violet stained nissel bodies from similar tissue sections of subfields CA3, CA1, and dentate gyrus respectively.
Adrenalectomy had no effect on G protein α-subunit levels or distribution. Two other studies examined the effects of corticosterone on G protein α-subunit levels in the hippocampus. The increase in $G_s$ and $G_o$ α-subunit levels that we observed with high corticosterone concentrations was also reported by Wolfgang et al., (1995) using animals subjected to restraint stress. Gannon et al., (1994) reported that neither adrenalectomy nor corticosterone treatment altered the levels of $G_s$ or $G_i$ α-subunits in the hippocampus. We also observed no change in the α-subunit levels of $G_{11}$ and 2 with adrenalectomy or basal concentrations of corticosterone. We did detect an increase in the α-subunit levels of $G_s$ with basal corticosterone concentrations and this increase may not have been detected by Gannon due to important differences in the treatment paradigms. In Gannon’s study, the animals were sacrificed at the peak of the diurnal surge when both MR and GR are occupied in the sham animals. In contrast, our animals were sacrificed during the trough of the diurnal surge when only a small fraction of GR is occupied. The time of sacrifice maybe a very important experimental parameter to take into account since both GR and MR mRNA levels show circadian variation (Holmes et al., 19995a).

The effects of glucocorticoids on G protein expression have been examined in other tissue with various results. Adrenalectomy decreased $G_i$ and increased $G_s$ α-subunit
levels in the rat aorta when compared to sham (Haigh et al., 1990). In the cerebral
cortex, corticosterone replacement decreased G\textsubscript{i} and increased G\textsubscript{\textalpha} subunit levels when
compared to adrenalectomy (Saito et al., 1989). Finally, in the liver, dexamethasone
(which selectively activates GR) increased the \alpha-subunit levels of both G\textsubscript{\textalpha} and G\textsubscript{i} (Kawai
and Arinze, 1993). It is clear from the various effects of glucocorticoids on G protein
levels in different tissue types that the effects of glucocorticoids are tissue specific.

Examining the effects of glucocorticoids on G protein levels may help to determine
the mechanism by which glucocorticoids modulate the responses associated with
different G protein-linked neurotransmitter receptors. For example, it is known that
glucocorticoids modulate neurotransmitter stimulated cAMP accumulation in both
periphery and central nervous systems (reviewed by Harrelson and McEwen, 1987). For
example, adrenalectomy increases NA stimulated cAMP accumulation in the limbic
forebrain and frontal cortex (Mobley and Sulser, 1980a,b). Adrenalectomy also increased
cAMP accumulation in response to vasoactive intestinal peptide but decreased the same
response to histamine (Harrelson et al., 1987). In the hippocampus, adrenalectomy
increased the NA stimulated cAMP accumulation (Roberts et al., 1984; Harrelson et al.,
1987) without altering \beta-adrenergic receptor binding (Roberts et al., 1984) or forskolin-
stimulated adenylyl cyclase activity (Mobley et al., 1983; Harrelson et al., 1987; Gannon
and McEwen, 1990). The administration of corticosterone reversed the effects of
adrenalectomy in the hippocampus (Roberts et al., 1984) and cortex (Mobley et al.,
1983) while even higher levels of corticosterone attenuated the NA response below control levels in the hippocampus (Gannon and McEwen, 1990). We observed no change in G protein α-subunit levels with ADX. Therefore, the glucocorticoid-induced effects on NA stimulated cAMP accumulation are not occurring by directly altering neurotransmitter receptor binding or G protein levels. Adrenalectomy may be altering the coupling between signal transduction components.

Unlike the noradrenergic receptors, several studies have shown that glucocorticoids modulate 5-HT₁A receptor expression in the hippocampus. Despite some conflict on the effects of glucocorticoids among the various subfields (reviewed by Chaouloff, 1995), adrenalectomy appears to increase 5-HT₁A receptor binding (Mendelsohn and McEwen, 1992a,b; Chalmers et al., 1993; Kuroda et al., 1994; Tejani-Butt and Lablow, 1994; Zhong and Ciaranello, 1995) and mRNA levels (Chalmers et al., 1993; Meijer and de Kloet, 1994; Zhong and Ciaranello, 1995). The effects of adrenalectomy on 5-HT₁A expression and binding is reversed with corticosterone.

In the present study, ADX did not alter the α-subunit levels of Gᵢ and Gₒ, the G proteins probably linked to the 5-HT₁A receptor in subfield CA3 (Okuhara et al., 1994). Therefore, neither the smaller EC₅₀ value nor the larger GTPγS evoked current observed in cells from ADX treated animals reported in Chapter IV is due to changes in Gᵢ or Gₒ α-subunit levels. Furthermore, the HCT-induced increase in Gᵢ or Gₒ α-subunit levels observed in the present investigation also does not account for the larger EC₅₀
value or smaller GTPγS evoked current observed in cells from HCT treated animals reported in Chapter IV. The increase in G protein α-subunit levels may be a compensatory response to changes in the receptor signal transduction system. Therefore, corticosteroids may be altering the 5-HT_{1A} receptor signal transduction system by modulating the kinetic properties of the potassium channel linked to the receptor, coupling between the G protein and potassium channel, or G protein activity.

There are several reasons why changes in G protein levels and neurotransmitter receptor responses may not coincide. Neurotransmitter receptors, G proteins, and effector molecules are all targets for post-translational modifications, such as phosphorylation (Bushfield et al., 1990; Akiho et al., 1993; Girault, 1993; Yamane and Fung, 1993; Levitan, 1994; Morris et al., 1994; Siegelbaum, 1994; Levistre et al., 1995; Neer, 1995; Pfeifer et al., 1995; Premont et al., 1995). Post-translational modifications may alter the coupling of receptor, G proteins, and effector molecules and therefore their ability to function (reviewed by Girault, 1993; Yamane and Fung, 1993; Levitan, 1994; Siegelbaum, 1994; Neer, 1995; Premont et al., 1995). Future studies will address issues of the phosphorylation state of the G protein signal transduction components.

Another reason why it is difficult to correlate changes in G protein levels with changes in receptor-mediated responses is the potential interaction of the different G protein subunits and effector molecules. It is clear the βγ-subunits as well as
α-subunits modulate the activity of the various adenylyl cyclase isoforms (reviewed by Birnbaumer and Birnbaumer, 1995) and ion channel activity (Kofuji et al., 1995).

Although glucocorticoids do regulate β-subunit levels in the rat aorta (Haigh et al., 1990), adipocytes (Ros et al., 1989), and liver (Kawai and Arinze, 1993), they do not regulate β-subunit expression in the rat cortex (Saito et al., 1989). Glucocorticoid effects on βγ-subunits in the hippocampus are not known.

In conclusion, G proteins are associated with a large number of biochemical and physiological processes including hormone, neurotransmitter, and growth factor receptor mediated responses. The steroid hormone corticosterone alters the responses of neurotransmitter receptors which are linked to G proteins. In the present study, high concentrations of corticosterone increased the G protein α-subunit levels of Gs, Go, and Gila and 2 in the hippocampus. Our results provide important information towards understanding how corticosterone modulates neurotransmitter receptor-mediated responses in the hippocampus.
CHAPTER VII
DISCUSSION

Major Findings

The hippocampus contains the highest density of corticosteroid receptors in the brain, which leads to the suggestion that corticosteroids may have a profound influence on hippocampal signal processing. Previous investigations have demonstrated that corticosteroids modulate hippocampal pyramidal cell membrane electrophysiological characteristics and responses elicited by the activation of different neurotransmitter receptors (Joëls and de Kloet, 1992; Beck et al., 1994; Birnstiel and Beck, 1995; Beck et al., 1996). However, these findings are limited to the cells in subfield CA1. The effects of corticosteroids on neuron cell properties for other hippocampal regions (i.e., dentate gyrus and subiculum) and subfields (i.e., CA2 and CA3) are not known. Information on the actions of corticosteroids in all regions of the hippocampus is essential to determine how corticosteroids modulate signal processing in the hippocampus. Therefore, it was the goal of this dissertation to examine the actions of corticosteroids on cell membrane electrophysiological characteristics and the 5-HT\textsubscript{1A} receptor signal transduction system in subfield CA3 pyramidal cells. There are two basic hypotheses for this dissertation. First, corticosteroids influence cell membrane
electrophysiological properties in subfield CA3 pyramidal cells. Second, corticosteroids modulate the response elicited by the activation of the 5-HT$_{1A}$ receptor. Furthermore, corticosteroids modulate the 5-HT$_{1A}$ receptor-mediated response by altering different components (i.e., receptor, G protein, potassium channel) of the 5-HT$_{1A}$ receptor signal transduction system.

One major finding of this dissertation is that chronic corticosteroid treatment influenced pyramidal cell membrane electrical properties in subfield CA3. Corticosteroid treatment shifted the ratio of nonburst and burst firing neurons and the action potential duration in subfield CA3. The effects of corticosteroids on the cell firing mode and action potential duration are not likely to be due to changes in cell viability. The cell membrane potential, resistance, and time constant were the same in all treatment groups and similar to those reported in naive animals (Beck et al., 1992). The effects of corticosteroids on the cell firing mode and action potential duration may be secondary to changes in CA3 cell morphology. However, it unlikely that changes in cell morphology would only affect the cell firing mode and action potential duration.

The alteration in cell firing pattern and action potential duration may have a significant effect on the synaptically encoded signals sent from subfield CA3 to CA1. Shifting the firing pattern of subfield CA3 cells represents a prominent and critical change in the pattern of communication between subfields CA1 and CA3. Further experiments are required to determine the ion channels and mechanism underlying the
corticosteroid-induced changes in cell firing pattern and action potential duration. It is also important to determine if the ratio of burst and nonburst firing cells changes during the diurnal surge of corticosteroids.

Another major finding of this dissertation is that chronic corticosteroid treatment altered the 5-HT concentration-response curve characteristics for the 5-HT1A receptor on subfield CA3 pyramidal cells. Furthermore, corticosteroids altered the 5-HT1A receptor signal transduction system at sites (i.e., G protein) downstream of the receptor. The corticosteroid-induced changes in the 5-HT concentration-response curve characteristics for the 5-HT1A receptor may be due to changes in G protein function. The smaller EC50 and greater Emax value observed in cells from ADX treated animals was also accompanied by an increase in the current evoked by the activation of G proteins with GTPγS. Similarly the larger EC50 and smaller Emax value observed in cells from HCT treated animals was accompanied by a decrease in the current evoked by the activation of G proteins. However, the ADX- and HCT-induced effects are not directly due to changes in G protein Gi1 and 2 or Go α-subunit levels. Based on Western blot analysis, ADX treatment had no effect while HCT treatment increased Gi1 and 2 and Go α-subunit levels when compared to SHAM.

Based on these results, there are several possible mechanisms by which corticosteroid treatment may be altering the 5-HT1A receptor system. Corticosteroids may influence the kinetic properties of the potassium channel linked to the 5-HT1A
receptor. Corticosteroid may also modulate G protein function by altering the coupling between G protein and potassium channels, GTP hydrolysis, or the exchange rate of GDP for GTP. These changes in G protein activity or ion channel kinetics may occur through post-translational modification mechanisms (i.e., phosphorylation).

Based on the results from this dissertation and those previously reported by collaborating colleagues (Beck et al., 1994, 1996), it can be concluded that corticosteroids also modulate neural signal processing in the hippocampus by differentially modulating neuron membrane properties in subfields CA1 and CA3. Adrenalectomy and MR activation have opposite effects on action potential duration in subfield CA1 and CA3 cells. There was no difference in the 5-HT concentration-response curve characteristics for the 5-HT1A receptor in cells from SHAM and ADX treated animals in subfield CA1. In contrast, the EC50 value was shifted in cells from ADX compared to SHAM treated animals in subfield CA3. The different effects of corticosteroids on neuron membrane electrical properties in subfields CA1 and CA3 further supports the critical roles that the different hippocampal subfields have on HPA axis activity. One future study that may help to determine the roles of hippocampal subfields CA1 and CA3 on HPA axis activity is examining the effects of stimulating subfields CA1 and CA3 in different treatment groups (i.e. HCT and MR) on corticosterone plasma levels.
Synaptic Activity

The new findings resulting from this dissertation work provide important information on the action of corticosteroids on neural signal processing in the hippocampus. Earlier investigations examined the action of corticosteroids on synaptically driven field potentials in subfield CA1. Stimulating the Schaffer collaterals arising from subfield CA3 pyramidal cells evokes action potentials in CA1 pyramidal cells that summate and produce a corresponding population spike (Andersen et al., 1971). The population spike amplitude in subfield CA1 is decreased in hippocampal sections from adrenalectomized compared to adrenally-intact animals (Rey et al., 1987a,b; Doi et al., 1991; Joëls and Fernhout, 1993). The adrenalectomy-induced effect was reversed when the slice was perfused with basal physiological concentrations of corticosterone. Consistent with the decrease in population spike amplitude, a study using intracellular recording techniques reported that the probability for inducing synaptically driven action potentials was decreased in adrenalectomized compared to adrenally-intact animals (Joëls and de Kloet, 1993). However, the decrease in firing probability was not accompanied by a decrease in the excitatory postsynaptic potential (EPSP) amplitude (Joëls and de Kloet, 1993). Recently, it was demonstrated that the EPSP and inhibitory postsynaptic potential (IPSP) both are decreased in cells from adrenalectomized compared to adrenally-intact animals (Birnstiel and Beck, 1995).
The decrease in population spike amplitude may be explained by the change in pyramidal cell nonbursting to burst firing ratio observed in this dissertation work. A shift in the pyramidal cell’s firing pattern in subfield CA3 may decrease the population spike amplitude evoked in subfield CA1. However, this is only a conjecture since the relationship between subfield CA3 nonburst and burst firing cells with the EPSP and population spike amplitude in CA1 cells is not known.

Several important questions have developed from this dissertation. First, what are the mechanisms underlying the shift in the ratio of nonburst to burst firing cells? Since the current(s) underlying burst firing have not been fully characterized, this is a difficult question to address. Second, how is the ratio of nonburst to burst firing cells in subfield CA3 related to the population spike, or EPSP, recorded in subfield CA1? Finally, does corticosteroid treatment also shift the ratio of nonburst to burst firing cells in other regions of the hippocampus? The answers to these questions will provide more insight into the mechanism by which corticosteroids alter neuronal signal processing in the hippocampus.

**Chronic Corticosteroid Treatment**

Normally, plasma corticosteroid concentrations fluctuate according to circadian rhythm or acute stress (reviewed by Dallman *et al.*, 1987). However long-term increases in plasma corticosteroid concentrations are observed during chronic stress and senescence and are accompanied by changes in hippocampal physiology (Sapolsky
1985; Sapolsky et al., 1985a, 1986; Stein-Behrens et al., 1994). Therefore, investigators have focused on elucidating the mechanisms underlying the changes in hippocampal physiology observed with long-term exposures to high corticosteroid concentrations.

Briefly, chronically high levels of corticosteroids increase the vulnerability of hippocampal CA3 neurons to cell damage (Sapolsky 1985; Sapolsky et al., 1985a; Stein-Behrens et al., 1994). An alteration in Ca\(^{2+}\) homeostasis is proposed as one factor that may increase the vulnerability of hippocampal CA3 neurons to cell damage. Increasing the excitability of neurons may elevate intracellular Ca\(^{2+}\) concentrations enough to damage a cell (Landfield and Eldridge, 1994). However, HCT treatment did not selectively alter any of the cell membrane properties examined in this dissertation work. Therefore, a two week chronic treatment with high concentrations of corticosterone does not appear to lead to changes in membrane electrical properties that influence neuron excitability in subfield CA3. The effects of HCT treatment on Ca\(^{2+}\) homeostasis or isolated Ca\(^{2+}\) currents were not examined in this dissertation.

An increase in CA3 pyramidal cell excitability may occur from alterations in the 5-HT\(_{1A}\) receptor-mediated response observed in this dissertation work. A decrease in the maximum current elicited by the activation of 5-HT\(_{1A}\) receptors was observed in cells from HCT compared to SHAM treated animals. Physiologically, the HCT-induced change decreases the inhibitory response of CA3 pyramidal cells to 5-HT,
which may lead to an increase in the cell’s response to excitatory inputs. This question may be addressed by using extracellular recording techniques and examining the effects of HCT treatment on the response of CA3 cells to the stimulation of 5-HT inputs (i.e., raphe).

**G protein-linked Receptor Signal Transduction System**

Previous investigators have demonstrated that corticosteroids influence the hippocampal 5-HT system in at least two ways. First, corticosteroids alter the response elicited by the activation of either the 5-HT₁A (Joëls et al., 1991, Joëls and de Kloet, 1992; Beck et al., 1996) or 5-HT₄ receptors (Birnstiel and Beck, 1995). Second, corticosteroids modulate the number of 5-HT₁A receptor binding sites and mRNA expression (reviewed by Chaouloff, 1995). Based on the results from this dissertation work, it was shown that corticosteroids also alter the 5-HT₁A signal transduction system at site(s) downstream from the receptor. This observation bears an interesting question. Are other G protein-linked receptor signal transduction systems altered by corticosteroids?

Similar to the results in this dissertation work, the β-adrenergic receptor-mediated response in the hippocampus is also increased in adrenalectomized animals compared to adrenally-intact animals. Adrenalectomy increases NA stimulated cAMP accumulation (Roberts et al., 1984; Harrelson et al., 1987) without altering β-adrenergic receptor
binding (Roberts et al., 1984) or forskolin-stimulated adenylyl cyclase activity (Mobley et al., 1983; Harrelson et al., 1987; Gannon and McEwen, 1990).

No changes in G protein α-subunit levels with ADX were observed in this thesis work. Therefore, the adrenalectomy-induced effect on NA stimulated cAMP accumulation is not occurring by directly altering neurotransmitter receptor binding or G protein levels. Adrenalectomy may be altering the coupling between signal transduction components. In support of this hypothesis, it was observed in this thesis work that the EC₅₀ value for the 5-HT₁₅ receptor-mediated response was smaller (i.e. 5-HT was more potent) in cells from ADX compared to SHAM animals. Furthermore, the current elicited by the activation of G proteins with GTPγS was also greater in cells from ADX compared to SHAM animals. Therefore, adrenalectomy may alter the coupling or activity of signal transduction components downstream of the receptor for either the β-adrenergic or 5-HT₁₅ receptors.

The Hippocampus and HPA Axis Feedback

Finally, the results from this dissertation work provide some insight into how the hippocampus processes its feedback signal to the HPA axis. MR activation is important for maintaining the cell’s firing pattern, action potential duration, and sensitivity to 5-HT in subfield CA3 pyramidal cells. Since MR activation modulates pyramidal cell characteristics as well as the 5-HT₁₅ receptor-mediated response, the
activation of MR influences any input signals arriving at subfield CA3 cells.

In contrast, chronic activation of MR and GR together, had no selective effects on cell membrane electrophysiological characteristics but did decrease the inhibitory response of CA3 pyramidal cells to 5-HT. Therefore, the feedback signal from the hippocampus during chronic MR and GR activation may depend on the attenuation of 5-HT mediated responses in the hippocampus.

This dissertation work has contributed new and important information towards elucidating the mechanism by which corticosteroids modulate neural signal processing in the hippocampus. It is now clear that corticosteroids have different effects on cell membrane characteristics and 5-HT$_{1A}$ receptor-mediated responses in subfields CA1 and CA3. Furthermore the selective actions of corticosteroids in the hippocampus may have important ramifications on the development of the HPA axis feedback signal and hippocampus related behaviors.
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October 8, 1996

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Dayanithi G, Antoni FA (1989) Rapid as well as delayed inhibitory effects of glucocorticoid hormones on pituitary adrenocorticotropic hormone release are mediated by type II glucocorticoid receptors and require newly synthesized messenger ribonucleic acid as well as protein. Endocrinology 125: 308-313.


Mendelson SD, McEwen BS (1992) Quantitative autoradiographic analyses of the time course and reversibility of corticosterone-induced decreases in binding at 5-HT$_{1A}$ receptors in rat forebrain. Neuroendocrinology 56:881-888.


Miles R, Wong RK (1986) Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. J Physiol (Lond) 373:397-418.


Okuhara DY, Beck SG (1994) 5-HT\textsubscript{1A} receptor linked to inward-rectifying potassium current in hippocampal CA3 pyramidal cells. J Neurophysiol 71:2161-2167.

Okuhara DY, Beck SG, Muma NA (1996a) Corticosterone alters G protein \(\alpha\)-subunit levels in the rat hippocampus. Brain Res Accepted

Okuhara DY, Beck SG (1996b) Corticosteroids alter pyramidal cell electrophysiological characteristics in hippocampal subfield CA3. Submitted

Okuhara DY, Lee JM, Beck SG, Muma NA (1996c) Differential immunohistochemical labeling of \(G_\text{\alpha}\), \(G_{\text{i1}}\) and \(G_{\text{i2}}\), and \(G_\text{\alpha}\) \(\alpha\)-subunits in rat forebrain. Synapse 23:246-257.


Sanchez ER, Meshinchi S, Schlesinger MJ, Pratt WB (1987a) Demonstration that the 90-kilodalton heat shock protein is bound to the glucocorticoid receptor in its 9S nondeoxynucleic acid binding form. Mol Endocrinol 1:908-912.


Tejani-Butt SM, Labow DM (1994) Time course of the effects of adrenalectomy and corticosterone replacement on 5-HT_{1A} receptors and 5-HT uptake sites in the hippocampus and dorsal raphe nucleus of the rat brain: an autoradiographic analysis. Psychopharmacology (Berl) 113:481-486.


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The dissertation is, therefore, accepted in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

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