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The Neurochemical Effects of Several Carboxylated Tetrahydroisoquinolines

by

Jerome James Hannigan

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

June

1983

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ACKNOWLEDGEMENTS

I would like to acknowledge Dr. Michael Collins for his role in training me to be a research scientist. His supervision has been helpful in pursuing the answers to the questions addressed by this dissertation. I wish to thank Dr. Byron Anderson for encouraging me to pursue a career in Biochemistry. I have come to share his love for science. I could not have survived graduate school without the help and guidance of the faculty of the Department of Biochemistry. To my fellow students I owe a debt of gratitude. Graduate school has been a time of hard work and many frustrations and many joys. I wish to thank Cindy Weiner for her friendship and assistance during her time at Loyola. My fellow students, especially Bill Kennedy and Thomas Origitano have encouraged me and provided friendship which I treasure. Without their help none of this would have been possible. I wish to thank my mother for her love and for teaching me to work hard and to persevere.

I wish to thank my wife Lynn for her constant support and love throughout this time. I also wish to thank the members of the Department of Pediatrics at Loyola, especially Dr. Craig Anderson for the support that has been given to me during the preparation of this dissertation.

I also wish to thank Fred and Lorraine Meding and their sons Dave and Paul whose support has been there when I needed it, always. I deeply appreciate all that you have done for me.

VITA

Jerry Hannigan was born on April 14, 1951. He is a graduate of Transfiguration grade school in Wauconda, Il. and of Carmel High School for Boys in Mundelein, Il. In 1969, he enrolled at Loyola University of Chicago and received a B.S. degree in 1973. He worked as a research assistant at the Department of Biochemistry at Northwestern Medical School in Chicago in 1974. In 1975 he was enrolled in the Graduate School of Loyola University of Chicago in the Department of Biochemistry. He received a NIH Traineeship in Clinical Chemistry and worked under the direction of Dr. Michael Collins. Since May 1980 he has been working as a research assistant in the Department of Pediatrics at Loyola where a faculty position of Assistant Professor is awaiting Jerry after the fullfillment of the requirements for the degree of Doctor of Philosophy. On June 27,1981 Jerry wed Lynn Worley.

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LIST OF ABBREVIATIONS

ACH	acetaldehyde
alpha-M-dopa	alpha-methyl-dopa
AMMT	alpha-methyl-meta-tyrosine
1-carboxy-THP	1-carboxy-tetrahydropapaveroline
CNS	central nervous system
COMT	catechol-O-methyltransferase
1-CSAL	1-carboxy-salsolinol
3-CSAL	3-carboxy-salsolinol
DA	dopamine
DDC	dopa decarboxylase
DHIQ	dihydroisoquinoline
DNLCA	desoxynorlaudanosoline carboxylic acid
DOPAC	3,4-dihydroxyphenylacetic acid
DOPET	3,4-dihydroxyphenylethanol
5-HIAA	5-hydroxyindole acetic acid
5-HT	serotonin
HPLC	high performance liquid chromatography
HPLC/EC	high performance liquid chromatography with electrochemical detection
HVA	homovanillic acid
6-M-3-CSAL	6-methoxy-3-carboxy-salsolinol
7-M-3-CSAL	7-methoxy-3-carboxy-salsolinol
6-M-SAL	6-methoxy-salsolinol
7-M-SAL	7-methoxy-salsolinol
MAO	monoamine oxidase
NLCA	norlaudanosoline carboxylic acid
SAM	s-adenosylmethionine
THBC	tetrahydro-beta-carboline
THIQ	tetrahydroisoquinoline
THP	tetrahydropapaveroline

CHAPTER I

INTRODUCTION

The overall objective of this dissertation study was to ascertain the effects in rats of peripherally administered carboxylated 1,2,3,4-tetrahydroisoquinolines (THIQs) on the levels of biogenic amines in certain brain regions. Furthermore, the principal metabolic routes of specific carboxylated THIQs were elucidated, particularly whether decarboxylation was apparent. The central nervous system (CNS) uptake of the THIQs and their major metabolites were measured and related to changes in the levels of the bio- genic amines.

A. Background:

Condensation products derived from neurotransmitters and carbonyl compounds have been detected in the disease states of alcoholism (1,2), Parkinson's disease(3), and phenylketonuria(4). Since endogenous levels of these condensation products have been reported, a new emphasis has been placed upon the pharmacology, origin, and metabolism of these compounds.

The condensation products can be separated into two main groups: the THIQs and the tetrahydro-beta-carbolines (THBCs). The THIQs are condensation products of phenethylamines and carbonyl compounds. The THBCs are condensation products of indoleamines and carbonyl compounds. There are at least four basic sources of the carbonyl compounds which are available to condense with these neurotransmitters. The first source is acetaldehyde (AcH) which is derived from ethanol oxidation. A list of several AcH condensation products is found in Table 1. The second is formaldehyde which can come from methanol or from the tetrahydrofolate linked metabolism of one carbon fragments. Table 2 contains a list of these condensation products. The third source is pyruvic acid which is a normal product of glucose metabolism. The fourth source is an actual carbonyl metabolite of the neuroamine itself. Table 3 shows specific examples of this type of condensation product.

B. The presence of condensation products in mammals.

Acetaldehyde condensation products with dopamine (DA) and l-Dopa are presented in Table 1. The dopamine and AcH condensation product, salsolinol (SAL), has been found to be in rat brain(5), human cerebrospinal fluid(6), adrenals(7), and urine(8). Its mono-O-methylated metabolite 7-O-methyl-SAL (7-M-SAL) has been found in cerebrospinal

Table 1

ACETALDEHYDE CONDENSATION PRODUCTS

Parent Compound	Product
1-DOPA	1-Methyl-3-carboxy-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (3-CSAL)
Dopamine	1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (Salsolinol;Sal)
3-O-Methyl-Dopa	1-Methyl-3-carboxy-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (6M-3-CSAL)
4-O-Methyl-Dopa	1-Methyl-3-carboxy-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline (7M-3-CSAL)
Serotonin	1-Methyl-6-hydroxy-1,2,3,4-tetrahydro-beta-carboline (1-Methyl-6-hydroxy-THBC)
5-Methoxytryptamine	1-Methyl-6-methoxy-1,2,3,4-tetrahydro-beta-carboline (1-Methyl-6-Methoxy-THBC)

Table 2
FORMALDEHYDE CONDENSATION PRODUCTS

Parent Compound	Product
1-DOPA	3-Carboxy-norsalsolinol (3-Carboxy-6,7-dihydroxy-tetrahydroisoquinoline)
Dopamine	Norsalsolinol (6,7-dihydroxy-tetrahydroisoquinoline)
Serotonin	6-Hydroxy-tetrahydronorharmane
5-Methoxytryptamine	6-Methoxy-tetrahydronorharmane
Tetrahydropapaveroline	Tetrahydroprotoberberine (THB)

Table 3

NEUROAMINE DERIVED CONDENSATION PRODUCTS

Parent Amine	Carbonyl Compound	Product
Dopamine	3,4,-dihydroxyphenylacetaldehyde	Tetrahydropapaveroline (THP; norlaudanosoline)
Dopamine	3,4-dihydroxyphenylpyruvic acid	1-Carboxy-Tetrahydropapaveroline (1-Carboxy-THP)
Dopamine	Phenylacetaldehyde	3',4'-desoxynorlaudanosoline
Dopamine	Phenylpyruvic acid	3',4'-desoxynorlaudanosoline-1-carboxylic acid (DNLCA)

fluid (6). Large amounts of 7-M-SAL have been found in the urine of alcoholics undergoing detoxification(8).

Barker et al (9) have reported whole rat brain levels of 6,7-dihydroxy-THIQ, the DA and formaldehyde adduct. It is speculated that one carbon groups linked to tetrahydrofolate or S-adenosyl methionine are the source of the formaldehyde for this condensation product. The condensation product of DA and pyruvic acid is 1-carboxy-SAL (1-CSAL). The condensation product of l-Dopa and AcH is 3-carboxy-SAL (3-CSAL) which is a geometric isomer of 1-CSAL. The THIQs derived from formaldehyde, AcH, or pyruvic acid are known as "simple" THIQs.

"Complex" THIQs are derived from the condensation of catecholamines and catecholamine derived aldehydes. The product of the condensation of DA and 3,4-dihydroxyphenylacetaldehyde is tetrahydropapaveroline (THP), which is a probable intermediate in the synthesis of morphine by the poppy (10-14). The complex THIQ derived from DA and 3,4-dihydroxyphenylpyruvic acid is 1-carboxy-THP.

THBC formation following ethanol intake has been reported by several investigators. McIsaac (15) administered radiolabeled 5-methoxytryptamine and ethanol or AcH to rats which had been pretreated with iproniazid and disulfuram. He found the formation of urinary radiolabeled

6-methoxy-1-methyl-1,2,3,4-THBC in the rats. Dajani and Saheb (16) administered 5-hydroxytryptophan and radiolabeled ethanol to rats that were treated with inhibitors of monoamine oxidase (MAO) and aldehyde dehydrogenase inhibitors. Using thin-layer chromatography as a means of detection the researchers believe that they found 6-hydroxy-1-methyl-THBC in 24 hour urine samples from the treated rats. Dajani and Saheb failed to rule out the possibility that the spot identified was the formaldehyde adduct of serotonin, 6-hydroxy-THBC, which has been demonstrated to be a normal constituent of platelets. Rommelspacher et al. (17) have shown that 6-hydroxy-THBC, the derivative of serotonin and formaldehyde is present in rat brain and platelets and is excreted by human volunteers ingesting 5-hydroxytryptophan (5-HTP). The condensation product of tryptamine and formaldehyde, THBC, has been found in platelet rich human plasma (18). Bidder et al (19) found material in human platelets which was characterized as 1-methyl-beta-carboline (Harmane), which is the fully aromatised beta-carboline. 3-carboxy-harman has been found in the urine of cows which were fed silage (20). Evidence has been accumulating showing that THBC is a normal constituent of both rat brain and adrenals. The 6-methoxy congener of THBC is readily quantifiable in rat adrenals (21).

In vitro THBC formation is much slower than 6,7-dihydroxy-THIQ in the uncatalyzed process. However, the ratio of endogenous THBC to the 6,7-dihydroxy-THIQ in rat brain is orders of magnitude greater. This fact has lead Barker et al (22) to speculate that the formation of formaldehyde derived THBCs in brain is an enzyme related or catalyzed process. The pyruvic acid derived beta-carbolines have not previously been described in the literature, but have been detected in a spinal fluid sample taken at death from a patient suffering from Leigh's disease (23) and in the spinal fluid of alcoholic monkeys (24).

C. Overview of neuroamine synthesis and metabolism.

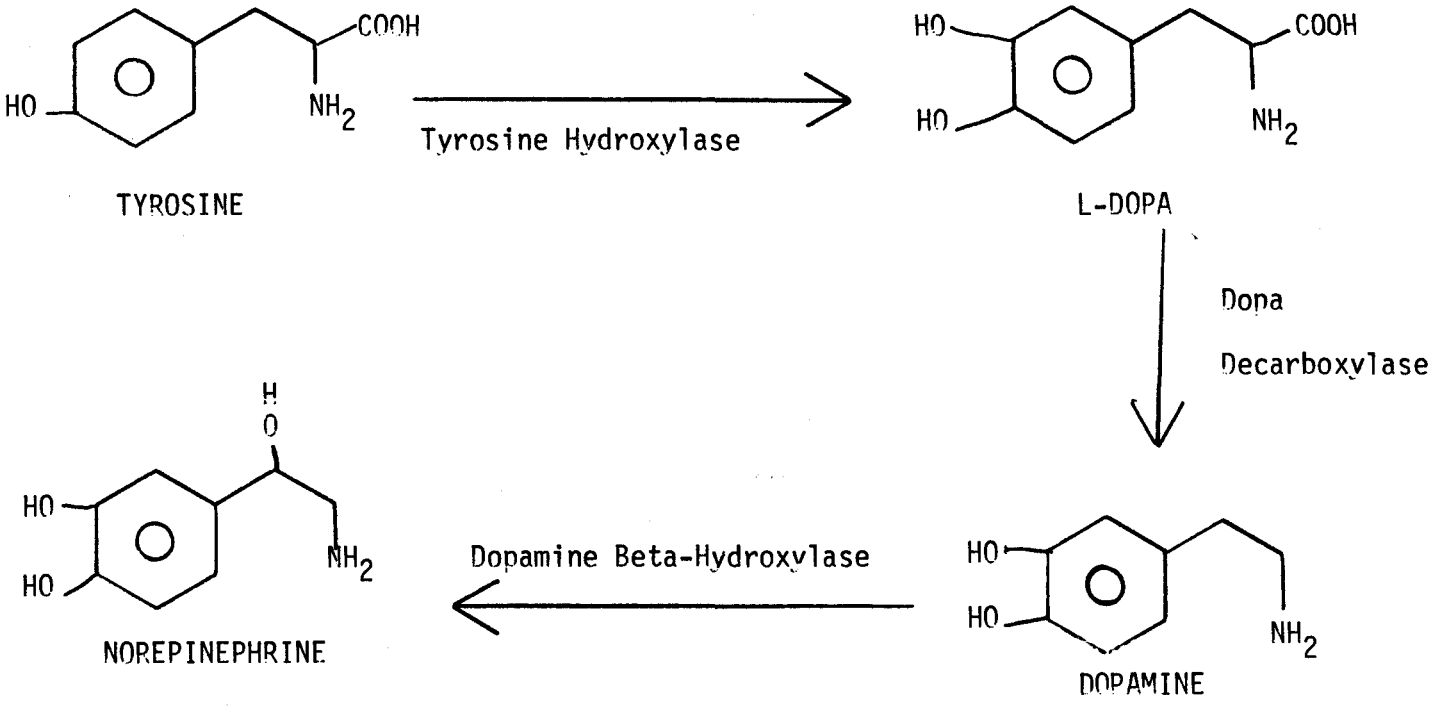
The condensation products introduced thus far are derived from the neurotransmitters DA, norepinephrine (NE), and serotonin (5-HT). An overview of the substrates and enzymes involved in the syntheses and metabolism of these neurotransmitters is necessary for an understanding of the neuropharmacology and metabolism of these condensation products.

Tyrosine is the essential dietary amino acid necessary for the synthesis of the catecholamines DA and NE. Tyrosine is hydroxylated by the enzyme tyrosine hydroxylase yielding the amino acid L-Dopa. L-Dopa is subsequently decarboxylated

by Dopa decarboxylase (DDC) which is also known as Aromatic Amino Acid Decarboxylase. The product of this reaction is the neurotransmitter DA. For dopaminergic neurons, this is the end point of the synthesis scheme. In noradrenergic neurons DA is hydroxylated by the enzyme dopamine-beta-hydroxylase to yield NE (Figure 1).

The metabolism and clearance of DA is accomplished via the processes of oxidation, O-methylation, and/or conjugation. Dopamine is oxidized by the enzyme monoamine oxidase (MAO) to 3,4-dihydroxyphenylacetaldehyde (dopaldehyde). The oxidation of a biogenic amine to an acid is a two step process with the enzyme monoamine oxidase (MAO) taking care of the first step. There are two known isozymes of MAO: MAO A and MAO B. The preferred substrates for MAO A are 5-HT and NE. Phenethylamine and benzylamine are the preferred substrates for MAO B. Tryptamine, tyramine, and dopamine are metabolized by both forms of the enzyme. Based upon the redox-status of the cells and the relative affinities of the substrate for the two subsequent enzymes, dopaldehyde is either further oxidized to 3,4-dihydroxyphenylacetic acid (DOPAC) by the enzyme aldehyde dehydrogenase or is reduced by the enzyme aldehyde reductase to form 3,4-dihydroxyphenethanol (DOPET). Typically though, DOPAC is the major product of DA oxidation. The catechol moiety of

Figure 1: Catecholamine Synthesis



TYROSINE

L-DOPA

NOREPINEPHRINE

DOPAMINE

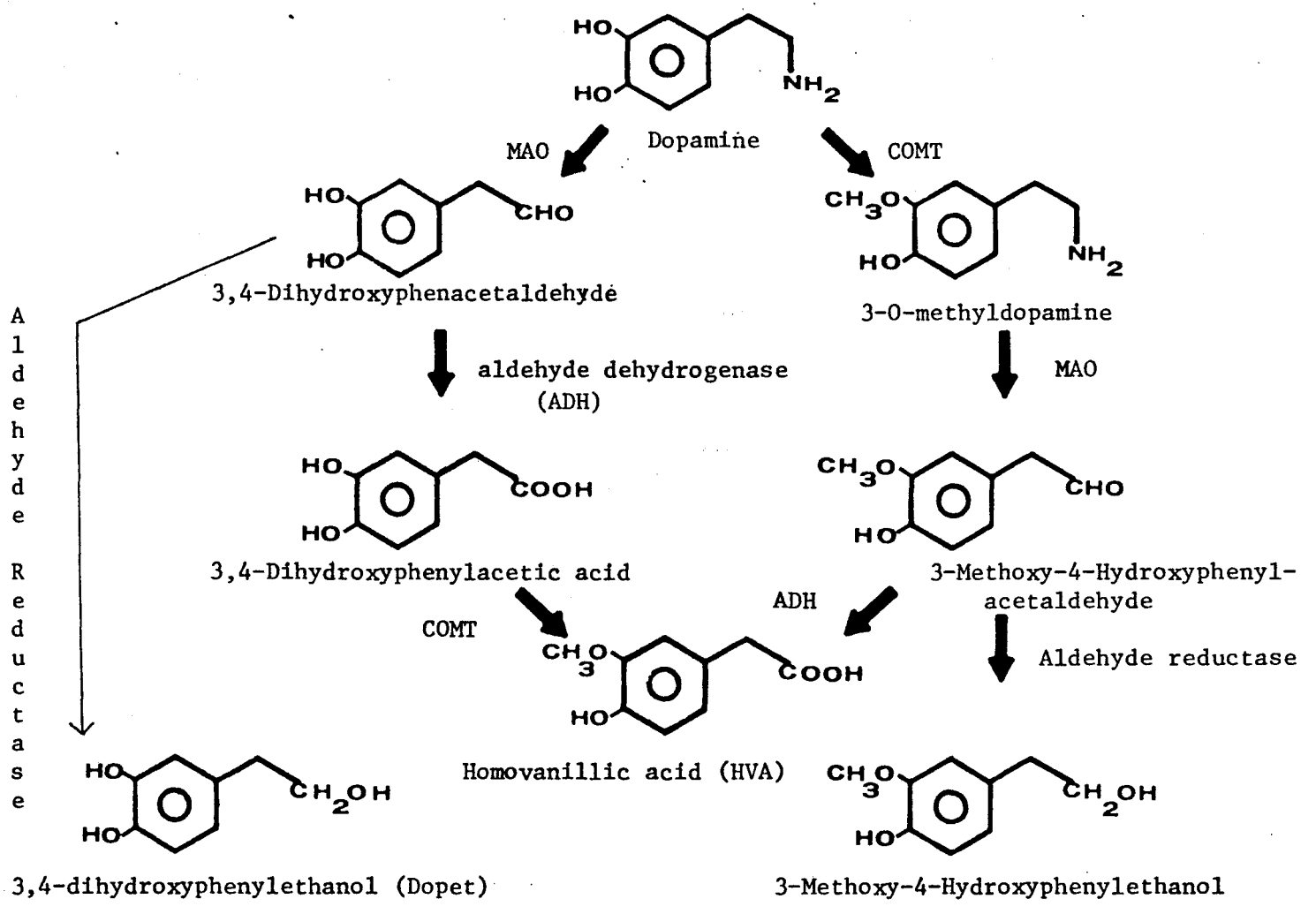
CAs and DOPAC can be O-methylated by the enzyme catechol-O-methyltransferase (COMT) (Figure 2).

1. O-methylation of the catecholamines.

Enzymatic mono-O-methylation of the CAs DOPAC or DOPA is performed by the enzyme catechol-O-methyl-transferase (COMT) (25). S-Adenosylmethionine (SAM) is the methyl donor. Magnesium is required for the reaction. Predominantly, the hydroxyl group meta- to the alkyl- side chain of the substrate is O-methylated.

O-Methylation of DOPAC is stereospecific at the 3-position of the ring and yields 3-Methoxy-4-hydroxyphenylacetic acid (Homovanillic Acid; HVA). O-methylation of DA is also stereoselective at the 3-position of the ring and produces 3-Methoxy-4-hydroxyphenethylamine (3-M-DA). This O-methylated product can be oxidized by MAO and this product, 3-Methoxy-4-hydroxy-phenyl acetaldehyde is a substrate for both aldehyde dehydrogenase and aldehyde reductase. Oxidation by aldehyde oxidase predominates yielding HVA. In the corpus striatum of the adult rat the nanomole/gram quantities of DA, DOPAC, and HVA are 56.9, 11.6, and 3.8 respectively.

Figure 2: Dopamine Metabolism


Dopamine
MAO
COMT
3,4-Dihydroxyphenacetaldehyde
3-O-methyldopamine
aldehyde dehydrogenase (ADH)
MAO
3,4-Dihydroxyphenylacetic acid
3-Methoxy-4-Hydroxyphenyl-acetaldehyde
COMT
ADH
Aldehyde reductase
Homovanillic acid (HVA)
Aldehyde Reductase
3,4-dihydroxyphenylethanol (Dopet)
3-Methoxy-4-Hydroxyphenylethanol

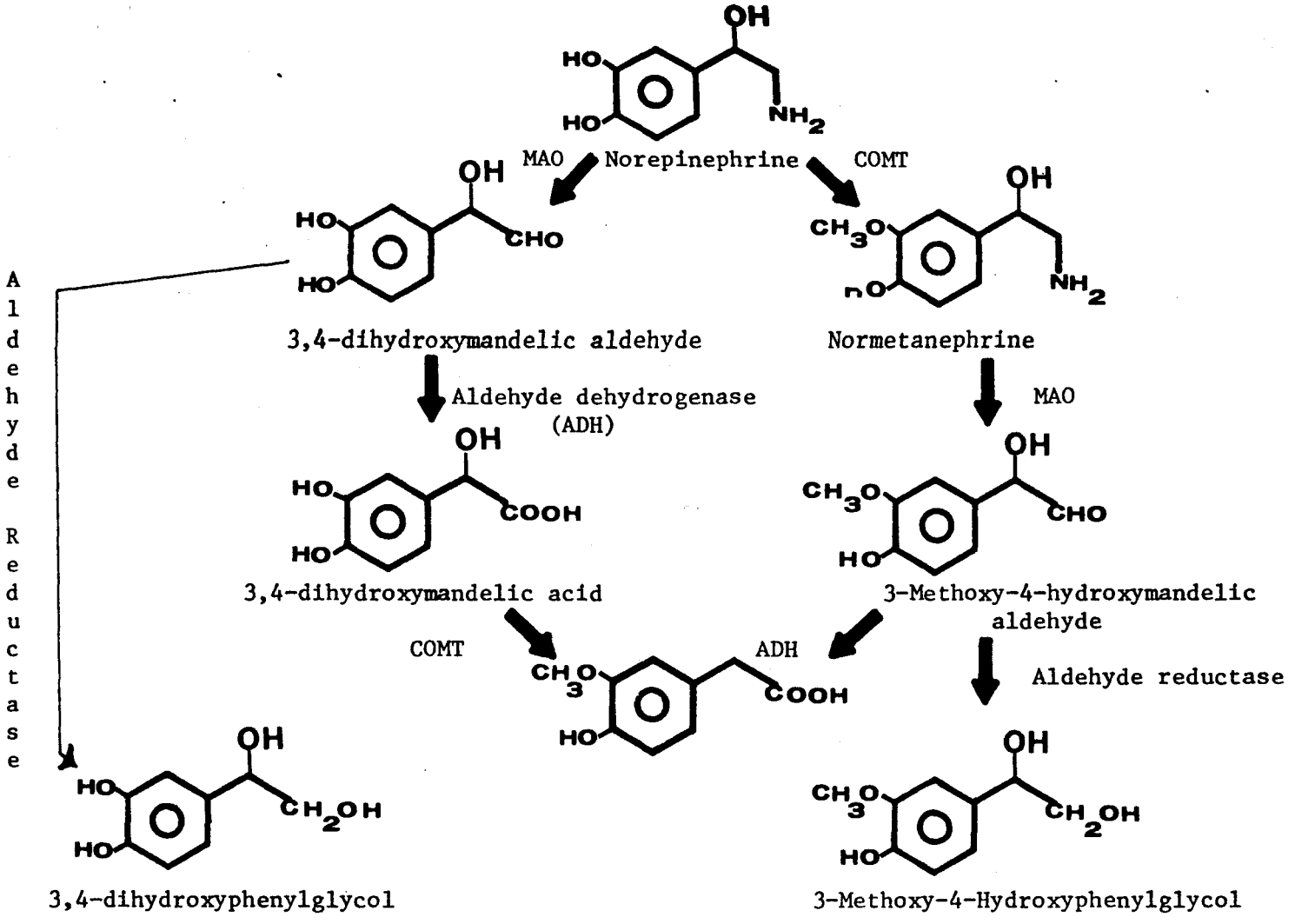
2. Norepinephrine synthesis and metabolism.

As seen earlier, the synthesis of NE requires all of the enzymes necessary for DA synthesis. Similiarly, the metabolism of NE shares many of the enzymes which oxidize and O-methylate DA. Oxidation of NE by MAO yields 3,4-dihydroxymandelic aldehyde. Reduction of 3,4-dihydroxymandelic aldehyde by aldehyde reductase produces 3,4-dihydroxyphenylglycol; whereas oxidation by aldehyde oxidase produces 3,4-dihydroxymandelic acid which in turn can be O-methylated by COMT giving 3-methoxy-4-hydroxy-mandelic acid. Direct O-methylation of NE produces normetanephrine which is a substrate for MAO. Oxidation of normetanephrine produces 3-methoxy-4-hydroxymandelic aldehyde which can be oxidized by aldehyde oxidase to give 3-methoxy-4-hydroxymandelic acid or it can be reduced by aldehyde reductase to 3-methoxy-4-hydroxyphenylglycol. These pathways of NE metabolism are summarized in Figure 3.

3. Serotonin synthesis and oxidation.

The synthesis of the neurotransmitter 5-HT begins with the amino acid tryptophan. Tryptophan is hydroxylated by the enzyme tryptophan hydroxylase giving 5-hydroxytryptophan (5-HTP). 5-HTP is decarboxylated to 5-HT by the enzyme

Figure 3: Norepinephrine Metabolism


Norepinephrine
MAO
COMT
3,4-dihydroxymandelic aldehyde
Normetanephrine
Aldehyde dehydrogenase (ADH)
MAO
3,4-dihydroxymandelic acid
3-Methoxy-4-hydroxymandelic aldehyde
COMT
ADH
Aldehyde reductase
Aldehyde Reductase
3,4-dihydroxyphenylglycol
3-Methoxy-4-Hydroxyphenylglycol

aromatic amino acid decarboxylase. 5-HT is oxidized by MAO yielding 5-hydroxyindole acetaldehyde which is further oxidized to 5-hydroxyindole acetic acid (5-HIAA). Reduction of 5-hydroxyindole acetaldehyde would yeild 5-Hydroxytryptophol. 5-HIAA is the predominant end product of 5-HT metabolism.(Figure 4).

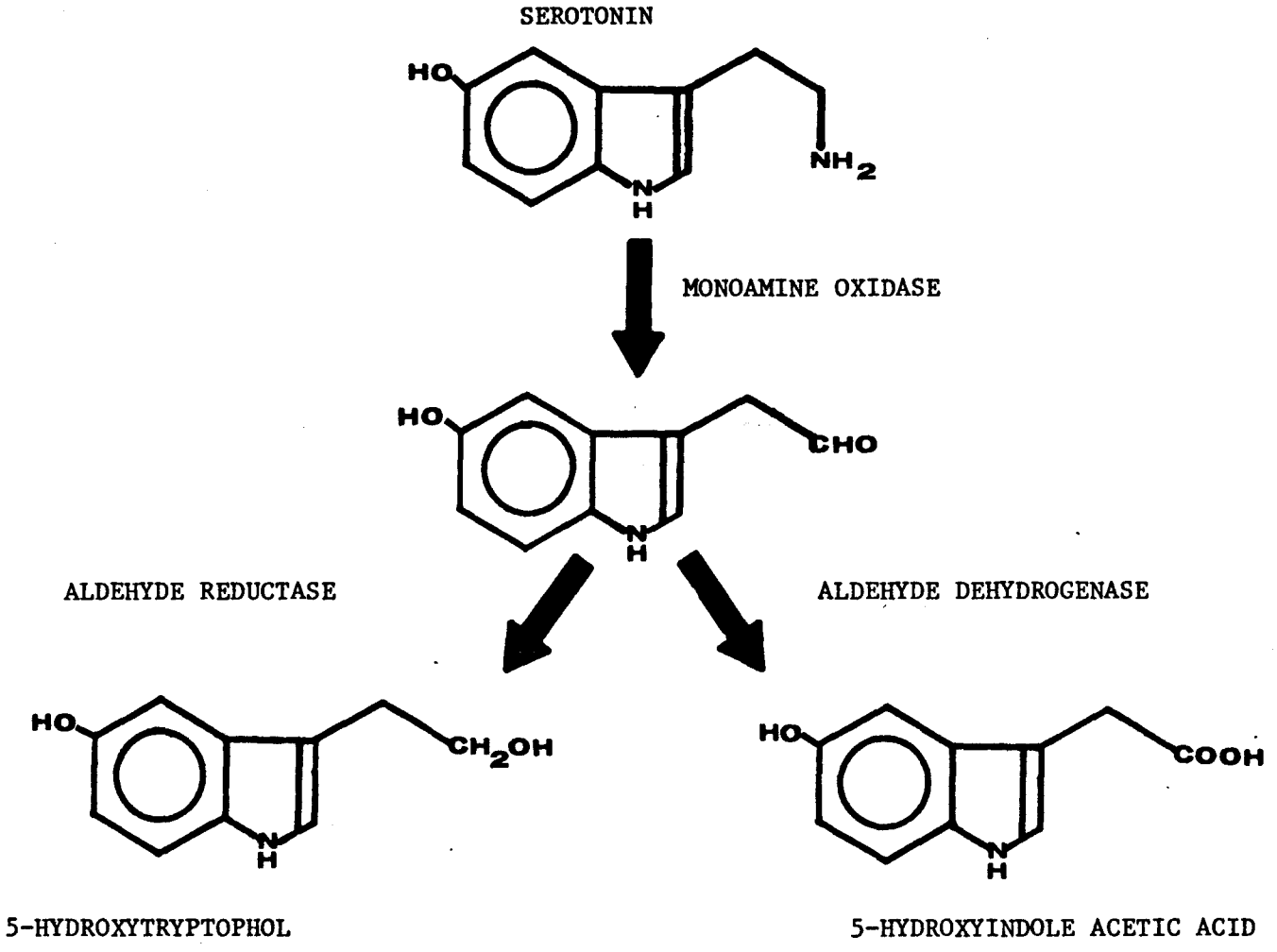
4. O-methylation of hydroxyindoles.

The hydroxyindoleamine 5-HT can be O-methylated by the magnesium dependent enzyme indole-O-methyl-transferase. SAM is the methyl donor. Most of the brain indole-O-methyl-transferase is located in the pineal gland.

D. Review of the relevant literature.

The condensation products mentioned are neuroamine derived. Both THIQs and THBCs are taken up into synaptosomes and stored and thus can be looked upon as "false neurotransmitters". Cohen has studied the <u>in vivo</u> uptake and release of THIQs by adrenergic nerves (26). For this reason investigators have researched the interactions of these condensation products with the enzymes of synthesis and metabolism of the parent neuroamines.

Figure 4: Serotonin Metabolism


SEROTONIN
HO
NH2
N
H
MONOAMINE OXIDASE
HO
CHO
N
H
ALDEHYDE REDUCTASE
ALDEHYDE DEHYDROGENASE
HO
CH2OH
N
H
HO
COOH
N
H
5-HYDROXYTRYPTOPHOL
5-HYDROXYINDOLE ACETIC ACID

Coscia's research group has examine in detail the biochemical effects of two condensation products: desoxynorlaudanosolinecarboxylic acid (DNLCA-the condensation product of DA and phenylpyruvic acid) and norlaudanosolinecarboxylic acid (NLCA-the condensation product of DA and 3,4-dihydroxyphenylpyruvic acid). DNLCA was found to be a potent inhibitor of catecholamine metabolism in adrenal medulla explants whereas NLCA was not (27). They have found DNLCA to be a good inhibitor of tyrosine hydroxylase (28) and phenylethanolamine N-methyltransferase (29) whereas NLCA is not. Collins and Weiner (30) have shown that various catecholic THIQs are potent in vivo inhibitors of tyrosine hydroxylase. 3-CSAL was found to be as potent as NE at feed-back inhibiting the activity of tyrosine hydroxylase (the rate limiting enzyme of dopamine synthesis). Neither SAL nor 3-CSAL have any effect in vitro upon liver dopa decarboxylase activity (DDC) (30). Catecholic THIQs are good substrates for COMT and therefore are competitive inhibitors of this enzyme (31). THP can be biotransformed by the inclusion of a methyl-bridge to form tetrahydroprotoberberines by rat liver enzymes. The tetrahydroprotoberberines are also substrates for COMT (32). THIQs are in vitro and in vivo inhibitors of MAO (31,33,34). The norlaudanosoline carboxylic acids (condensation products of DA and phe-

nylpyruvic acid or phenylacetaldehyde) are known inhibitors of adrenal dopamine-beta-hydroxylase(35).

3-CSAL possesses some interesting pharmacological properties. Ethanol narcosis has been shown to be potentiated by the DA and L-dopa derived THIQ 3-CSAL (36). 3-CSAL has been shown to produce ethanol discrimination in rats (37) and has been shown to be as potent as Demerol in producing analgesia in the rat (38).

SAL and 7-M-SAL, as mentioned previously, have been detected in mammals. The exact source of this brain SAL is not known. A dietary source is not favored because i.p. SAL (20 mg/kg i.p. in rats) does not penetrate the blood brain barrier (39).

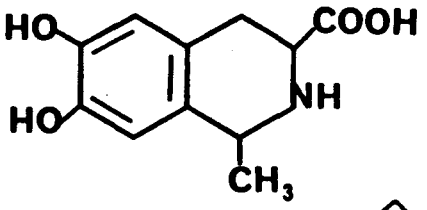
The possibility of 3-CSAL being taken up into the central nervous system and subsequently decarboxylated to SAL is one of the questions addressed in this dissertation. Carboxylated THIQs have been shown to enter the brain from the periphery (4) and it is speculated that the uptake is via the neutral amino acid transport system.

Decarboxylation of 3-CSAL could occur via either an oxidative or non-oxidative pathway (See Figure 5). Oxidative decarboxylation of 3-CSAL would yield 1-methyl-6,7-dihydroxy-dihydroisoquinoline (1-methyl-6,7-dihydroxy-DHIQ). Non-oxidative decarboxylation of 3-CSAL would yield SAL.

Figure 5: Oxidative versus Non-Oxidative Decarboxylation of 3-CSAL.

OXIDATIVE VERSUS NON-OXIDATIVE

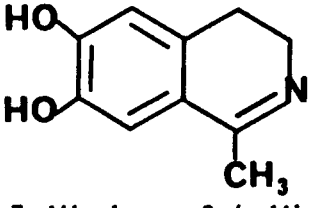
DECARBOXYLATION OF 3-CSAL



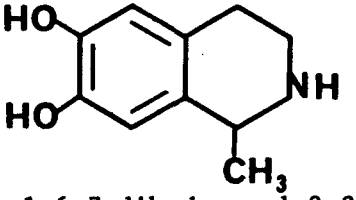
3-CSAL

OXIDATIVE

NON-OXIDATIVE



1-Methyl-6,7-dihydroxy-3,4-dihydroisoquinoline

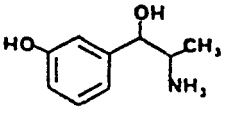


1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydro-isoquinoline (Sal)

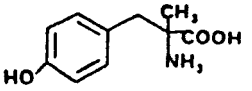
A number of amino acid analogues have been shown to be taken up by brain tissue and are non-oxidatively decarboxylated, yielding the corresponding neuroamine analogues (See Figure 6). For example, the L-Dopa analogue, alpha-methyldopa (alpha-M-dopa) has been found in brain tissue when given peripherally. Acute administration of alpha-M-dopa lead to a brain accumulation of alpha-methyldopamine and alpha-methylnorepinephrine (40,41,42). Brunner et al (43) gave alpha-M-dopa chronically to rats. Again an accumulation of alpha-methyldopamine and alpha-methylnorepinephrine was seen. Alpha-methyl-meta-tyrosine (AMMT) given peripherally at a dose of 100 mg/kg intra-peritoneally (i.p.) is taken up into the brain where it is decarboxylated and beta-hydroxylated to form metaraminol. Maximal striatal brain levels of AMMT were seen at 1 hour after the dose was given (43).

In contrast to the above examples, alpha-methyl-para-tyrosine (AMPT) is not decarboxylated <u>in vivo</u>. Following the administration of 80 mg/kg of AMPT to guinea pigs only AMPT was found in the heart and brain tissues of these animals. The tissues were analyzed for alpha-methyl-tyramine and alpha-methyl-norsynephrine content 1,2,4,6 and 8 hours after the AMPT dose was given. Of the decarboxylated products 0.2 to 0.3 ug/g could have been detected but were not.

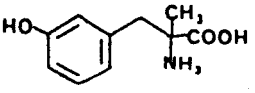
Figure 6: Examples of Amino Acid Analogues.



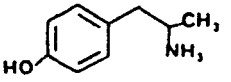
Metaraminol



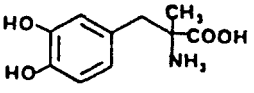
Alpha-methyl-para-tyrosine



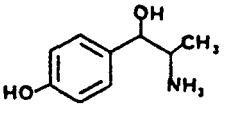
Alpha-methyl-meta-tyrosine



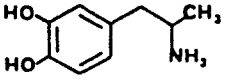
Alpha-methyl-tyramine



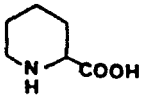
Alpha-methyl-Dopa



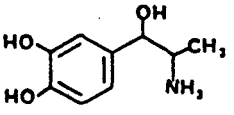
Alpha-methyl-Norsynephrine



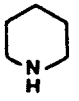
Alpha-methyl-dopamine



Pipecolic Acid



Alpha-methyl-Norepinephrine



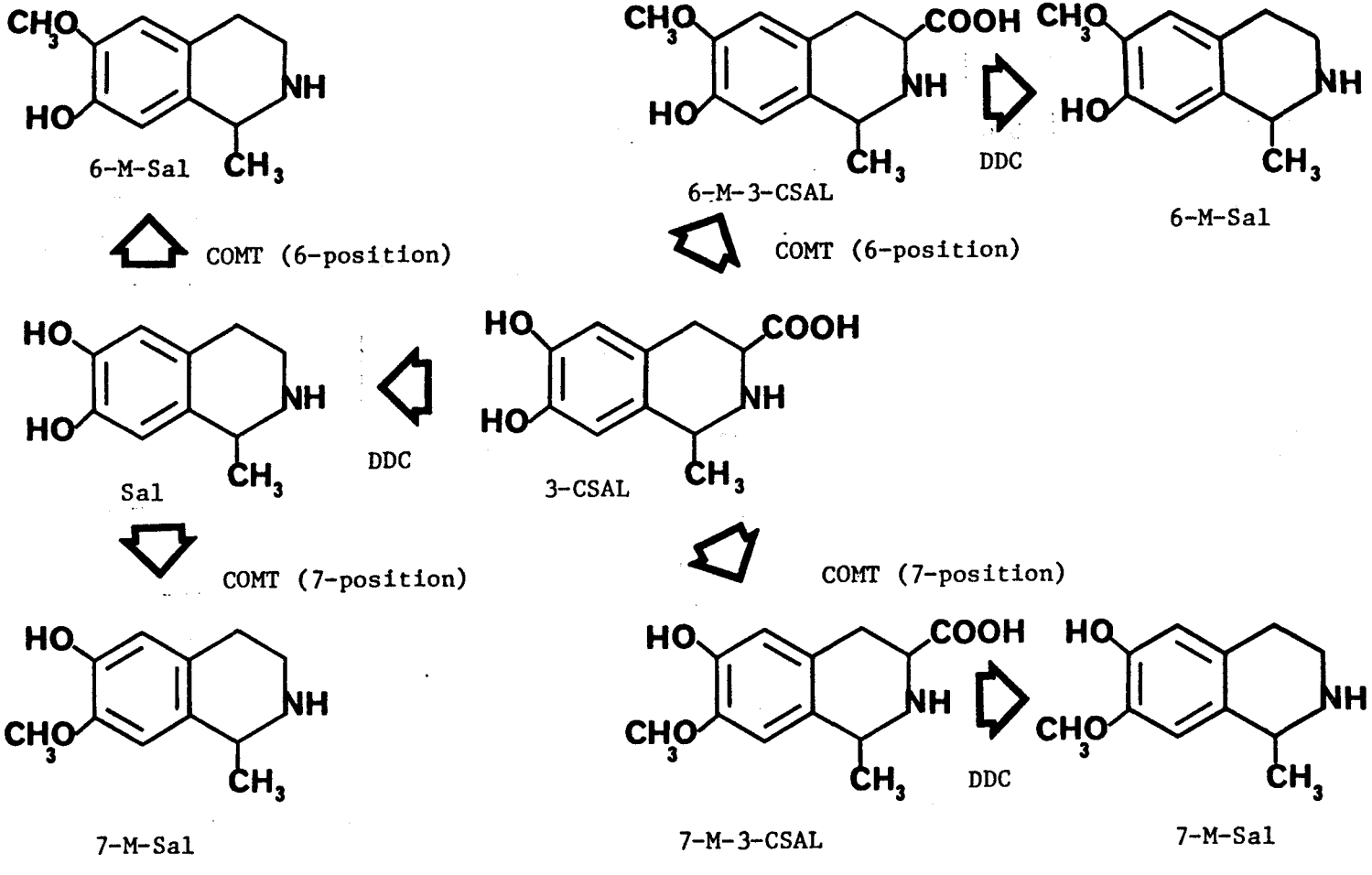
Piperidine

All of the above mentioned examples are compounds having primary amines. 3-CSAL is a cyclized secondary amine. Pipecolic acid, like 3-CSAL is an imino acid, i.e., it has a carboxylic acid moiety attached to a ring which contains a secondary amine. In mouse brain, pipecolic acid is decarboxylated to give piperidine (44,45). It is possible that carboxylated-THIQs might be substrates for this enzyme as well as for aromatic amino acid decarboxylase.

The possible metabolic routes of 3-CSAL by brain tissue are depicted in Figure 7. It is possible that 3-CSAL would be O-methylated at either the 6- or 7- position prior to decarboxylation. 3-CSAL itself was shown to be a poor inhibitor of DDC and therefore a poor substrate (30). However 6-M-3-CSAL and 7-M-3-CSAL might well be suitable substrates for DDC producing 6-M-SAL and 7-M-SAL respectively.

Figure 7: Some Possible Metabolic Routes for 3-CSAL

POSSIBLE METABOLIC PATHWAYS FOR 3-CSAL



In order to document the actual metabolism of 3-CSAL by rats in vivo it was necessary to synthesize de novo all the possible metabolites of 3-CSAL seen in Figure 7. Secondly, the weak cation-exchange resin Bio-Rex 70 (46,47) was utilized to isolate 3-CSAL and its possible metabolites as well as the neurotransmitters and their metabolites from tissue samples. Although the strong cation-exchange resin Dowex 50, X-4 has been used by a number of researchers to isolate the neurotransmitters and their metabolites (48, 49,50,51,52,53,54), typically these eluents were directly used in a fluorometric assay. Since the compounds bind tightly to Dowex (especially the THIQs), either large volumes of acid or high molar concentrations of the acids were necessary for elution.

For gas chromatography and HPLC, it is necessary to concentrate the samples. Upon lyophilization of these eluents, substantial oxidation of the samples is seen. Therefore Bio-Rex 70 is the preferred preparative column since small volumes of dilute acid is sufficient for isolation of the amine fraction with good recovery upon lyophilization.

Appropriate methods of liquid chromatography with electrochemical detection were also developed to provide resolution and quantitation of all of the compounds of

interest within a reasonable time-frame. Chromatographic methods in the literature describe separation of the catecholamines by HPLC with cation-exchange (55,56,57,58) or HPLC by reverse-phase chromatography with ion-pairing (59,60,61,62,63,64). These methods were not utilized for the measurement of the biogenic amines and their respective acid metabolites in the tissues samples for this dissertation for two reasons: 1) NE and 3-CSAL consistently co-chromatographed using the buffers described in the literature and 2) 5-HT's retention time was so great as to severely decrease both peak height and sample throughput.

E. Tissue samples analyzed and experimental strategy.

Three brain regions were chosen to be analyzed for the metabolism of and effects of 3-CSAL: the hypothalamus which is rich in NE, 5-HT, and contains DA innervation; the corpus striatum which is nearly devoid of NE terminals but has the highest DA content of any brain region and has modest 5-HT innervation from the raphe nucleus; and the hippocampus which has moderate levels of NE and 5-HT but practically no DA terminals (65,66).

The levels of the THIQs in these individual tissues will be related to the type of innervation of the individual

tissues, and to the levels of the neurotransmitters NE, DA, 5-HT and their respective acid metabolites. If changes in neurotransmitter content were produced by 3-CSAL, they could be due to release of neurotransmitters from storage sites (displacement) or due to an inhibition of the respective neurotransmitter's synthesis or clearance. Experiments in which rats are pretreated with an inhibitor of dopa decarboxylase would reveal if changes in the neurotransmitter levels was due to increased synthesis of 5-HT, i.e. precursor amino acids would accumulate to a greater extent in the 3-CSAL treated animals versus the appropriate controls. Two well characterized inhibitors of DDC are R04-4602 which in low doses (50 mg/kg) only inhibits DDC peripherally (however 800 mg/kg provides total DDC inhibition) (67,68,69,70) and NSD-1015 which provides total (peripheral and central) inhibition of DDC when given in low doses (71,72). A carboxylated THIQ dose of 50 mg/kg for one hour prior to sacrifice was chosen based on the results reported by the investigators who gave rats i.p. doses of the amino acid analogues described earlier. Typically, maximal brain levels of the injected drug were seen at the one hour time point.

Although 3-CSAL is catecholamine derived and it is most likely that its neurochemical effects would be upon the

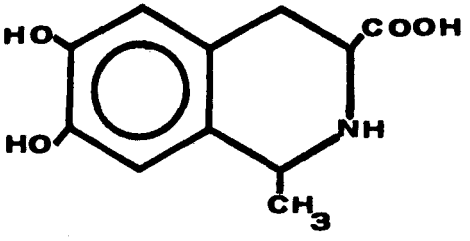
catecholamines, its effects upon 5-HT and 5-HIAA levels in the various tissues were determined, predicated upon the fact that AMMT, which is also catecholamine derived has been shown to lower brain 5-HT (73).

It was decided to compare aspects of the metabolism and neurochemical effects of 3-CSAL to two other carboxylated-THIQs, 1-CSAL and 1-carboxy-THP, on regional neurotransmitters (Figure 8). These THIQs are representative of two other alkaloid types, the first being the condensation product of DA and pyruvic acid. This compound could be formed in diseases in which pyruvic acid accumulates, such as subacute necrotizing encephalitis (Leigh's Disease)(74). The second is formed in the disease state of Phenylketonuria (PKU).

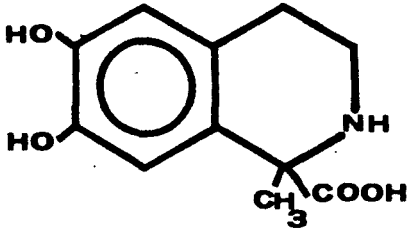
F. HPLC with electrochemical detection.

High performance liquid chromatography with electrochemical detection (HPLC/EC) is the preferred technique for measuring the compounds which have been discussed thus far. HPLC/EC was first described by Kissinger et al in 1973 (75). The electrochemical detector maintains a voltage applied across a 1 microliter volume flow cell through which

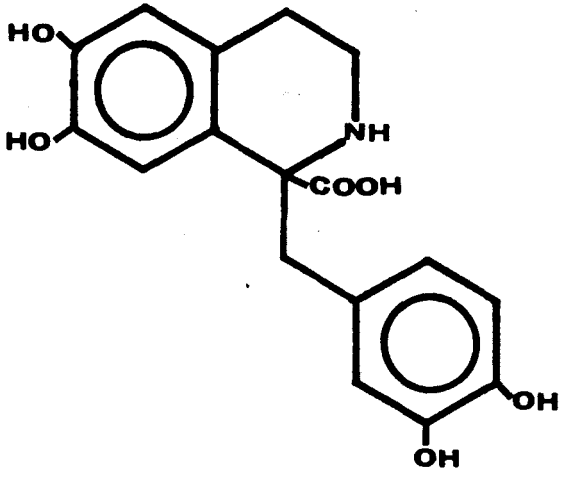
Figure 8: Carboxylated THIQs Studied.



3-CSAL



1-CSAL



1-COOH THP

the column eluant passes. For oxidative HPLC/EC, a positive voltage is applied across the flow cell and a standing electron current is maintained. This is known as the background current. When an oxidizable compound passes into the detector cell, the compound gains or absorbs electrons (oxidation), which results in a corresponding drop in the current reaching the cathode of the detector. A potentiometer measures the drop in the current across the cell and compares it to a reference voltage set up by a silver/silver chloride reference cell. The potentiometer then converts this drop in current to a voltage output which in turn drives the chart recorder or integrator.

The detector response for individual compounds relies heavily upon several parameters: the intrinsic ease of oxidation of the individual compounds; the voltage applied to the cell; the amount of time the compound spends in contact with the detector; and the ability of the eluting buffer to conduct current and transfer charge.

The catecholamines, 5-HT, plus their precursors and acid metabolites are among the compounds with the best response to electrochemical detection. Similiarly, the condensation products derived from these compounds are easily detected by HPLC/EC. The magnitude of the voltage applied across the detector cell influences the detector response.

For each compound there is a specific voltage which gives maximum detector response. The HPLC/EC is an inefficient detector using a flow rate of 1 ml/min, in that approximately only 10% of the sample is irreversibly oxidized and yet the signal to noise ratio for the detector is very large thereby permitting great sensitivity. Much of the lack of efficiency is due to the geometry of the detector (lack of a large surface area), but equally important is the amount of time that the compound is in the detector. If the oxidation is a slow process, then increased time spent in the detector would permit increased amount of sample oxidized and therefore detected. Also, of great consideration is the intrinsic electrical conductivity of the eluting buffer. Electrons must reach the compound in order to be captured. The citrate/phosphate buffers introduced by Kissinger are very electrochemically active in the sense that they conduct current without generating an appreciable background noise (intrinsic resistivity) at the voltages used for optimal measurement of the compounds of interest. It is interesting to note that a 0.1 M sodium phosphate monobasic buffer provides a good medium for electrochemical detection whereas a 0.1 M ammonium phosphate monobasic buffer, pH 5.0 yields poor sensitivity for the compounds of interest as the background noise by far exceeds the signal reaching the detector

when this buffer is used. Therefore it is critical that the buffer selected for any chromatography with electrochemical detection needs to both accomplish resolution of the compounds chromatographed as well as provide suitable conductivity for efficient electrochemical detection.

CHAPTER II

MATERIALS AND METHODS

A. Materials.

Dopamine HCl salt (Sigma)

Dopamine HBr salt (Aldrich)

Norepinephrine HCl salt (Sigma)

Epinephrine HCl salt (Sigma)

3,4-dihydroxybenzylamine HBr salt (Aldrich)

Normetanephrine HCl salt (Sigma)

Metanephrine HCl salt (Sigma)

Vanilmandelic Acid (Sigma)

Homovanillic Acid (Sigma)

3,4-Dihydroxyphenylacetic acid (DOPAC) (Sigma)

Serotonin Creatinine Sulfate Complex (5-HT) (Sigma)

5-Hydroxyindole acetic acid (5-HIAA) (Sigma)

3-O-methyl dopamine (Sigma)

Salsolinol HCl (de novo)

6-O-methyl-Salsolinol (de novo)

7-O-methyl-Salsolinol (de novo)

4-O-methyl-dopamine (Calbiochem)

3-CSAL (de novo)

L-Dopa (Sigma)

Acetaldehyde AR (Fischer)

4-O-methyl-Dopa (gift: John Daly, NIH)

3-O-methyl-Dopa (Sigma)

Pyruvic acid (Sigma)

Sodium phosphate monobasic monohydrate (Baker)

Sodium phosphate dibasic heptahydrate (Baker)

Sodium phosphate dibasic anhydrous (Baker)

EDTA (Fisher)

Ammonium phosphate monobasic (Baker)

Ammonium phosphate dibasic (Fisher)

1-Heptane sulfonic acid (Kodak)

Methanol (Kodak)

Ethyl acetate (Fisher)

Acetone (Baker)

Ammonium hydroxide concentrate (Scientific Products)

Hydrochloric acid AR (Scientific Products)

Perchloric acid, 60% reagent (Baker)

Nitric Acid concentrated (Scientific Products)

Acetic Acid, glacial (Baker)

n-Butanol (Baker)

B. Equipment

Aminco-Bowman Spectrophotofluorometer with
Ratio Photometer

Serval RC2 ultracentrifuge

FTS Flexi-Dry Lyophilizer

LC-2A Potentiometer

TL-5A Amperometric Detector

LC-19 Silver-Silver Chloride reference electrode

Bio Rad ODS-10 micron 250mm x 4mm analytical column

Model SR-204 Heath Single Pen Chart Recorder

TI-55 Calculator

Partisil-SCX 10 micron Strong Cation Exchange
250 x 4 mm analytical column

Bio-Rex 70 Weak Cation Exchange Resin

Isolab Minicolumns

Columns made from Pasteur pipets
plugged with glass wool

C. Methods

1. The Separation of the Neurotransmitter Metabolites Using Bio-Rex 70.

a. Properties of the Resin.

Bio-Rex 70 is a polyacrylic resin whose active moiety is a carboxylic acid functional group. The carboxylic acid functional group will form a salt linkage with primary amines only within a narrow pH range of 5.5-6.0. At this pH the acid moiety of the resin is unprotonated and therefore can form a salt linkage with a cationic species(76).

The weak cation-exchange characteristic of this resin permits the separation of three classes of functional groups of the molecules relative to the neurotransmitters: acid/ neutral metabolites; the precursor amino acids; and the amines. The acid metabolites of the neurotransmitters do not bind to the resin at all at pH 5.5 to 6.0, and they are removed in the void volume and water wash. There is a coulombic repulsion between the acid metabolites and the binding sites of the resin. The amphoteric molecules such as the amino acids bind to the resin, but only weakly. The amine moiety of the amino acid forms the salt linkage, but the presence of the acid moiety lessens the strength of that bond; they are eluted with 0.02 M sodium phosphate buffer, pH 6.5. The neuroamines themselves bind best to the resin (46) and they are eluted with acid.

b. Preparation of the Resin.

Bio-Rex 70, 200-400 mesh, sodium form was purchased from Bio-Rad Laboratories (Richmond, CA). Resin was added to a one-liter beaker, enough to occupy the 200 ml mark. The resin was then suspended in distilled water. The larger resin beads settled to the bottom of the beaker. The finer particles, still in suspension, were decanted off with the water. This process was repeated until the supernatant is clear, i.e. free of fine particles. The sizing of the resin ensures that the flow rates of the columns made from the resin will be more uniform and faster.

The resin was then stirred in 3 N ammonium hydroxide and allowed to settle. The resin was then washed with distilled water until the supernatant was neutral. Then the resin was stirred in 3 N hydrochloric acid (HCl). Again, it was washed with distilled water until the supernatant was neutral. Afterwards, 3 N sodium hydroxide was used to generate the sodium form of the resin. The resin was washed then with distilled water until the pH of the supernatant was less than 9.0.

Subsequently, the resin was washed with 0.1 M sodium phosphate buffer, pH 6.5, containing 0.1% EDTA. The washing was continued until the pH of the supernatant was 6.5. The pH of the supernatant was checked the next day to ensure

that it remained at 6.5. If it had increased, then the resin was washed with fresh buffer. This would be repeated daily until the pH of the supernatant remained at 6.5. This exhaustive washing gave better blanks for the fluorescent and chromatographic assays. Interferring substances, which otherwise would elute with the samples, were removed.

c. Column Preparation-The Fluorescent Studies.

Pasteur pipets were used to form mini-columns. Glass wool plugs were placed into the bottoms of the pipets and enough resin was added to form a 3 cm bed. Out of the population of pipet-columns thus formed, the ones with flow rates of plus or minus one standard deviation of the mean were chosen to be used in the experiments. Since the resin was stored in 0.1 M phosphate buffer, pH 6.5, the columns were rinsed with 25 ml distilled water prior to use.

d. Column Preparation-The HPLC Studies.

Isolab Quik-Sep Columns were used in some of the HPLC studies. They were filled with 500 ul of resin. The resin filled columns were rinsed with 25 ml distilled water prior to using.

2. Reagents.

Alkaline ascorbate: 100 mg L-ascorbic acid (Sigma) was dissolved in 1.0 ml of quartz-distilled water and 50 ml of 10 N sodium hydroxide was added.

Alkaline sulfite: 1.25 g sodium sulfate (Baker) was dissolved in 5 ml of quartz-distilled water, and 45 ml of 5 N sodium hydroxide was added.

Sodium periodate: 0.5% (w/v) sodium periodate (Baker) in quartz-distilled water.

Perchloric acid: 0.4 N (Baker)

Potassium carbonate:1 N (Baker)

Potassium ferricyanide: 0.25% (w/v) (Baker)

3. Buffers.

0.1 M phosphate, 0.1%EDTA, pH 6.5: 8.28 g sodium phosphate monobasic, monohydrate; 5.68 g Sodium phosphate dibasic, anhydrous; 10 ml 10% EDTA per liter of quartz distilled water.

0.02 M phosphate, 0.2% EDTA, pH 6.5: 1.66 g sodium phosphate monobasic, monohydrate, 1.14 g sodium phosphate dibasic, anhydrous, 20 ml 10% EDTA per liter quartz-distilled water.

0.5 M phosphate, pH 7.0 (Norepinephrine determination): 70.98 g sodium phosphate dibasic, anhydrous per liter of quartz-distilled water; 68.04 g potassium phosphate monobasic, anhydrous per liter of quartz-distilled water. 61.0 ml sodium phosphate solution was mixed with 39.0 ml of the potassium phosphate solution.

0.5 M phosphate, pH 7.0 (dopamine determination): A 1 M solution of potassium phosphate monobasic, anhydrous was adjusted to pH 7.0 with 1 N sodium hydroxide and diluted with quartz-distilled water to a concentration of 0.5 M.

Citrate 0.5 M, pH 4.0: A 1 M citric acid solution was adjusted to pH 4.0 with sodium hydroxide and diluted with quartz-distilled water to a concentration of 0.5 M.

4. Tissue Sample Preparation.

Animals were sacrificed by a blow on the head and decapitation. Brain regions were dissected out as per Iverson and Glowinski (77,78), immediately put into weigh boats and placed upon blocks of dry-ice. Brain region masses were determined using a 5-point Mettler Balance.

5. Extraction of the Biogenic Amines, THIQs and Their Metabolites from Tissues.

The samples were extracted either with perchloric acid or with ice-cold ethanol.

Acid extraction: The samples were placed into disposable test tubes (16 x 100 mm; SP-T1285-6) which contained 500 ul ice-cold 0.4 N perchloric acid (0.1% EDTA). Each sample was homogenized at maximal speed with a Tissue-Tek Homogenizer for 30 seconds. The homogenate was transferred to a Serval centrifuge tube. The homogenizer probe and dispo test tube were rinsed three times with the perchloric acid solution, and the rinses were added to the Serval centrifuge tube. The sample tubes were kept on ice throughout the procedure. The tubes were then transferred to the Serval RC-2 refrigerated centrifuge and spun for 15 minutes at 18,000 rpm (4 C). The supernatant was pipetted into 20 ml capacity scintillation vials. One of the control samples was split. To one half, 100 ng of each of the compounds of interest was added. To the other half, distilled water was added. This constituted a control for recovery after homogenization.

The pH of the perchloric acid supernatants were adjusted to 5.5-6.0. Initially, this was done coarsely to a pH of approximately 4.0, using 2.0 N potassium hydroxide and then carefully to pH 5.5-6.0 with 0.2 N potassium hydroxide.

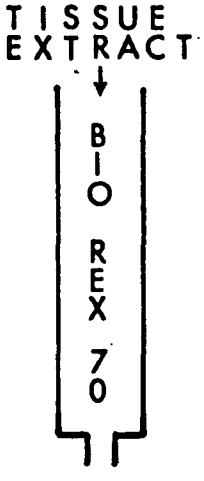
Since this was largely an ionic equilibrium, care had to be taken not to expose the sample to harsh alkaline conditions which could oxidize the compounds investigated. Perchlorate ions in excess of those necessary to precipitate the proteins were removed from solution by the potassium ions. The two species readily formed the sparingly soluble potassium perchlorate salt. It is very important to remove as much of the potassium perchlorate from solution as possibe. At 0 degrees centigrade the solubility of potasium perchlorate is 0.75 g/ 100 ml; whereas at 25 degrees centigrade 1.6 g of the salt can be dissolved in 100 ml. Failure to remove the potasium perchlorate in solution at 25 degrees or at 4 degrees will result in displacement of members of the amino acid fraction into the void volume. Precipitation of this salt was enhanced by placing the scintillation vials of pH adjusted solutions on a block of dry-ice. As soon as the solutions froze completely, they were removed from the dry-ice and allowed to thaw. The clear solution was pipetted away from the precipitate and applied to the pasteur-pipette or Isolab columns.

The effluent, plus a 1.0 ml distilled water wash, was collected and labelled the primary eluent. This fraction was frozen and lyophylized prior to analysis. The precursor amino acids were eluted using 1.5 ml of 0.02 M phosphate

buffer followed with 1.0 ml distilled water. This fraction was labelled the amino acid fraction and frozen at -20 degrees centrigrade until the fraction was analyzed. The amines were eluted with 1.5 ml of 0.5 N acetic acid. This fraction was labelled the amines and frozen at -20 degrees centrigrade until analyzed (Figure 9). Column blanks were derived from a column which had no tissue applied to it, but was otherwise processed as described above. Thus three blanks were generated: a primary eluent fraction blank, an amino acid fraction blank, and an amine fraction blank.

Ethanol extraction: The tissue samples were homogenized in 75% ice-cold ethanol (5 ml/g tissue). After homogenization and centrifugation as described above, the supernatants were diluted 1:1 with distilled water. When separation of the component fractions was necessary, the diluted ethanol extract was applied to Bio-Rex 70 columns. When chromatography with resolution of all of the compounds of interest was possible, the diluted ethanol solution was first lyophilized and then reconstituted with 0.1 N HCl and directly injected into the high performance liquid chromatograph.

Figure 9: Bio-Rex 70 Preparative Column Chromatography



BIO REX 70
ELUTION PROFILE

1) PRIMARY ELUENT: CONTAINS DOPAC, HVA, AND 5-HIAA.
2) AMINO ACID FRACTION: CONTAINS DOPA, TRYPTOPHAN, 5-HTP, TYROSINE, AND THE 3-CSALs.
3) AMINE FRACTION: CONTAINS NE, DA, 5-HT. SAL AND O-M-SAL ELUTE HERE.

6. Fluorometric analyses.

The fluorometric analyses for the amines were adapted from those employed by Holman et al (47).

a. Serotonin.

A 500 ul aliquot of the amine fraction was placed into a disposable test tube. To this 150 ul of 12 N HCl was added. The mixture was vortexed and the fluorescence was then read at an activation wavelength of 278 nm and an emission wavelength of 545 nm. External standards, reagent blanks and column amine fraction blanks were run each time with the samples. Stock solutions were made up in 3 N HCL and serially diluted with 3 N HCl to form the standards and the standard curve.

b. Norepinephrine: formation of the trihydroxy-indole chromophore.

To 200 ul of the 0.5 N Acetic acid eluent, 200 ul of 0.5 M sodium phosphate buffer, pH 7.0, was added. The pH was adjusted to a pH of 6.5-7.0, with approximately 600 ul of 1 M potassium carbonate. An addition of 20 ul of a fresh solution of 0.25% potassium ferric cyanide was placed into the tube; one minute later 200 ul of freshly prepared alkaline ascorbate was added; at 15-30 second thereafter, 600 ul of distilled water was added. The samples had to be measured within 3 to 6 minutes after the initiation of oxidation. The

fluorescence was monitored at an excitation wavelength of 336 nm and an emission wavelength of 502 nm. A column amine fraction blank was run at the same time.

c. Dopamine assay: generation of the dihydroxyindole chromophore.

To 200 ul of the 0.5 N acetic acid amine fraction, 100 ul of pH 7.0 sodium phosphate buffer was added. The pH was adjusted to 6.5-7.0 with 1 N potassium carbonate. Oxidation was initiated with 100 ul of a 0.5% (w/v) solution of sodium periodate. One minute later, 300 ul of the alkaline sulfite solution was added. Both the periodate and sulfite solutions were made up immediately before use. A 100 ul volume of 0.5 M sodium phosphate buffer, pH 4.0 was added, followed by 170 ul of 3 N phosphoric acid. The fluorescence was measured within ten minutes at an excitation wavelength of 316 nm and an emission wavelength of 370 nm. Stock solutions and serial dilutions were made up with 0.5 N acetic acid. A column amine fraction blank was run at the same time.

d. 5-hydroxy-indole acetic acid.

The native fluorescence of 5-HIAA was measured in 3 N HCl. The lyophylized primary eluents were reconstituted with 500 ul of 3 N HCl. The excitation wavelength was 290 nm with an emission wavelength of 545 nm. Standard solutions were made of 5-HIAA at a concentration of 1 mg/ml in dis-

tilled water serially diluted with 3 N HCl. A column blank primary eluant fraction was run at the same time.

e. Tryptophan analysis.

The tryptophan assay was adapted from the Bloxam and Warren (79) modification of the Dewey and Denkla method (80) utilizing an excitation wavelength of 373 nm and an emission wavelength of 452 nm. The assay was linear in sensitivity from 8 ng-4 ug. Tryptophan was converted to the fluorescent species, norharman. Glass-stoppered test tubes, which were scrupulously cleaned weekly with concentrated nitric acid overnight, were used in the assay.

Two ml of 10% trichloroacetic acid (w/v) was placed into the glass-stoppered test tube. A 100 ul aliquot from the amino acid fraction was added and the tube was vortexed. Then 100 ul of 0.0061 M ferric chloride in 10% trichloracetic acid was added. The test tube was placed into rapidly boiling water for one hour. Turbidity developed in some of the test tubes. These samples scattered the light of the incident fluorescent beam. To overcome this, the samples were centrifuged in a clinical centrifuge at 2,000 rpm for five minutes. The clarified samples were then read in the spectrophotofluorometer. A column amino acid fraction blank was run at the same time. Neither 3-CSAL nor SAL interfered with any of the fluorescence analyses described.

7. HPLC chromatography.

Quantitation of the compounds of interest was accomplished by means of an electrochemical detector (Bioanalytical Systems) and was based on peak heights. Kissinger et al have recently published a comprehensive review of neurochemical applications of HPLC with electrochemical detection (81). For this dissertation, two types of HPLC columns were utilized in the quantitation of the compounds of interest: a reverse-phase column using an ion suppression buffer and a strong cation exchange column.

Reverse-phase /ion suppression chromatography operates on the basis of relative hydrophobicity of the compounds of interest in the mobile phase used. The ionic character of the biogenic amines and their acid metabolites can be expressed or suppressed depending upon the pH of the mobile phase. The ionic strength of the mobile phase is not the predominant factor in this system. The retention times for all of the compounds of interest from a 0.1 M Sodium Phosphate buffer (pH 5.0), with 1 mM EDTA are given in Table 4. The O-methyl-SALs were not resolved and had relatively long retention times in this system (22.8 min.). Furthermore, the acid metabolites eluted in the same vicinity.

Table 4

HPLC RETENTION TIMES OF COMPOUNDS USING

A BioSil ODS 10 Column and 0.1 M Sodium Phosphate

Monobasic Monohydrate, 1.0 mM EDTA, pH 5.0.

COMPOUND	RETENTION TIME (MIN)
Norepinephrine	1.9
DHBA	3.9
Dopamine	5.2
DOPAC	6.8
5-HT	13.6
5-HIAA	17.6
HVA	20.1
Sal	8.4
6-M-Sal	22.8
7-M-Sal	22.8
1-CSAL	4.0
6-M-1-CSAL	12.8
7-M-1-CSAL	16.2
3-CSAL	4.4
6-M-3-CSAL	14.4
7-M-3-CSAL	16.0

The use of a strong cation-exchange HPLC column (Partisil-SCX) provided not only resolution of the 6- and 7-M-SALs, but also isolated these compounds away from the acid metabolites which now elute in the solvent front in this system. The ionic strength and pH chosen to chromatograph these compounds provided optimal response and elution times while preserving resolution of these compounds.

Since chromatography of the compounds was done utilizing a variety of parameters, for all cases, representative chromatograms show the conditions utilized. It is possible that only trace amounts of decarboxylated products might have been present in the samples. Therefore, the limits of sensitivity were determined for SAL, 7-O-M-SAL, and 6-O-M-SAL. The detector signal at twice background was 0.2 nanoamps. A plot of nanoamps versus nanograms was done for each of the compounds. The nanogram value at 0.2 nanoamps represents the limit of sensitivity for each of these compounds. The limits of detection were 2.0 ng/ml for SAL, 3.56 ng/ml for 6-O-M -SAL, and 5.88 ng/ml 7-O-M-SAL by cation exchange HPLC with electrochemical detection. When reverse-phase HPLC with electrochemical detection was employed, the limits of detection were 1.02 ng/ml SAL, 1.82 ng/ml 6-O-M-SAL, and 3.0 ng/ml 7-O-M-SAL.

The student's t-test was used in all of the statistical analyses. A p value of < 0.05 was considered to be statistically significant.

CHAPTER III

EXPERIMENTAL SECTION

All of the subjects were male Sprague-Dawley rats purchased from King Animal Laboratories (Madison, Wisc.). All of the experiments were initiated at 3 pm Central Standard Time and the route of administration of the drug in all instances was intraperitoneal injection of the drug in a sterile saline vehicle.

A. Fluorescent Studies.

1. Acute 50 mg/kg 3-CSAL.

Sprague-Dawley rats (300-400g) received a 50 mg/kg dose of 3-CSAL in a sterile saline vehicle. Saline alone was given to the control group. One hour later the animals were sacrificed. The corpus striatum and the hypothalamus were taken from each animal. Dopamine and norepinephrine levels were measured by fluorescence after being isolated using Bio-Rex 70 columns.

2. Acute 50 mg/kg 3-CSAL in animals pretreated with a peripheral decarboxylase inhibitor, RO4-4602 (Benserazide).

Sprague-Dawley rats (300-400g) were pretreated with a peripheral dopa decarboxylase inhibitor, RO4-4602, at a dose of 50 mg/kg. One hour later a 50 mg/kg dose of 3-CSAL was given to the experimental group. The control group received saline alone. Corpus striatum and hypothalamic tissues were assayed. Dopamine and norepinephrine were isolated using Bio-Rex 70 and measured fluorescently.

3. Acute 50 mg/kg 3-CSAL in animals pretreated with a total decarboxylase inhibitor.

Male Sprague-Dawley rats (300-400g) were pretreated with NSD-1015 at a dose of 100 mg/kg which provided total dopa decarboxylase inhibition. The NSD-1015 was administered in a sterile saline vehicle to both the experimental and the control groups. One hour later, the experimental group received a 50 mg/kg dose of 3-CSAL dose in a sterile saline vehicle. The control group received the sterile saline alone. One hour subsequent to this, both groups of animals were sacrificed. Corpus striatum and hypothalamic tissues were evaluated. Dopamine and norepinephrine were measured by the fluorescent technique after being isolated using Bio-Rex 70.

4. Dose-response study with 3-CSAL.

Doses of 50,150, or 400 mg/kg were given to groups of male Sprague-Dawley rats (210-270g). Control animals received saline alone. One hour later the animals were sacrificed. Dopamine, norepinephrine, and serotonin levels were measured in corpus striatum and hypothalamic tissue. The neurotransmitters were isolated using Bio-Rex 70 and measured by fluorescence.

5. Chronic 3-CSAL (50 mg/kg i.p.).

A 50 mg/kg dose of 3-CSAL was given to male Sprague-Dawley rats (112-152g) every eight hours for a total of five days. At the same times, the control group received sterile saline. One hour after the last injection, the animals were sacrificed. Corpus striatum and hypothalamic tissue were assayed. Dopamine, norepinephrine, serotonin, tryptophan, and 5-hydroxyindole acetic acid were measured fluorometrically after being isolated using Bio-Rex 70.

B. Animal HPLC studies.

1. 100 MG/KG 3-CSAL for 30 Minutes.

Rats (295-340g; 90 days old) were given a 100 mg/kg dose of 3-CSAL. Two animals were sacrificed at 15 minutes after the dose was given, and four were sacrificed at the 30 minute time point. Blood, caudate, and hypothalamic tissues were taken for analysis. The samples were fractionated using Bio-Rex 70 , lyophilized and stored at -20 degrees centigrade until assayed by HPLC.

2. A Single Dose Time Study.

Male Sprague-Dawley rats (250-350g; 90 days old) were given 3-CSAL (50 mg/kg). The control group was injected with the sterile saline alone. At time intervals of one hour, three hours, eight hours and twenty-four hours rats were sacrificed. The tissues were ethanol extracted. Extracts were lyophilized, reconstituted in 0.01 N HCl, and directly injected into the high performance liquid chromatograph. The corpus striatum was analyzed for its content of 3-CSAL and its O-methylated metabolites. Dopamine, DOPAC, HVA, serotonin, and 5-HIAA were also measured in this tissue. THIQ content was also determined in blood, heart and liver.

3. The effect of 3-CSAL on 5-HT synthesis.

Male Sprague-Dawley rats (214-407g; 2-10 months) were pretreated with 800 mg/kg RO4-4602 for one hour. The control group was then given sterile saline and the experimental group received 50 mg/kg 3-CSAL. One hour later the animals were sacrificed. Striatal tissues were extracted with ice-cold ethanol and placed on Bio-Rex 70 columns. The precursor amino acid fraction was collected and analyzed for 5-hydroxytryptophan content.

4. The relationship of age of the rat to 3-CSAL's effect upon serotonin.

a. Old rats.

Male Sprague-Dawley rats (392-455g; 10-12 months) were given 3-CSAL (50 mg/kg). The control group received sterile saline alone. Eight hours later the animals were sacrificed. Hypothalamic and striatal tissues were dissected and ethanol extracted. Extracts were lypholized, reconstituted in 0.01 N HCl and directly injected into the high performance liquid chromatograph.

b. Young rats.

Young rats (94-105g; 35 days old) were given 3-CSAL (50 mg/kg i.p.). The control group received the sterile saline vehicle alone. Control and experimental animals were sacrificed at 4 and 8 hours after the injections. The striatum and hypothalamus were dissected, extracted with ice-cold ethanol, and placed upon Bio-Rex 70 to isolate the amine fraction. DA and 5-HT content were determined.

5. RO4-4602 and 3-CSAL.

The peripheral decarboxylase inhibitor, benserazide (RO4-4602; 50 mg/kg), was given to male Sprague-Dawley rats (196-205g; 50 days old) one hour before they were given a 50 mg/kg dose of 3-CSAL. The animals were sacrificed one hour after the 3-CSAL was given. Control rats received a 50 mg/kg dose of RO4-4602 and were sacrificed two hours later. Striatal, hypothalamic, and hippocampal samples were extracted

with 75% ethanol and analyzed by HPLC for dopamine, serotonin, DOPAC, HVA, and 5-HIAA.

6. NSD-1015 and 3-CSAL.

Male Sprague-Dawley rats (200-214g; 52 days old) were pretreated with NSD-1015 (100 mg/kg). One hour later, 3-CSAL (50 mg/kg) was given to the experimental group. The control animals received NSD-1015 only. One hour later, they received a saline injection. Two hours after the first injection, the rats were sacrificed. The corpus striatum, hypothalamus, and hippocampus tissues were extracted with 75% ethanol and analyzed for their dopamine, DOPAC, HVA, serotonin, and 5-HIAA content by direct injection onto the HPLC.

7. The effect of other carboxylated-THIQs.

Male Sprague-Dawley rats (140-165g; 42 days old) were divided into three groups. The first group received 1-carboxy-THP at a dose of 50 mg/kg. The second group received a 50 mg/kg dose of 1-CSAL. The third group received the saline vehicle alone. One hour later the animals were sacrificed. Corpus striatum, hypothalamus, and hippocampus tissues were analyzed. Dopamine, DOPAC, HVA, serotonin, and 5-HIAA were quantitated. Levels of 1-CSAL and 1-carboxy-THP were determined for the respective groups of rats.

CHAPTER IV

RESULTS

A. Levels of the THIQs in the tissues analyzed.

1. 3-CSAL levels-acute studies.

a. 100 MG/KG 3-CSAL for 30 minutes.

For rats given a 100 mg/kg i.p. dose of 3-CSAL and sacrificed at time points of 15 and 30 minutes, the blood levels of 3-CSAL and 6-O-M-3-CSAL, and 7-O-M-3-CSAL are given in Table 5. The 3-CSAL blood levels were significantly higher ($p<0.01$) at the 30 minute time point when compared to the 15 minute time point. The O-methylated 3-CSAL levels were not different between the groups at the time points sampled. The Bio-Rex 70 amine fraction from the blood samples were reconstituted with 250 ul of mobile phase and 100 ul were placed onto a cation-exchange column. No 6- or 7-O-M-SAL content was apparent from the chromatogram (Figure 10). Figure 11 shows the same blood sample spiked with 25 ng 7-M-SAL (60 ng/ml blood).

3-CSAL and O-methylated 3-CSALs were measured in the striatum. 3-CSAL alone was measured in the hypothalamus

Table 5

BLOOD LEVELS OF CARBOXYLATED THIQs AT TWO TIME POINTS FOLLOWING

100 MG/KG I.P. DOSE OF 3-CARBOXY-SAL (NG/ML ± S.E.M)

295-340 g rats (approximately 72 days old)

TIME	3-Carboxy-Sal	6-M-3-Carboxy-Sal	7-M-3-Carboxy-Sal	
15 MIN	553.0 ± 18.7	914.6 ± 532.7	887.3 ± 173.0	(n=2)
30 MIN	* 1,223.5 ± 836.0	808.9 ± 493.5	1,224.6 ± 610.6	(n=4)

p 0.01 compared to the 15 minute 3-CSAL level

Figure 10 : Representative Chromatogram
Blood Sample of an Animal Given 100 mg/kg 3-CSAL
for 30 minutes.

Sample: Bio-Rex 70 Amine Fraction; 100 ul of a 250 ul sample.

Column: Partisil-SCX (10 microns)

Buffer: 12.61 g Citric Acid, 3.45 g Ammonium Phosphate Monobasic, monohydrate and 88 ml 1 N Ammonium Hydroxide per liter distilled water.

Conditions: 1 ml per minute; 5 nA/V.

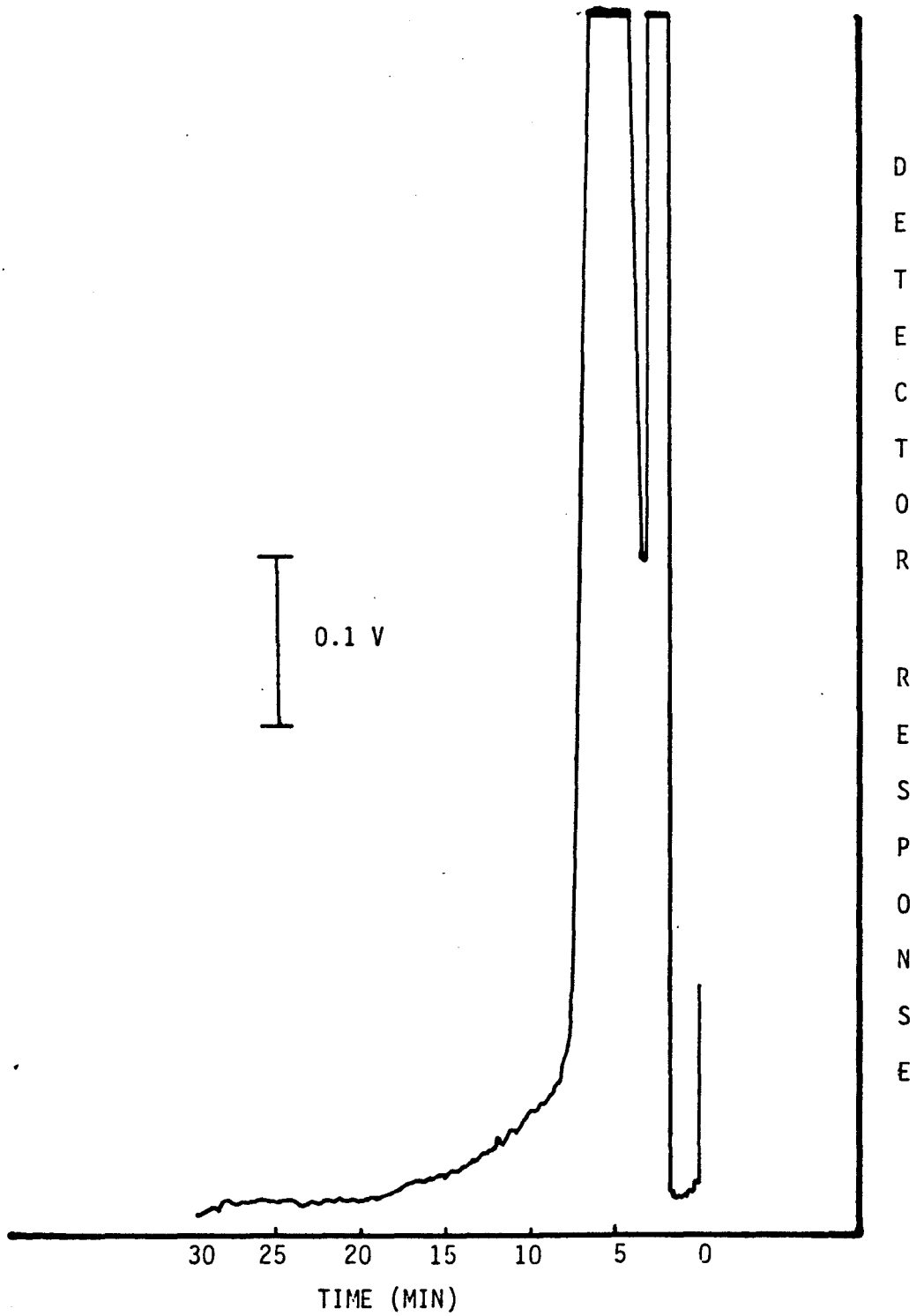

0.1 V
DETECTOR RESPONSE
30
25
20
15
10
5
0
TIME (MIN)

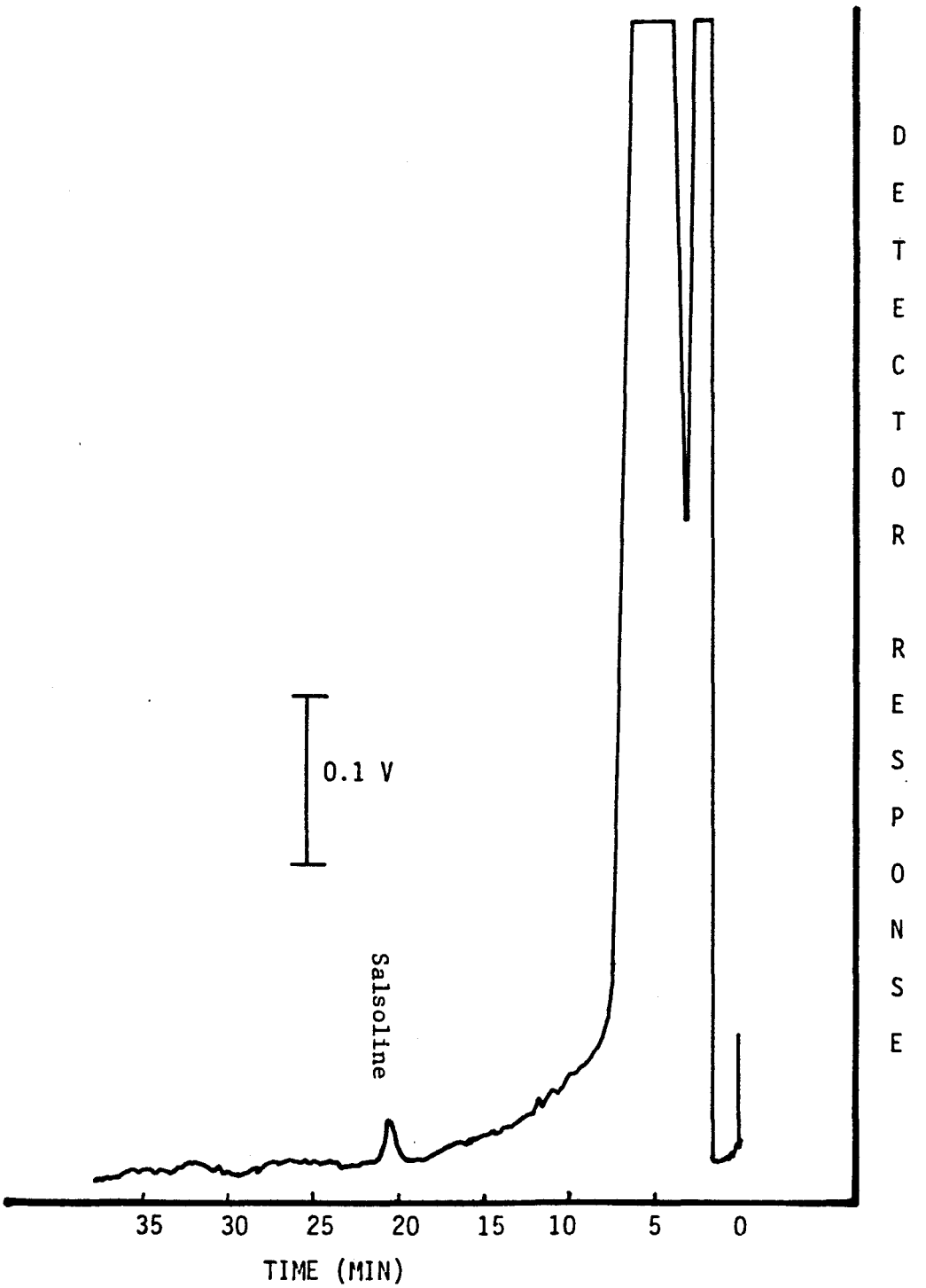
Figure 11: Blood Sample of an Animal Given 100 mg/kg 3-CSAL for 30 Minutes; Spiked with Salsoline.

Sample: 100 ul of a 250 ul sample of The Amine Fraction from Bio-Rex 70.

Column: Partisil-SCX (10 micron) 250 mm x 4 mm.

Buffer: 12.61 g Citric Acid; 3.45 g Ammonium Phosphate Monobasic, monohydrate; 88 ml Ammonium Hydroxide per liter distilled water.

Conditions: 1 ml per minute; 5 nA/V.


0.1 V
Salsoline
35
30
25
20
15
10
5
0
TIME (MIN)
DETECTOR RESPONSE

since the HPLC column lost its ability to resolve 6- and 7-M-3-CSAL after the blood and caudate samples were run on the HPLC. This was most likely due to irreversible adsorption of tissue components. The brain levels are given in Table 6. The striatal tissue contained a mean level of 2.27 ug/g 3-CSAL whereas the hypothalamic tissue contained 4.64 ug/g 3-CSAL at the 30 minute time point. The amine fractions from the same tissue samples were chromatographed on both cation exchange and reverse-phase columns.

Figure 12 shows the cation-exchange chromatogram of one-fifth of the amine fraction of a caudate of an animal given 100 mg/kg 3-CSAL for 30 minutes. No O-M-SAL was seen in the sample. Figure 13 shows the same brain sample spiked with 6-M-SAL and 7-M-SAL. Figure 14 shows the chromatogram of one-fifth of the same caudate extract on a reverse-phase column. No SAL was seen to be present in the sample. When the sample was spiked with 25 ng of SAL (625 ng/g SAL), the SAL chromatographed separately from the peaks for dopamine and 5-HT. The SAL did not co-migrate with any endogenous peaks in these tissues (Figure 15).

Table 7 shows the levels of 3-CSAL and its two mono-O-methyl metabolites in three brain regions one hour after 3-CSAL injection in untreated rats and in rats pretreated with peripheral or central decarboxylase inhibitors. It is

Table 6

BRAIN LEVELS OF CARBOXYLATED THIQs AT 30 MINUTES FOLLOWING A 100 MG/KG I.P. DOSE OF 3-CSAL

UG/G + S.E.M.

TISSUE	3-CSAL	6- and 7-M-3-CSAL
CAUDATE	2.27 ± 0.3	0.39 ± 0.23
HYPOTHALAMUS n=4	4.64 ± 0.4	not measured

Figure 12: Absence of 6- or 7-M-Sal in the Striatum of a Rat Given 100 mg/kg 3-CSAL.

Sample: Amine Fraction from Bio-Rex 70; 100 ul of a 500 ul fraction.

Column: Rsil-Cation Exchange Resin (10 micron) 50 mm x 4mm.

Buffer: 44.14 g Citric Acid, 12.08 g Ammonium Phosphate Monobasic Monohydrate, 308 ml 1N Ammonium Hydroxide brought to a final volumn of 1 liter with distilled water.

Conditions: flow rate of 1 ml per minute; 5 nA/V.

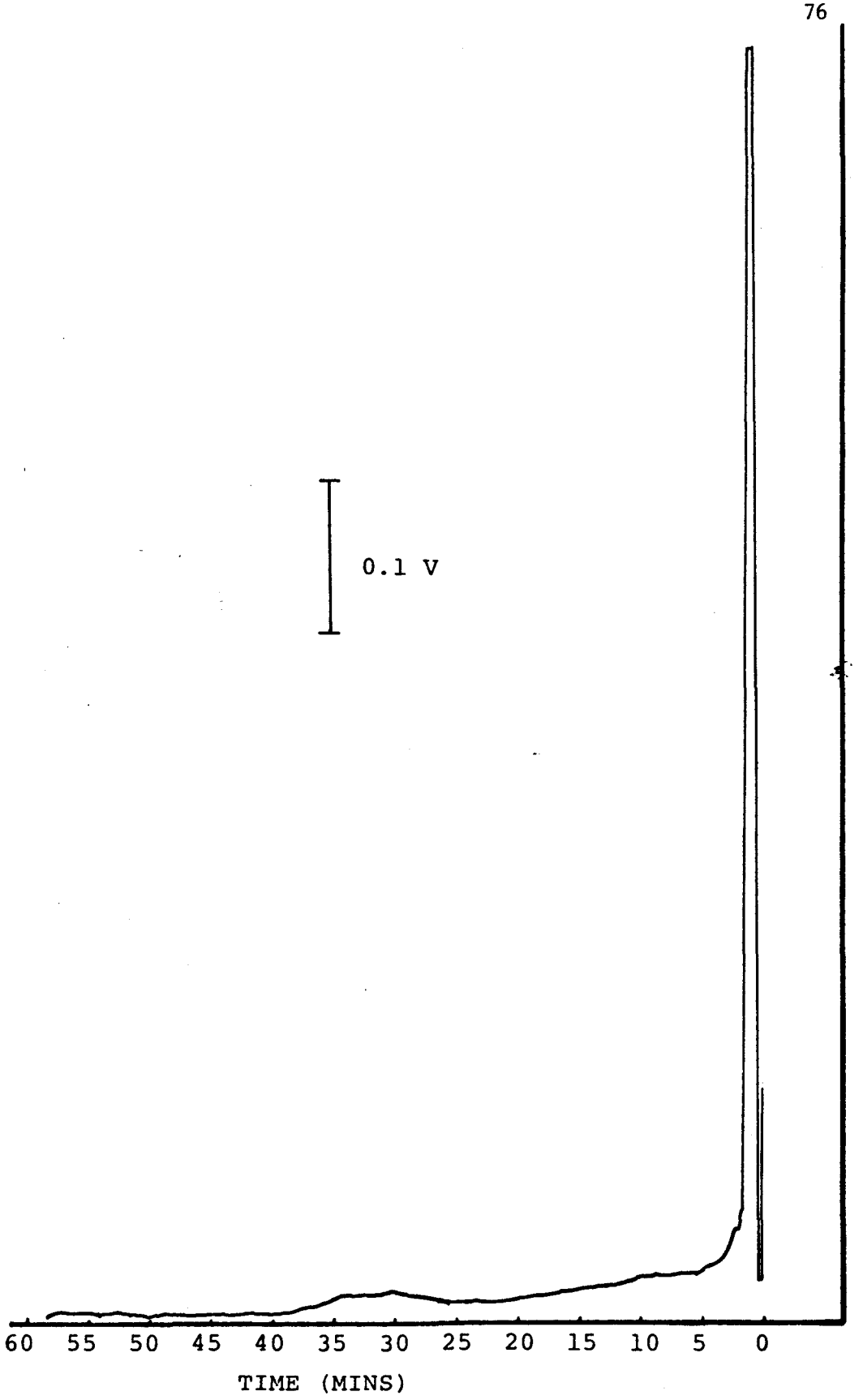

0.1 V
60 55 50 45 40 35 30 25 20 15 10 5 0
TIME (MINS)

Figure 13: Caudate Spiked with IsoSal and Salsoline (25 ng each)

Sample: Amine Fraction from Bio-Rex 70; 100 ul of a 500 ul fraction.

Column: Rsil-Cation Exchange (10 micron) 50 mm x 4 mm.

Buffer: 44.14 g Citric Acid, 12.08 g Ammonium Phosphate Monobasic Monohydrate, 308 ml 1 N Ammonium Hydroxide brought to a final volume of 1 liter with distilled water.

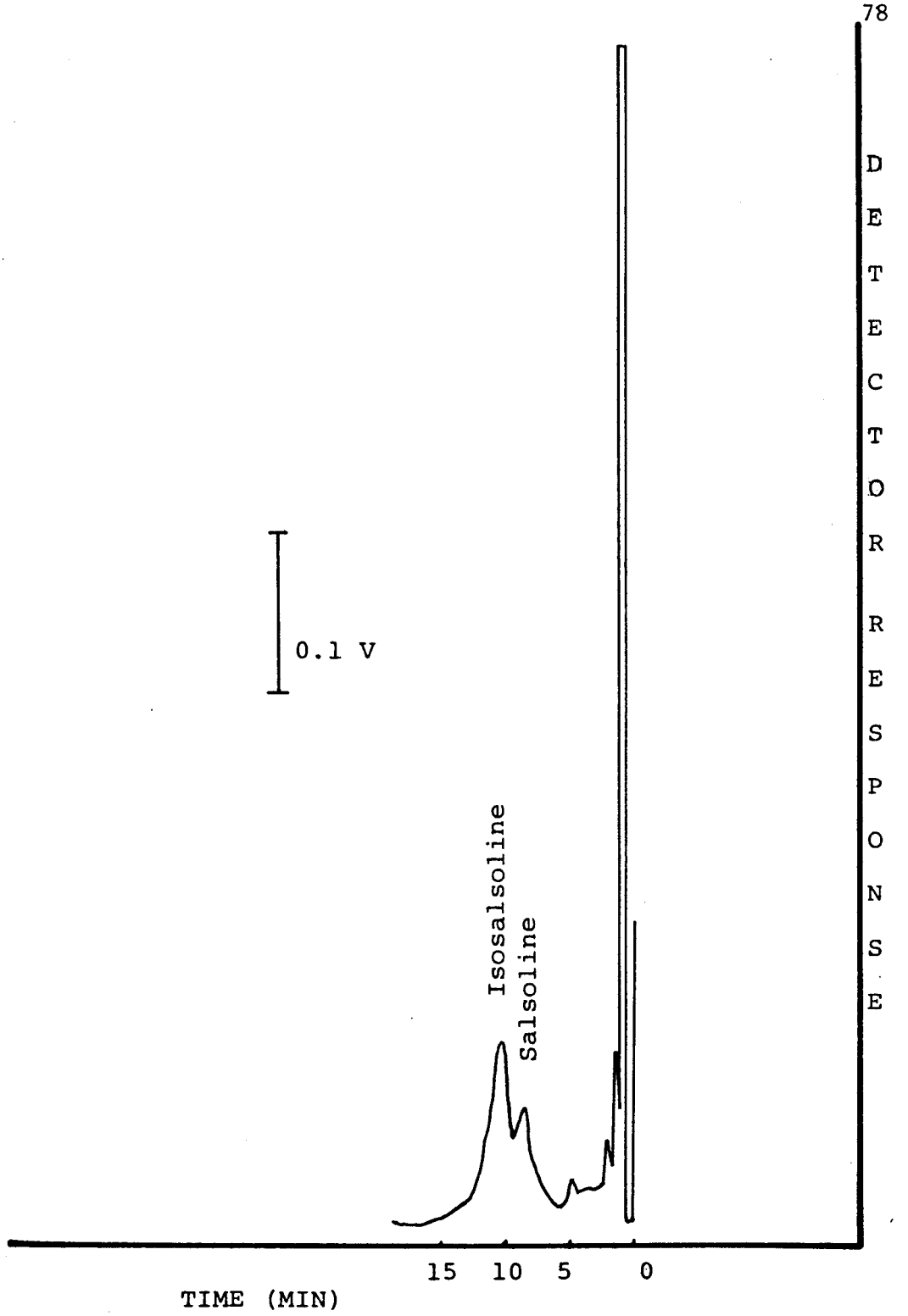

0.1 V
Isosalsoline
Salsoline
DETECTOR RESPONSE
15
10
5
0
TIME (MIN)

Figure 14: Caudate Amine Fraction of an Animal Given 100 mg/kg 3-CSAL for 30 minutes.

Sample: Amine Fraction from Bio-Rex 70; 100 ul of a 500 ul fraction.

Column: 0.1 M Sodium Phosphate Monobasic Nonohydrate, 1.0 mM EDTA, pH 5.0.

Conditions: Flow rate of 1 ml per minute; 10 nA/V.

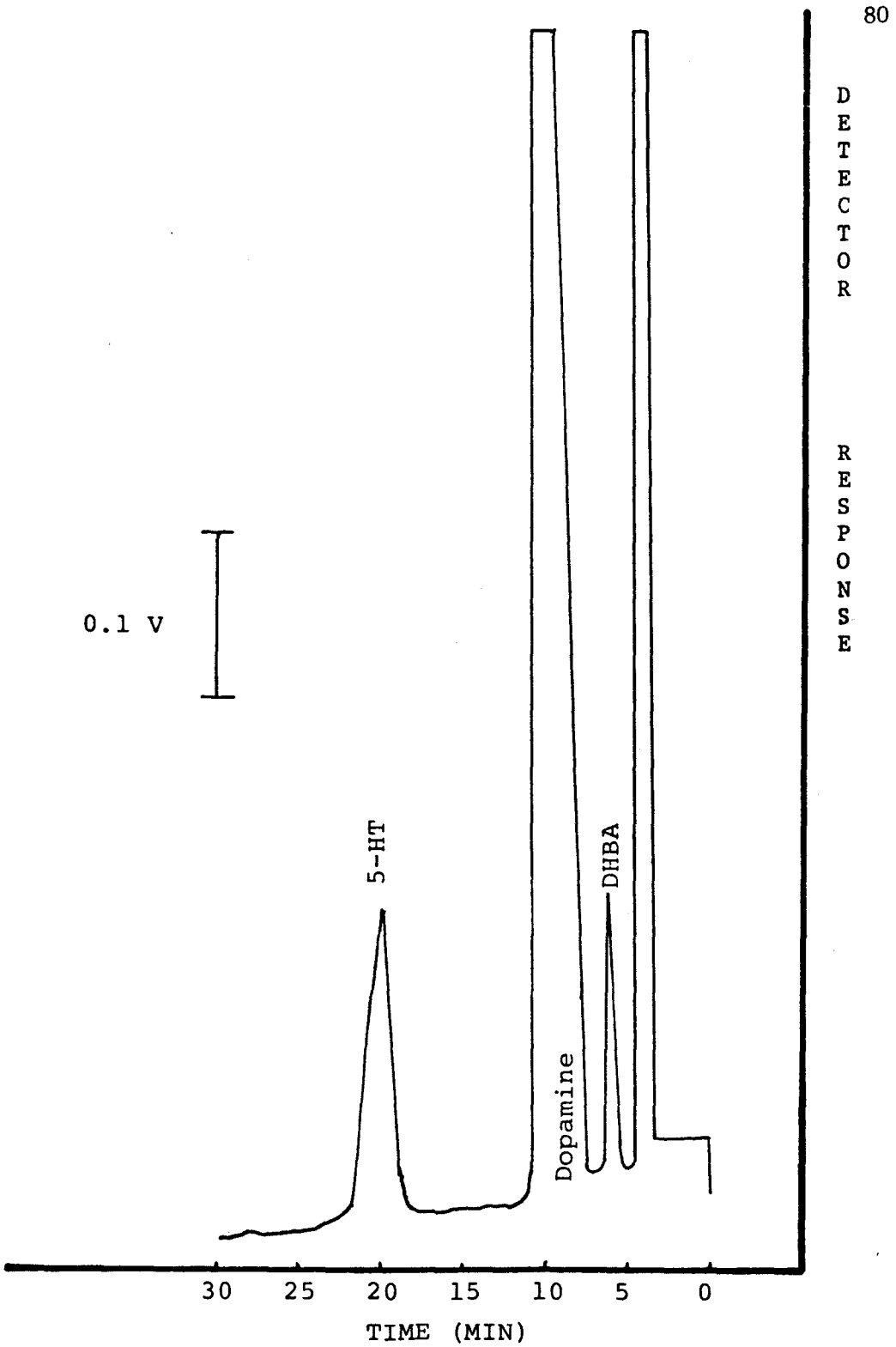

DETECTOR RESPONSE
0.1 V
5-HT
Dopamine
DHBA
30
25
20
15
10
5
0
TIME (MIN)

Figure 15 : Bio-Rex 70 Amine Fraction from a Caudate of a Rat given 100 mg/kg 3-CSAL; Spiked with 25 ng Sal.

Sample: 100 ul of 1000 ul of the Amine Fraction from Bio-Rex 70.

Column: BioSil- ODS 10 250 mm x 4 mm.

Buffer: 0.1 M Sodium Phosphate Monobasic Monohydrate, 1.0 mM EDTA, pH 5.0.

Conditions: Flow rate of 1 ml per minute; 10 nA/V.

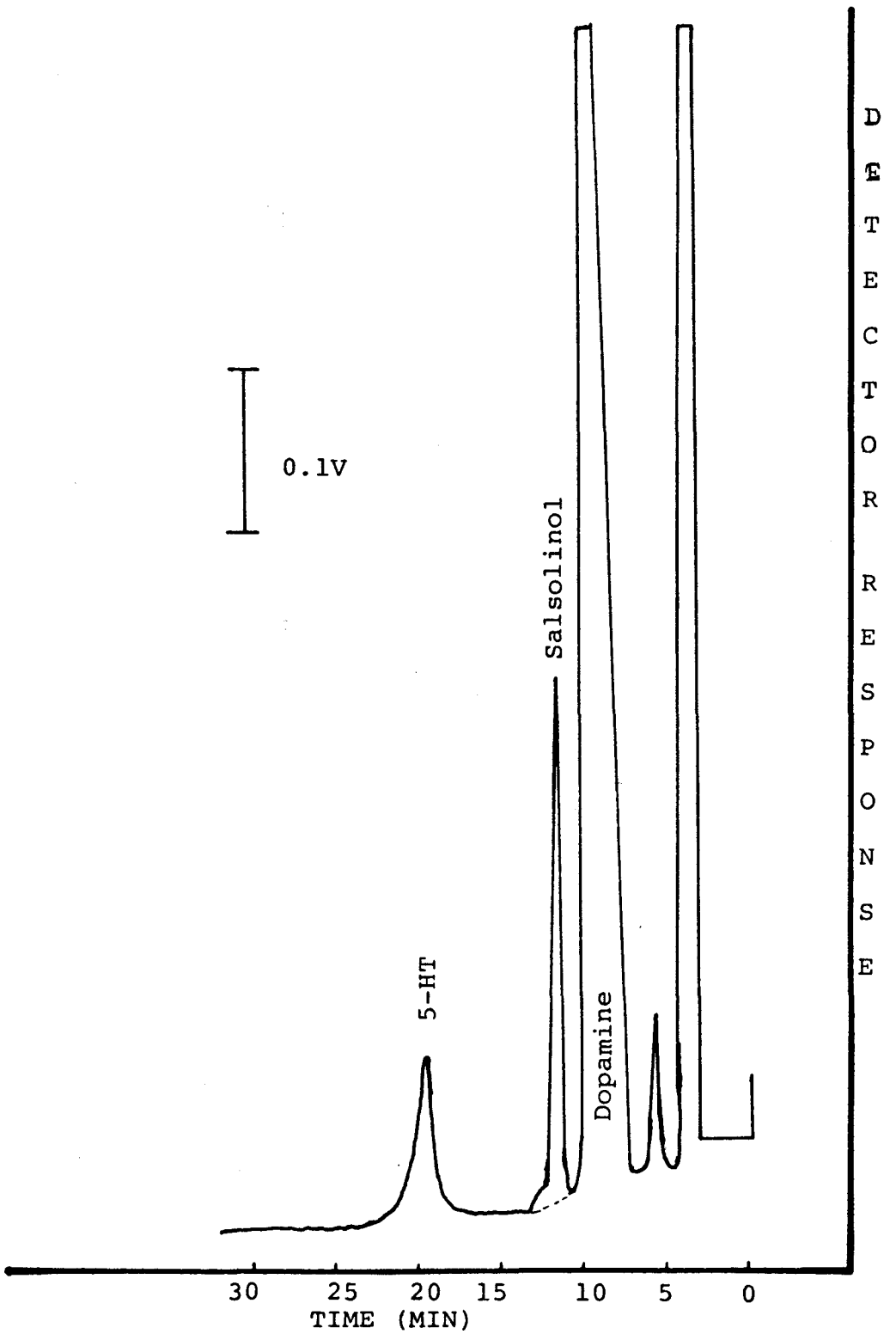

0.1V
5-HT
Salsolinol
Dopamine
DETECTOR RESPONSE
30
25
20
15
10
5
0
TIME (MIN)

Table 7

REGIONAL BRAIN 3-CARBOXY-SAL AND O-METHYLATED 3-CARBOXY-SAL ISOMERS ONE HOUR AFTER 3-CARBOXY-SAL (50 MG/KG I.P.).

UG/G ± S.E.M.

BRAIN REGION	3-CSAL	6M3CSAL	7M3CSAL
CORPUS STRIATUM			
CONTROL	0.75 ± 0.23	0.52 ± 0.10	0.69 ± 0.17
RO4-4602	0.20 ± 0.04	0.19 ± 0.06	0.14 ± 0.04
NSD-1015	0.14 ± 0.06	0.03 ± 0.03	0.25 ± 0.05
HYPOTHALAMUS			
CONTROL	0.20 ± 0.02	2.07 ± 0.34	1.62 ± 0.15
RO4-4602	0.29 ± 0.05	1.30 ± 0.23	1.36 ± 0.11
NSD-1015	0.23 ± 0.03	1.73 ± 0.22	1.74 ± 0.11
HIPPOCAMPUS			
CONTROL	0.18 ± 0.02	0.07 ± 0.04	0.15 ± 0.06
RO4-4602	0.11 ± 0.02	0.09 ± 0.02	0.09 ± 0.02
NSD-1015	0.11 ± 0.04	0.12 ± 0.04	0.14 ± 0.02

apparent that there is decreased brain THIQ content when the inhibitors RO4-4602 and NSD-1015 were given.

2. Other carboxylated THIQ levels-acute experiments.

Tissue levels of 1-CSAL and 1-carboxy-THP are given in Table 8. The hypothalamic 1-carboxy-THP content was very high when compared to the other to carboxylated THIQs administered. This was possibly due to the relative lipophilic nature of this compound when compared to the others.

3. THIQ contents between 1 and 24 hours after a single dose of 3-CSAL (50 mg/kg i.p.).

Large sample sizes of heart (0.9 $\pm$ 0.1 g) and liver (1.5 $\pm$ 0.3 g) and blood (1.5 ml) were taken to ensure that if decarboxylation products were present, they could be quantitated. The large sample sizes, however, interfered with 3-CSAL quantitation because they resulted in corresponding large solvent peaks. Therefore despite a good detector sensitivity for 3-CSAL, it could not be assayed in these tissues. The retention times for 6-methoxy-3-CSAL and 7-methoxy-3-CSAL were sufficiently removed from the larger peaks and were therefore quantitated.

Table 8

BRAIN CARBOXYLATED THIQ LEVELS FOLLOWING A 50 MG/KG DOSE I.P.

CARBOXYLATED THIQ	UG/G + S.E.M.
1-CSAL (n=4)	
CORPUS STRIATUM	0.32 ± 0.09
HYPOTHALAMUS	0.56 ± 0.05
HIPPOCAMPUS	0.55 ± 0.04
1-CARBOXY-THP (n=4)	
CORPUS STRIATUM	0.96 ± 0.29
HYPOTHALAMUS	6.48 ± 1.75
HIPPOCAMPUS	3.10 ± 1.07

a. Rat serum O-methylated 3-CSALs.

As seen in Table 9 and Figure 16, the highest O-methylated 3-CSAL levels were seen at the 1 hour time point. The rate of disappearance of the serum O-methylated-3-CSALs was greater between the 1 hour to 3 hours than between the 3 and 8 hours. Small amounts of O-methylated 3-CSALs were present at the 24 hour time point.

b. Rat liver O-methylated metabolites.

Table 10 and Figure 17, display the time course for levels of liver O-methylated 3-CSALs after the single (50 mg/kg) dose of 3-CSAL. The THIQs persisted up to the 24 hour period of time. No SAL or 7-M-SAL was seen in any of the livers analyzed. At the 30 minute time point the liver 6-methoxy-3-CSAL content was more than twice the 7-methoxy-3-CSAL content ($p<0.001$). The one and three hour 6-methoxy-3-CSAL contents were not significantly greater than the 7-methoxy-isomers. The 8 and 24 hour levels of liver 6-methoxy-3-CSAL were significantly greater than the 7-methoxy-3-CSAL levels. Figure 18 shows a chromatogram of 6- and 7- methoxy-3-CSAL in liver tissue.

Table 9

RAT SERUM O-METHYLATED 3-CSAL LEVELS AT VARIOUS TIMES

AFTER 3-CSAL (50 MG/KG I.P.) NG/ML ± S.E.M.

TIME POINT	6-M-3-CSAL	7-M-3-CSAL
30 MINUTES	993.20 ± 264.50	1089.45 ± 373.00
1 HOUR	552.75 ± 155.94	662.32 ± 210.62
3 HOURS	162.79 ± 102.40	152.35 ± 82.37
8 HOURS	38.45 ± 7.18	30.50 ± 10.08
24 HOURS	7.16 ± 5.14	18.35 ± 13.84

n=4 per time point

Figure 16: Rat serum O-Methylated THIQ Levels

3-CSAL Dose-Time Study.

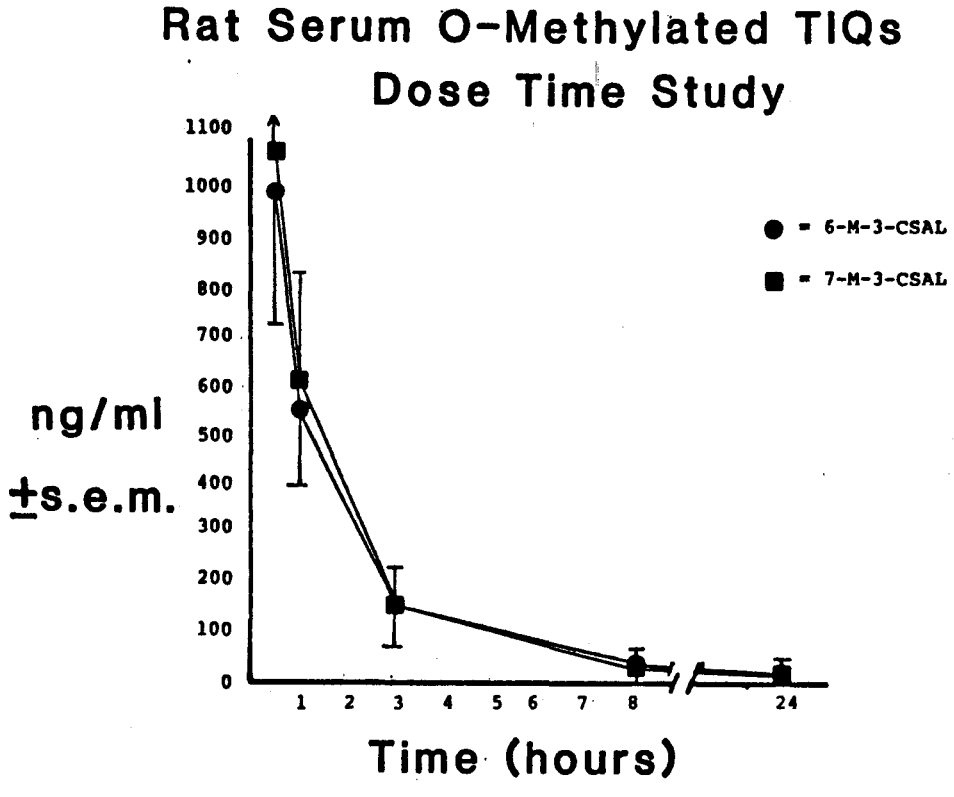

Rat Serum O-Methylated TIQs
Dose Time Study
ng/ml
±s.e.m.
1100
1000
900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
24
Time (hours)
● = 6-M-3-CSAL
■ = 7-M-3-CSAL

Table 10

RAT LIVER O-METHYLATED 3-CSAL LEVELS AT VARIOUS TIMES

AFTER 3-CSAL (50 MG/KG I.P.) UG/G ± S.E.M.

TIME POINT	6-M-3-CSAL	7-M-3-CSAL
30 MINUTES	2.21 ± 0.43	1.03 ± 0.37
1 HOUR	1.44 ± 0.64	1.08 ± 0.76
3 HOURS	0.99 ± 0.04	0.79 ± 0.38
8 HOURS	0.59 ± 0.68	0.10 ± 0.12
24 HOURS	0.19 ± 0.13	0.04 ± 0.03

n=4 per time point

Figure 17: Rat Liver THIQ Levels

3-CSAL Dose-Time Study.

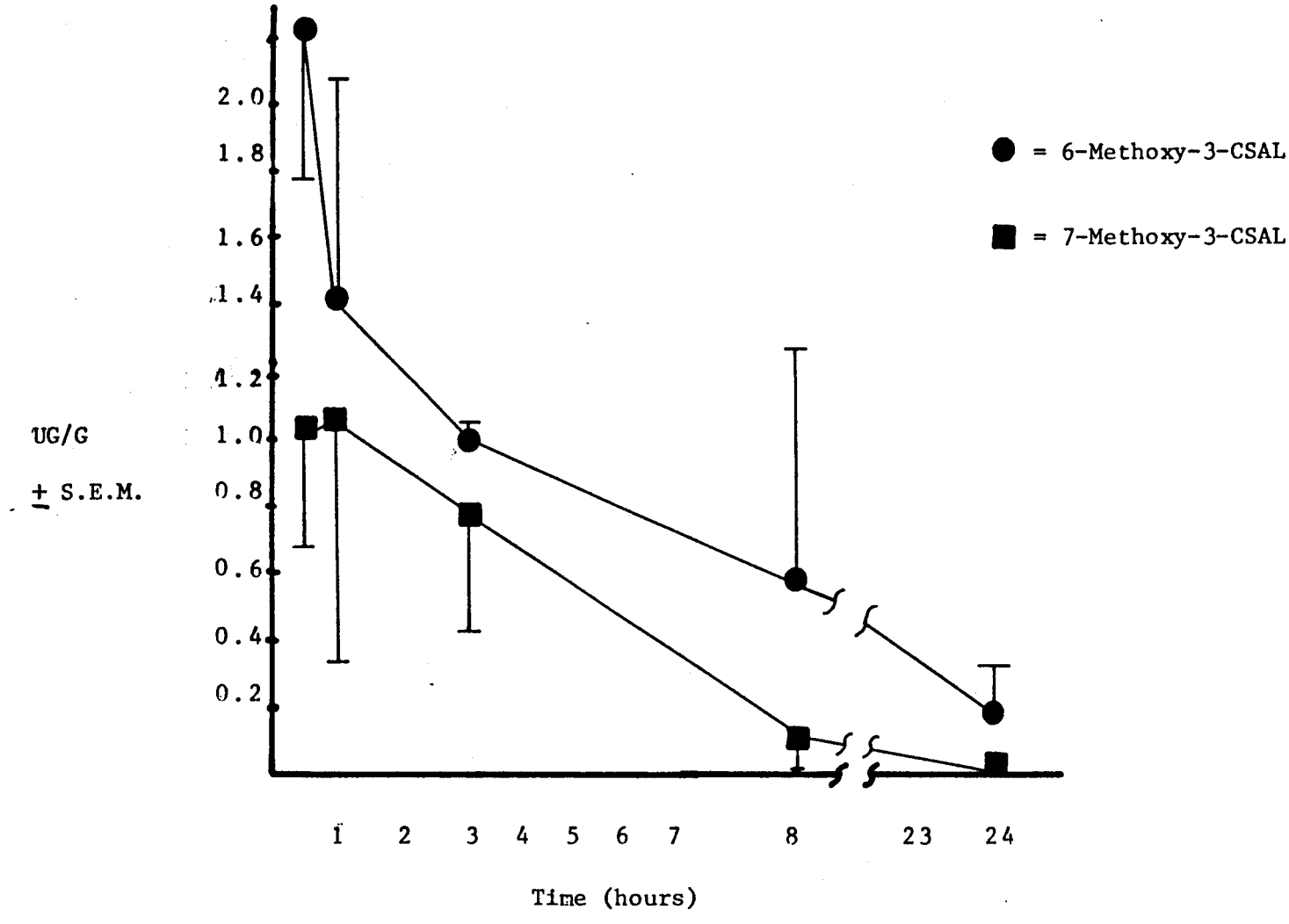

2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
UG/G
± S.E.M.
● = 6-Methoxy-3-CSAL
■ = 7-Methoxy-3-CSAL
1 2 3 4 5 6 7 8 23 24
Time (hours)

Figure 18: Chromatogram of a liver amino acid fraction from an animal given 100 mg/kg 3-CSAL for 30 minutes.

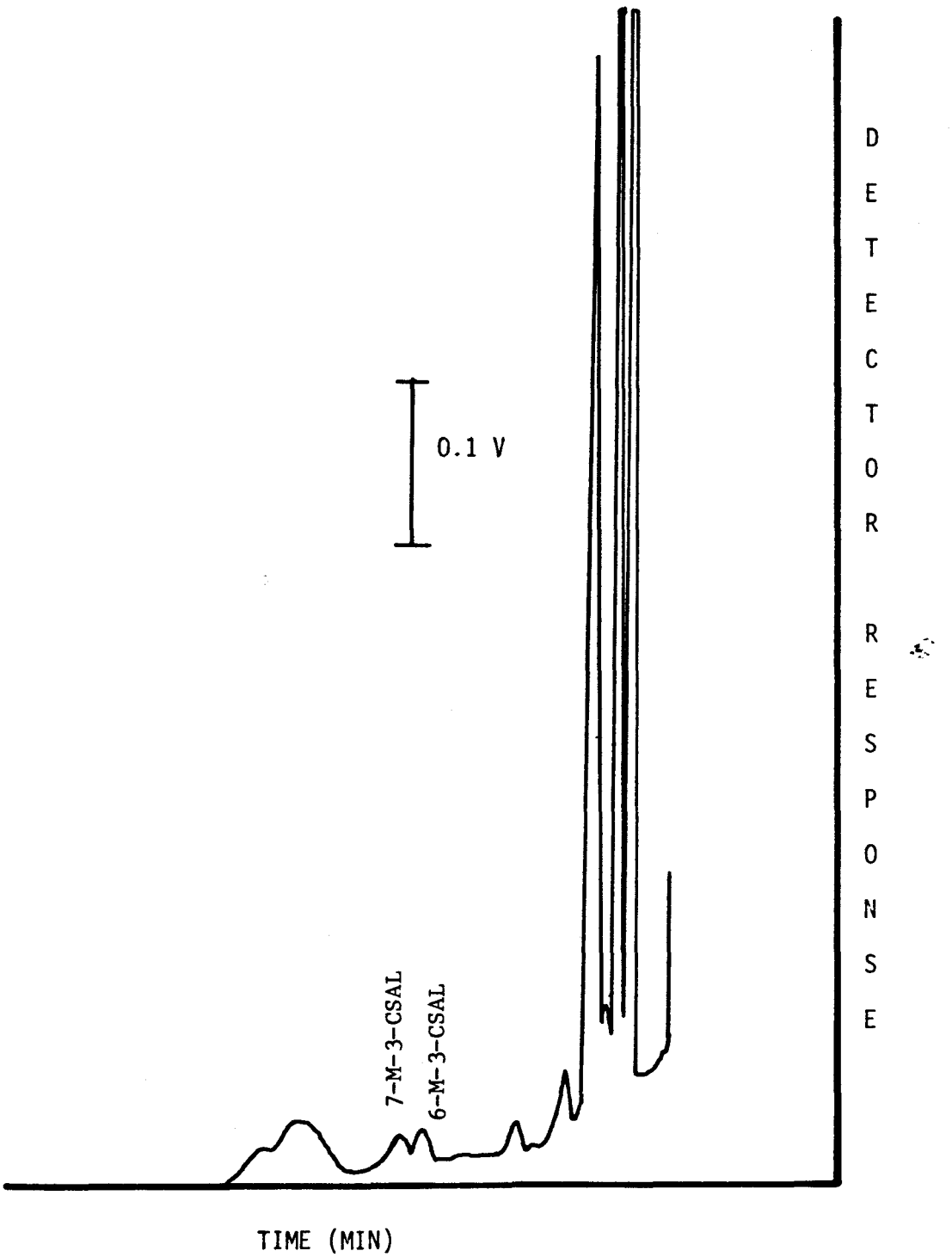
Column: Biosil ODS-10 250mm x 4mm.

Buffer: 0.1 M Sodium phosphate monobasic, monohydrate pH 5.0.; 1.0 mM disodium EDTA.

Detector setting: 5 nA/V.

Flow rate: 1 ml/minute.

Sample: 100 ul of a 500 ul 0.02 M Sodium phosphate eluant from Bio-Rex 70.


0.1 V
7-M-3-CSAL
6-M-3-CSAL
DETECTOR RESPONSE
TIME (MIN)

There was no evidence of SAL or 6- or 7-methoxy-SAL in the rat livers despite the fact that the liver is rich in decarboxylases.

c. Rat heart tissue O-methylated 3-CSALs.

Table 11 and Figure 19 show the levels of O-methylated 3-CSALs in heart tissue following a single dose of 3-CSAL. The levels of the THIQs in the heart were lower than that found in the liver. Neither SAL nor 7-M-SAL was found to be present in the heart tissue. The 30 min, 3,8, and 24 hour levels of 6- and 7-M-3-CSALs were not statistically different. However at 1 hour there was 73% more 6-M-3-CSAL than 7-M-3-CSAL ($p<0.01$).

d. Striatal levels of 3-CSAL and its two O-methylated metabolites after 3-CSAL (50 mg/ kg i.p.).

The presence of striatal 3-CSALs was documented in Table 12 and Figure 20. The levels were highest at the 1 hour time point. Based upon the levels at the three hour and eight hour time points, the half-life of 3-CSAL was approximately 120 minutes. Also, there was no evidence of reductive decarboxylation products in this tissue.

Table 11

RAT HEART LEVELS OF O-METHYL-3-CSAL ISOMERS AT VARIOUS TIMES

FOLLOWING 3-CSAL (50 MG/KG I.P.) NG/G ± S.E.M.

TIME POINT	6-M-3-CSAL	7-M-3-CSAL
30 MINUTES	32.63 ± 15.99	27.95 ± 12.76
1 HOUR	49.07 ± 18.60	28.44 ± 7.35
3 HOURS	9.67 ± 0.26	10.01 ± 0.90
8 HOURS	13.01 ± 0.74	11.50 ± 0.65
24 HOURS	0.00 ± 0.00	0.00 ± 0.00

n=4 per time point

Figure 19: Rat Heart O-Methylated THIQ Levels

3-CSAL Dose-Time Study.

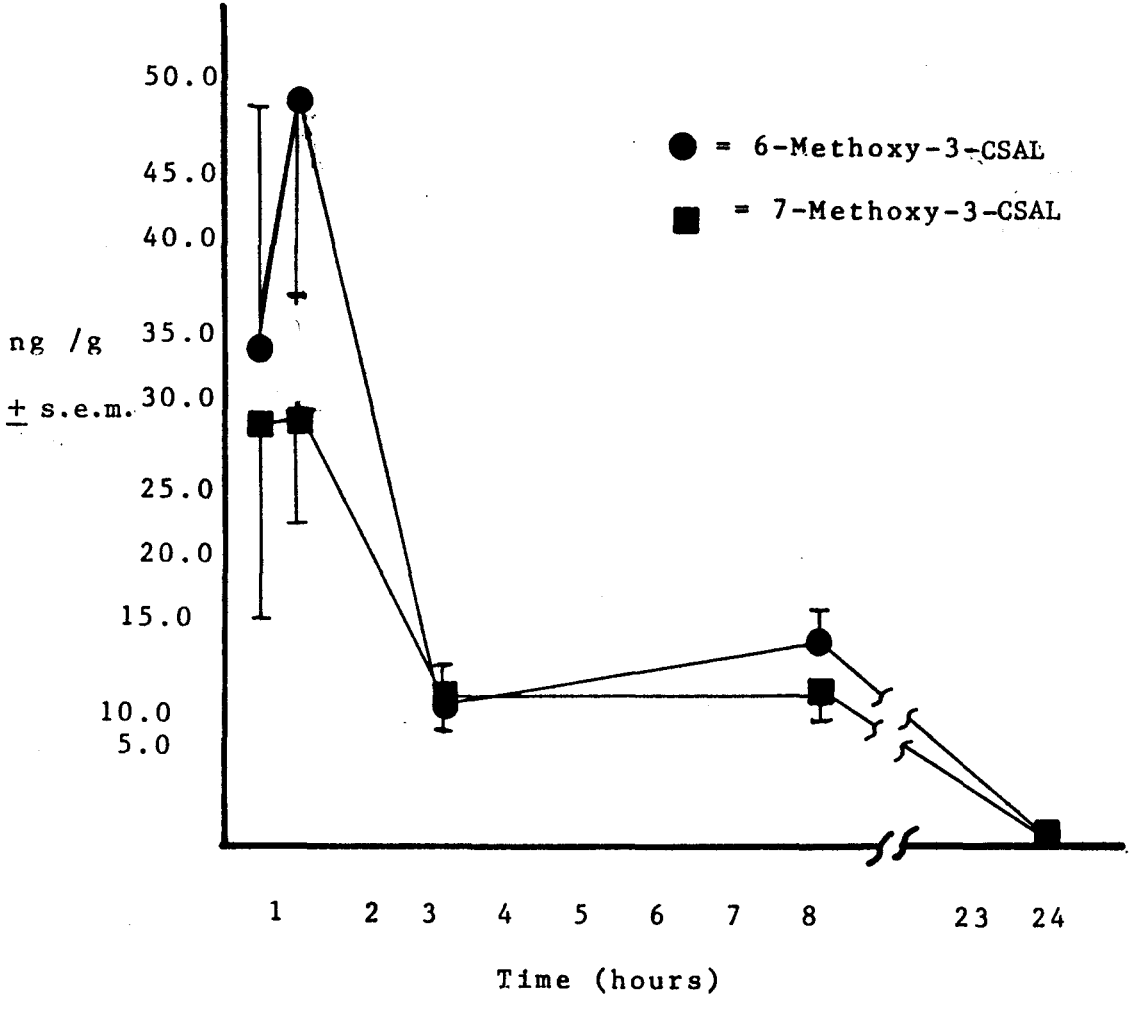

50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
ng /g
± s.e.m.
● = 6-Methoxy-3-CSAL
■ = 7-Methoxy-3-CSAL
1
2
3
4
5
6
7
8
23
24
Time (hours)

Table 12

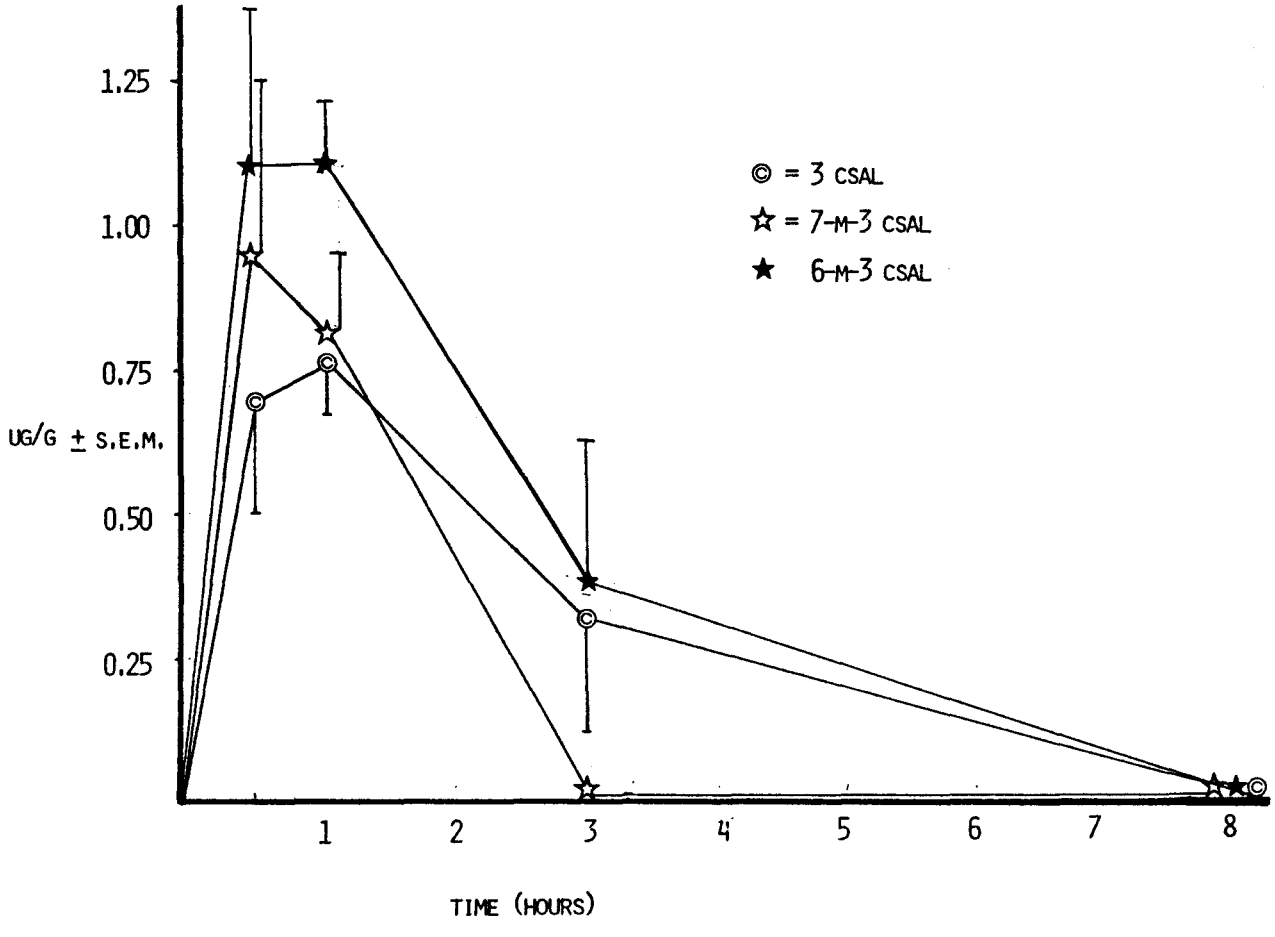
STRIATAL 3-CSAL AND O-METHYL ISOMERS AT VARIOUS TIMES

FOLLOWING 3-CSAL (50 MG/KG I.P.) UG/G ± S.E.M.

TIME POINT	3-CSAL	6-M-3-CSAL	7-M-3-CSAL
30 MINUTES	0.69 ± 0.21	1.13 ± 0.36	0.96 ± 0.31
1 HOUR	0.76 ± 0.08	1.15 ± 0.09	0.81 ± 0.08
3 HOURS	0.33 ± 0.18	0.38 ± 0.22	0.00 ± 0.00
8 HOURS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
24 HOURS	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

n=4 per time point

Figure 20: Striatal THIQ levels After i.p. 3-CSAL.


Striatal TIQ Levels After i.p. 3cSAL (50 mg/kg)
1.25
1.00
0.75
UG/G ± S.E.M.
0.50
0.25
◎ = 3 CSAL
☆ = 7-M-3 CSAL
★ 6-M-3 CSAL
1
2
3
4
5
6
7
8
TIME (HOURS)

B. The effects of carboxylated THIQs on 5-HT and 5-HIAA.

1. 3-CSAL acute experiments-fluorescent studies.

a. The effect of 3-CSAL on striatal 5-HT levels.

The three i.p. doses investigated were 50, 150, and 400 mg/kg. As seen in Table 13, striatal serotonin was significantly increased for all three doses as compared to the control values (+70%; +71%; +90%). Hypothalamic serotonin levels were somewhat depressed but the changes were not significant at the $p<0.05$ level.

b. One-hour pretreatment with the peripheral dopa-decarboxylase inhibitor, Benserazide (RO4-4602).

When 3-CSAL was given to rats pretreated with RO4-4602, striatal serotonin was elevated 336% when compared to the control levels but 5-HIAA levels were not different from the controls. There was no significant difference between experimental and control hypothalamic serotonin or 5-HIAA. The hippocampus showed no difference in serotonin or 5-HIAA content levels. See Table 14.

Table 13

THE EFFECT OF ACUTE 3-CSAL (50 MG/KG I.P.) ON

SEROTONIN LEVELS IN RAT STRIATUM AND HYPOTHALAMUS UG/G ± S.E.M.

3-CSAL DOSE	STRIATUM	HYPOTHALAMUS
CONTROL	0.52 ± 0.08	1.53 ± 0.15
50 MG/KG	0.89 ± 0.16 *	1.05 ± 0.07
150 MG/KG	0.90 ± 0.14 *	1.29 ± 0.14
400 MG/KG	0.99 ± 0.11 *	1.41 ± 0.19

* denotes $p < 0.01$ compared to controls.

Rats 210-270g; 60 days old.

Measured by Fluorescence.

n=6 per group

Table 14

THE EFFECT OF 3-CSAL AND RO4-4602 ON REGIONAL BRAIN SEROTONIN AND 5-HIAA

UG/G ± S.E.M.

TISSUE	5-HT	5-HIAA
CORPUS STRIATUM		
EXPERIMENTAL	4.38 ± 0.95 *	0.91 ± 0.06
CONTROL	1.30 ± 0.43	0.88 ± 0.11
HYPOTHALAMUS		
EXPERIMENTAL	0.39 ± 0.08	0.99 ± 0.21
CONTROL	0.45 ± 0.22	0.78 ± 0.10
HIPPOCAMPUS		
EXPERIMENTAL	0.34 ± 0.01	0.55 ± 0.03
CONTROL	0.38 ± 0.01	0.53 ± 0.03

* denotes $p \leq 0.01$ compared to controls.

Rats 300-400g; 100 days old. n=4 per group

Measured by Fluorescence.

c. One-hour pretreatment with a total (central and peripheral) dopa decarboxylase inhibitor: NSD-1015.

Rats pretreated with sufficient NSD-1015 to totally inhibit decarboxylation had significantly elevated striatal serotonin levels one hour after 3-CSAL when compared to rats which were given the inhibitor followed by saline. Striatal 5-HIAA was significantly depleted when compared to the control group.

Hypothalamic serotonin was significantly elevated but 5-HIAA was not significantly changed when compared to controls For the hippocampus, there was no significant difference between the serotonin or 5-HIAA for the experimental and the control groups (Table 15).

Table 15

THE EFFECT OF 3-CSAL AND NSD-1015 ON REGIONAL BRAIN LEVELS OF 5-HT AND 5-HIAA (UG/G ± S.E.M.).

TISSUE	5-HT	5-HIAA
CORPUS STRIATUM		
EXPERIMENTAL	5.96 ± 0.59 **	0.54 ± 0.05 *
CONTROL	2.29 ± 0.21	0.83 ± 0.07
HYPOTHALAMUS		
EXPERIMENTAL	0.58 ± 0.05 *	1.04 ± 0.11
CONTROL	0.41 ± 0.04	1.06 ± 0.06
HIPPOCAMPUS		
EXPERIMENTAL	0.39 ± 0.02	0.29 ± 0.02
CONTROL	0.43 ± 0.06	0.31 ± 0.07

** denotes $p < 0.01$ compared to controls.

* denotes $p < 0.05$ compared to controls.

Rats 300-400g; 100 days old. n=4 per group

Measured by Fluorescence.

d. The effect of chronic 3-CSAL (5 days)
on regional brain 5-HT and 5-HIAA levels.

Following the chronic administration of 3-CSAL, striatal serotonin was significantly depleted to 48% of control. The serotonin precursor amino acid, tryptophan was significantly elevated by 21% over the control value. 5-HIAA was not significantly different between groups.

In the hypothalamic tissue, serotonin was significantly depleted to 82% of the control but there was no significant difference in tryptophan content between groups The hypothalamic 5-HIAA levels were significantly reduced in the experimental group by 24%. See Table 16.

Table 16

RAT STRIATAL 5-HT AND 5-HIAA AT VARIOUS TIMES

AFTER 3-CSAL (50 MG/KG I.P.) UG/G ± S.E.M.

TIME POINT	5-HT	5-HIAA
CONTROL	0.44 ± 0.04	0.52 ± 0.04
30 MINUTES	0.66 ± 0.11 *	0.54 ± 0.07
1 HOUR	1.01 ± 0.30 *	0.76 ± 0.07 *
3 HOURS	1.32 ± 0.51 *	0.43 ± 0.06
8 HOURS	2.08 ± 0.60 *	0.72 ± 0.09 *
24 HOURS	0.53 ± 0.03 *	0.97 ± 0.08 **

** denotes $p < 0.01$ compared to controls.

* denotes $p < 0.05$ compared to controls.

n=4 per time point

2. HPLC determinations.

a. The time course effect of a single dose 50 mg/kg i.p. of 3-CSAL on 5-HT and 5-HIAA.

The effects of a single 50 mg/kg dose of 3-CSAL was followed at the time points of 30 min., 1, 3, 8, and 24 hours. Striatal serotonin was significantly elevated up to 8 hours afer 3-CSAL was given. At 24 hours, serotonin approached control values but it was still significantly higher (20% over control).

Striatal 5-HIAA was elevated slightly at 30 min. The change however was not statistically significant. At the one hour time point there was a significant increase in the striatal 5-HIAA. The three hour time point showed a non-significant decrease in the 5-HIAA levels when compared to controls. At 24 hours there was again a significant increase in the 5-HIAA levels of the experimental group when compared to the controls. See Table 17 and Figure 21.

Table 17

THE EFFECT OF CHRONIC 3-CSAL ON TRYPTOPHAN, SEROTONIN, AND 5-HIAA IN RAT STRIATUM AND HYPOTHALAMUS UG/G ± S.E.M.

COMPOUND	STRIATUM	HYPOTHALAMUS
TRYPTOPHAN		
CONTROL	3.27 ± 0.08	5.06 ± 0.79
EXPERIMENTAL	3.96 ± 0.17	3.88 ± 0.36
SEROTONIN		
CONTROL	0.33 ± 0.01 *	0.37 ± 0.01
EXPERIMENTAL	0.16 ± 0.02	0.30 ± 0.01
5-HIAA		
CONTROL	0.40 ± 0.05	0.77 ± 0.04
EXPERIMENTAL	0.45 ± 0.06	0.59 ± 0.01

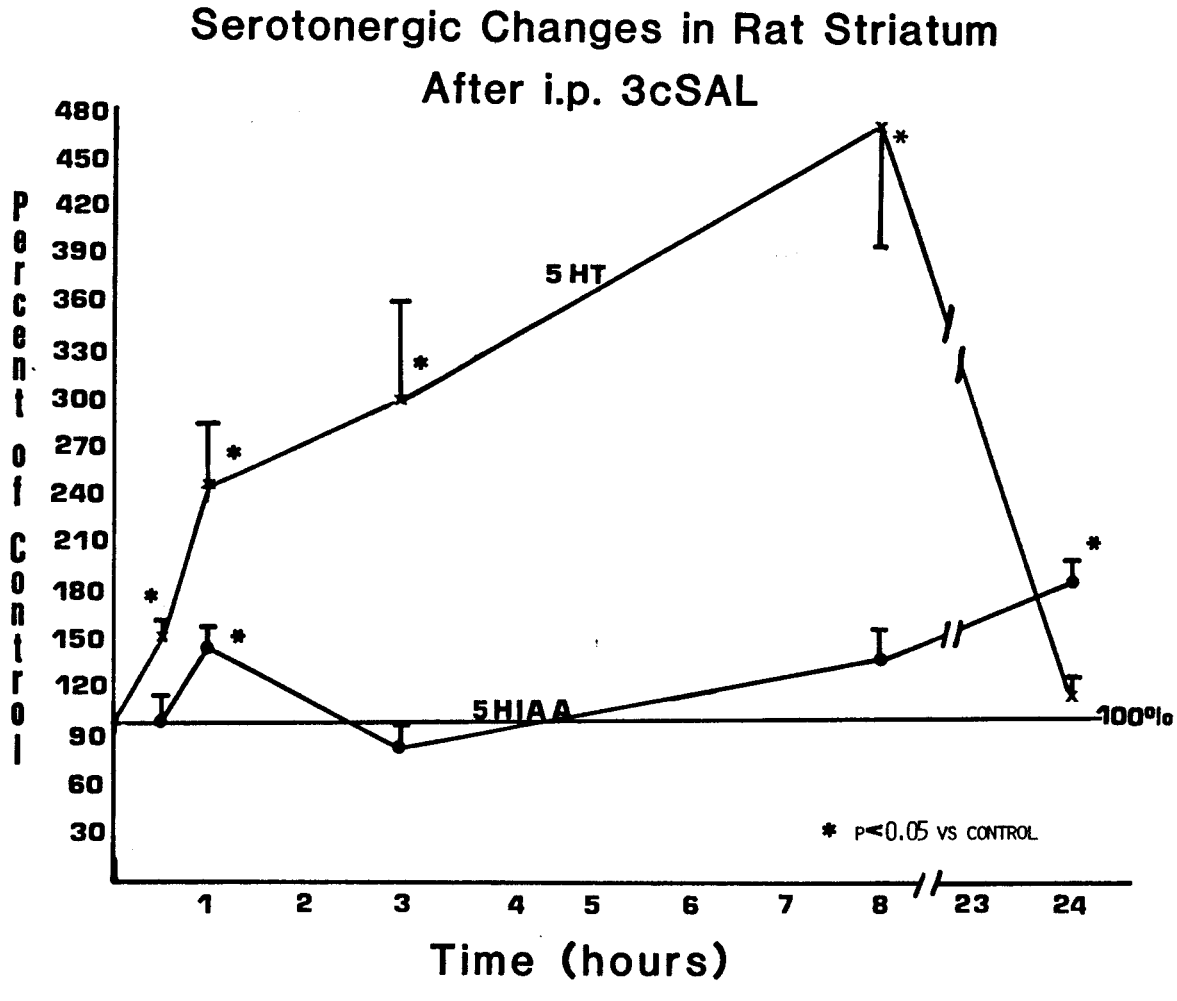
* denotes $p < 0.01$ compared to controls.

Rats 112-152g; 40 days old. n=12 per group

Measured by Fluorescence.

Figure 21: Striatal Serotonergic Changes

3-CSAL Dose-Time Study.


Serotonergic Changes in Rat Striatum
After i.p. 3cSAL
Percent of Control
480
450
420
390
360
330
300
270
240
210
180
150
120
90
60
30
5 HT
5HIAA
100%
* P<0.05 VS CONTROL
1
2
3
4
5
6
7
8
23
24
Time (hours)

b. The effect of 3-CSAL on 5-HT synthesis.

If 3-CSAL increased the turnover of 5-HT, then a total inhibition of aromatic amino acid decarboxylase by RO4-4602 would lead to an increased accumulation of 5-HTP. Table 18 demonstrates that 3-CSAL does not increase the synthesis of 5-HT in decarboxylase inhibited rats.

c. The age-dependent increase of striatal 5-HT by 3-CSAL.

As can be seen in Tables 19 and 20, 3-CSAL did not have a significant effect upon striatal or hypothalamic 5-HT content 8 hours after it was given to the older rats. In fact the only significant difference between the groups was that the HVA content was significantly different between groups. When the younger rats were given 3-CSAL, striatal 5-HT levels were significantly elevated at 4 hours after the dose was given. Striatal 5-HT was further elevated at the 8 hour time point ($p<0.01$) (Table 21). Striatal DA levels were not affected at either of the time points investigated. The hypothalamus showed no significant difference in either DA or 5-HT content.

Table 18

STRIATAL 5-HYDROXYTRYPTOPHAN LEVELS FOLLOWING TOTAL AROMATIC AMINO ACID DECARBOXYLATION INHIBITION BY 800 MG/KG RO4-4602 FOLLOWED BY 3-CSAL

GROUP	5-HYDROXYTRYPTOPHAN (UG/G ± S.E.M.).	
CONTROL	18.00 ± 4.12	n=5
EXPERIMENTAL	22.71 ± 3.42	n=7

Rats 214-407g; 6-10 months of age.

Measured by HPLC.

Table 19

THE EFFECTS OF 3-CSAL 50 MG/KG I.P. FOR EIGHT HOURS UPON THE CORPUS STRIATUM OF 10 MO OLD RATS UG/G ± S.E.M.

COMPOUND	CONTROL GROUP	EXPERIMENTAL GROUP
DOPAMINE	6.47 ± 1.52	6.67 ± 2.07
DOPAC	3.41 ± 0.82	1.82 ± 0.60
5-HT	0.44 ± 0.13	0.34 ± 0.08
5-HIAA	0.31 ± 0.22	0.43 ± 0.16
HVA	1.24 ± 0.15	0.57 ± 0.28 *

n=4 per group

* denotes $p < 0.01$ as compared to controls.

Rats 392-455g; 10-12 months of age.

Measured by HPLC.

Table 20

THE EFFECTS OF 3-CSAL 50 MG/KG I.P. FOR EIGHT HOURS

UPON THE HYPOTHALAMUS OF OLDER RATS

UG/G ± S.E.M.

COMPOUND	CONTROL GROUP	EXPERIMENTAL GROUP
DOPAMINE	0.28 ± 0.08	0.38 ± 0.14
DOPAC	0.18 ± 0.04	0.17 ± 0.04
5-HT	1.69 ± 0.39	1.66 ± 0.28
5-HIAA	0.63 ± 0.14	0.69 ± 0.15
HVA	0.19 ± 0.03	0.36 ± 0.05

n=4 per group

Rats 392-455g; 10-12 months of age.

Measured by HPLC.

Table 21

THE EFFECTS OF 3-CSAL 50 MG/KG I.P. FOR EIGHT HOURS

UPON THE CORPUS STRIATUM OF YOUNGER RATS UG/G ± S.E.M.

GROUP	DOPAMINE	5-HT		
4 Hour Saline	5.54 ± 0.65	0.63 ± 0.14		n=4
4 Hour 3-CSAL	4.99 ± 0.43	1.00 ± 0.09	*	n=5
8 Hour Saline	4.90 ± 0.54	0.78 ± 0.13		n=4
8 Hour 3-CSAL	4.97 ± 0.38	1.21 ± 0.05	*	n=5
Combined Controls	5.22 ± 0.39	0.69 ± 0.08		

* denotes $p < 0.01$ as compared to the time matched controls.

Rats 94-105g; 35 days of age.

Measured by HPLC.

d. The effect of other carboxylated THIQs on 5-HT and 5-HIAA.

1. One hour after 1-CSAL (50 mg/kg i.p.).

The dopamine-pyruvic acid condensation product, 1-CSAL, had a significant effect upon the striatal serotonergic system, resulting in significantly elevated 5-HT levels. 5-HIAA also was significantly elevated in this tissue.

1-CSAL had no effect on the hypothalamic serotonin content. However a 5-fold elevation of 5-HIAA was seen in this tissue.

Neither hippocampal 5-HT nor 5-HIAA was affected by the dose of 1-CSAL (Table 22).

2. One hour after 1-carboxy-THP (50 mg/kg i.p.).

1-carboxy-THP, the condensation product of dopamine and 3,4-dihydroxyphenylpyruvic acid, significantly increased both 5-HT and 5-HIAA content in the corpus striatum.

Both hypothalamic and hippocampal 5-HT and 5-HIAA contents were unaffected by this acute dose of 1-carboxy-THP. See Table 23.

Table 22

REGIONAL BRAIN 5-HT AND 5-HIAA LEVELS ONE HOUR FOLLOWING ACUTE 1-CSAL TREATMENT (50 MG/KG I.P.) UG/G ± S.E.M.

TISSUE	5-HT	5-HIAA
CORPUS STRIATUM		
EXPERIMENTAL	1.26 ± 0.16 *	1.32 ± 0.15 *
CONTROL	0.51 ± 0.02	0.72 ± 0.07
HYPOTHALAMUS		
EXPERIMENTAL	1.06 ± 0.10	1.56 ± 0.10 **
CONTROL	0.96 ± 0.04	0.30 ± 0.02
HIPPOCAMPUS		
EXPERIMENTAL	0.18 ± 0.02	0.20 ± 0.02
CONTROL	0.23 ± 0.02	0.23 ± 0.02

** denotes $p \leq 0.01$; * denotes $p < 0.05$ as compared to controls

n=4 per group
Rats 140-165g; 42 days of age.

Measured by HPLC.

Table 23

REGIONAL BRAIN 5-HT AND 5-HIAA LEVELS ONE HOUR FOLLOWING ACUTE 1-CARBOXY-THP TREATMENT (50 MG/KG I.P.) UG/G ± S.E.M.

TISSUE	5-HT	5-HIAA
CORPUS STRIATUM		
EXPERIMENTAL	1.42 ± 0.18 *	1.69 ± 0.14 *
CONTROL	0.51 ± 0.02	0.72 ± 0.07
HYPOTHALAMUS		
EXPERIMENTAL	0.90 ± 0.10	0.24 ± 0.04
CONTROL	0.96 ± 0.04	0.30 ± 0.02
HIPPOCAMPUS		
EXPERIMENTAL	0.27 ± 0.05	0.26 ± 0.05
CONTROL	0.23 ± 0.02	0.23 ± 0.02

* denotes $p < 0.05$ as compared to controls.

Rats 140-165g; 42 days of age. n=4 per group

Measured by HPLC.

C. The effects of carboxylated THIQs on the levels of catecholamines and their acid metabolites.

1. Acute fluorometric determinations.

a. Striatal and hypothalamic catecholamine content one hour after 3-CSAL.

One hour after the 50 mg/kg i.p. 3-CSAL dose, there was a non-significant decrease of striatal and hypothalamic dopamine. Hypothalamic norepinephrine levels were not statistically different between groups (see Table 24).

b. Striatal and hypothalamic content one hour after 3-CSAL (50 mg/kg i.p.) in Benserazide (RO4-4602) treated rats.

When preceded by a peripheral decarboxylase inhibitor, the 50 mg/kg dose of 3-CSAL lowered striatal dopamine to 52.3% of the level of the control group (Table 25). Hypothalamic dopamine was also significantly reduced, but not to the same degree. Hypothalamic norepinephrine content was not altered.

c. Striatal and hypothalamic content one hour after 3-CSAL (50 mg/kg i.p.) in NSD-1015 pretreated rats.

Table 24

REGIONAL CATECHOLAMINE LEVELS ON HOUR FOLLOWING ACUTE 3-CSAL TREATMENT (50 MG/KG I.P.) UG/G ± S.E.M.

TISSUE	LEVELS
CORPUS STRIATUM	
CONTROL DA	7.88 ± 0.76
EXPERIMENTAL DA	6.73 ± 1.45
HYPOTHALAMUS	
CONTROL DA	2.11 ± 0.25
EXPERIMENTAL DA	1.97 ± 0.98
CONTROL NE	2.38 ± 0.21
EXPERIMENTAL NE	2.52 ± 0.59

Rats 300-400g; 100 days old. n=5 per group

Measured by Fluorescence.

Table 25

REGIONAL CATECHOLAMINE LEVELS ONE HOUR FOLLOWING RO4-4602

AND ACUTE 3-CSAL TREATMENT (50 MG/KG I.P.) UG/G ± S.E.M.

TISSUE	LEVELS
CORPUS STRIATUM	
EXPERIMENTAL DA	4.31 ± 0.44 **
CONTROL DA	8.24 ± 0.50
HYPOTHALAMUS	
EXPERIMENTAL DA	2.12 ± 0.12 *
CONTROL DA	2.86 ± 0.09
EXPERIMENTAL NE	1.98 ± 0.19
CONTROL NE	2.62 ± 0.13

** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to controls

Rats 300-400g; 100 days of age. n=6 per group

Measured by Fluorescence.

In the presence of a total dopa decarboxylase inhibitor, 3-CSAL significantly reduced striatal dopamine levels when compared to rats given the inhibitor alone. Hypo- thalamic dopamine levels were not significantly affected. Hypothalamic norepinephrine content was not significantly affected (Table 26).

d. Striatal dopamine levels one hour following increasing doses of 3-CSAL (50, 150, or 400 mg/kg i.p.).

The effect of the 3-CSAL at the doses of 50, 150, or 400 mg/kg was investigated. As seen in Table 27, there was no significant effect upon striatal dopamine levels by any of the doses given.

2. HPLC determinations.

a. The effect of chronic 3-CSAL (50 mg/kg i.p., three times daily for 5 days) on catecholamines.

One hour following the final dose of 3-CSAL, the striatal and hypothalamic dopamine of the experimental group

Table 26

REGIONAL CATECHOLAMINE LEVELS FOLLOWING TREATMENT WITH A TOTAL DECARBOXYLASE INHIBITOR FOLLOWED BY ACUTE 3-CSAL (50 MG/KG I.P.) FOR ONE HOUR. UG/G ± S.E.M.

TISSUE	LEVELS
CORPUS STRIATUM	
CONTROL DA	7.32 ± 0.47 **
EXPERIMENTAL DA	4.74 ± 0.71
HYPOTHALAMUS	
CONTROL DA	1.97 ± 0.04
EXPERIMENTAL DA	2.05 ± 0.29
CONTROL NE	2.29 ± 0.24
EXPERIMENTAL NE	1.89 ± 0.21

** denotes $p < 0.01$ as compared to controls.

Rats 300-400g; 100 days of age. n=6 per group

Measured by Fluorescence.

Table 27

THE EFFECT OF ACUTE 3-CSAL (50 MG/KG I.P.) ON DOPAMINE LEVELS IN THE CORPUS STRIATUM UG/G ± S.E.M.

DOSE OF 3-CSAL	LEVELS
CONTROL	7.32 ± 0.47
50 MG/KG	7.29 ± 0.88
150 MG/KG	6.35 ± 0.53
400 MG/KG	6.65 ± 0.56

Rats 210-270g; 60 days of age.

Measured by Fluorescence.

n=6 per group

were essentially the same as that of the control group. Hypothalamic norepinephrine levels were unchanged (See Table 28).

b. Acute 3-CSAL studies-catecholamine and metabolite studies.

1. Striatal levels of DA, DOPAC, and HVA between 30 minutes and 24 hours after 3-CSAL (50 mg/kg i.p.).

The effect of a single 50 mg/kg dose of 3-CSAL was followed at the time points of 30 min., 1, 3, 8, and 24 hours. The levels of striatal dopamine, DOPAC, and HVA are listed in Table 29. There was no statistical difference in striatal dopamine levels at any of the times compared to the control group.

Thirty minutes post dosage, there was no significant difference in DOPAC levels as compared to the controls. Non-statistical increases in DOPAC levels were seen at one and three hours. Eight hours after the dose of 3-CSAL, striatal DOPAC was elevated greater than two-fold as compared to the controls The twenty-four hour DOPAC levels were also significantly elevated. The DOPAC elevation was greater at twenty-four hours than at eight hours.

Table 28

THE EFFECT OF CHRONIC 3-CSAL ON DOPAMINE AND NOREPINEPHRINE IN RAT STRIATUM AND HYPOTHALAMUS UG/G ± S.E.M.

DOPAMINE	STRIATUM	HYPOTHALAMUS
CONTROL	8.43 ± 0.73	2.86 ± 0.46
EXPERIMENTAL	8.38 ± 1.24	2.87 ± 0.40
NOREPINEPHRINE		
CONTROL	--	2.22 ± 0.16
EXPERIMENTAL	--	2.24 ± 0.31

The 3-CSAL was given every 8 hours for 5 days. Animals were sacrificed 1 hour after the last dose.

Rats 112-152g; 40 days of age.

Measured by Fluorescence. n=12 per group

Table 29

DA, HVA, AND DOPAC LEVELS IN THE CORPUS STRIATUM AT VARIOUS TIMES AFTER ACUTE 3-CSAL (50 MG/KG I.P.) UG/G ± S.E.M.

TIME POINT	DOPAMINE	DOPAC	HVA
CONTROL	7.13 ± 0.88	2.13 ± 0.09	1.13 ± 0.06
30 MINUTES	7.87 ± 1.24	2.03 ± 0.07	0.48 ± 0.19 **
1 HOUR	7.78 ± 0.61	3.08 ± 1.02	0.98 ± 0.44
3 HOURS	7.09 ± 1.95	2.82 ± 0.83	1.05 ± 0.07
8 HOURS	9.15 ± 2.32	4.50 ± 0.32 **	1.35 ± 0.06 *
24 HOURS	8.75 ± 1.17	5.19 ± 0.42 **	1.69 ± 0.51 *

** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to controls.

Rats 250-350g; 90 days of age.

Measured by HPLC. n=4 per group

The HVA levels were significantly lowered at the thirty minute time point as compared to the controls. At one and three hours, the HVA levels of the experimental groups were statistically the same as that for the control group. At eight hours the HVA levels for the experimental group was significantly elevated over that for the control group. The HVA levels were further elevated at twenty four-hours. See Figure 22.

2. Levels of DA, DOPAC, and HVA in striatum and hypothalamus following 3-CSAL (50 mg/kg i.p.) in Benserazide (RO4-4602) pretreated rats.

Striatal dopamine was significantly depleted in the experimental group. Striatal DOPAC levels were also significantly depleted, but HVA levels remained unchanged.

While the peripheral dopa decarboxylase inhibitor plus 3-CSAL had effects upon the dopaminergic system in the corpus striatum, no effects were seen in the hypothalamus: there was no significant effect on dopamine levels; HVA levels were non-significantly lowered (Table 30).

3. Animals given a total decarboxylase inhibitor followed by 3-CSAL.

Figure 22: Striatal Dopaminergic Changes

3-CSAL Dose-Time Study.

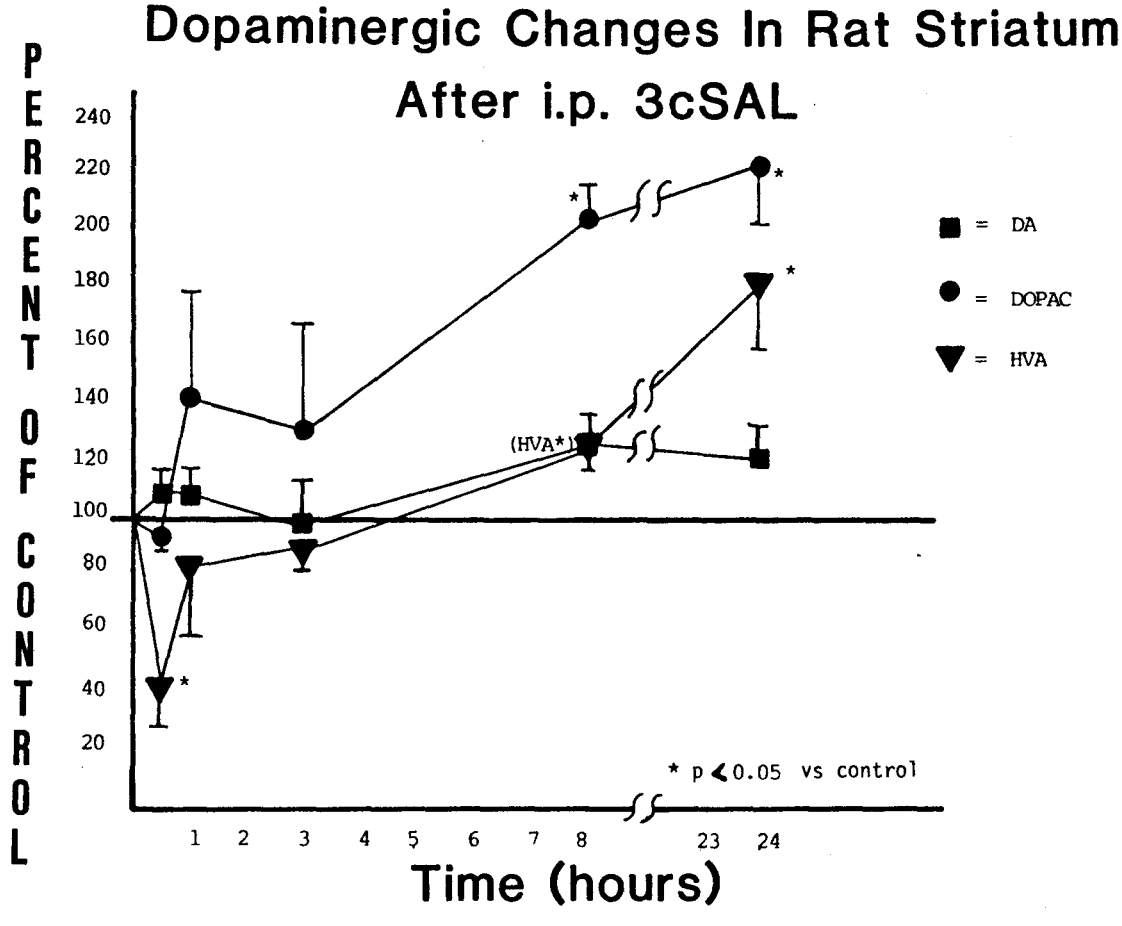

Dopaminergic Changes In Rat Striatum
After i.p. 3cSAL
PERCENT OF CONTROL
240
220
200
180
160
140
120
100
80
60
40
20
1 2 3 4 5 6 7 8 23 24
Time (hours)
= DA
= DOPAC
= HVA
(HVA*)
* p < 0.05 vs control

Table 30

THE EFFECT OF 3-CSAL AND RO4-4602 ON REGIONAL DOPAMINE, DOPAC, AND HVA LEVELS UG/G ± S.E.M.

TISSUE	DOPAMINE	DOPAC	HVA
CORPUS STRIATUM			
EXPERIMENTAL	3.68 ± 0.12 **	0.66 ± 0.06 *	0.48 ± 0.06
CONTROL	7.04 ± 0.33	1.09 ± 0.11	0.48 ± 0.07
HYPOTHALAMUS			
EXPERIMENTAL	1.60 ± 0.10	0.20 ± 0.03	0.18 ± 0.05
CONTROL	1.73 ± 0.18	0.15 ± 0.02	0.24 ± 0.06

** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to controls.

Rats 196-205g; 50 days of age.

Measured by HPLC. n=4 per group

Total dopa decarboxylase inhibition followed by 3-CSAL resulted in a significant depletion of dopamine levels in the corpus striatum. DOPAC levels for the experimental group were significantly reduced when compared to controls. Also, HVA levels were significantly less for the experimental group when compared to the controls.

The experimental treatment produced no significant difference in hypothalamic dopamine levels when compared to the controls; DOPAC levels were significantly elevated and there was a large non-significant increase in HVA levels. See Table 31.

4. The effect of 1-CSAL (50 mg/kg i.p.) on catecholamine and acid levels.

A 50 mg/kg i.p. dose of 1-CSAL had no effect upon striatal dopamine or DOPAC levels after one hour. However a significant effect ($p<0.01$) of 1-CSAL upon the striatal dopaminergic system is indicated by the increased HVA levels.

The hypothalamic dopaminergic system was more extensively affected by 1-CSAL. Hypothalamic dopamine and DOPAC were significantly elevated when compared to controls. Contrary to the striatum, hypothalamic HVA levels were not altered by acute 1-CSAL. See Table 32.

Table 31

TISSUE DOPAMINE AND ACID METABOLITES LEVELS FOLLOWING TOTAL DECARBOXYLASE INHIBITION AND 3-CSAL (50 MG/KG I.P.).

UG/G ± S.E.M.

TISSUE	DOPAMINE	DOPAC	HVA
CORPUS STRIATUM			
EXPERIMENTAL	5.90 ± 0.53 *	0.24 ± 0.03 **	0.31 ± 0.10 *
CONTROL	7.62 ± 0.43	0.36 ± 0.02	0.53 ± 0.04
HYPOTHALAMUS			
EXPERIMENTAL	1.68 ± 0.34	0.22 ± 0.02 *	2.80 ± 0.27
CONTROL	1.66 ± 0.16	0.16 ± 0.01	1.67 ± 0.75

** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to controls.

Rats 200-214g; 52 days of age. n=4 per group

Measured by HPLC.

Table 32

TISSUE DOPAMINE AND ACID METABOLITES FOLLOWING 1-CSAL

UG/G ± S.E.M.

TISSUE	DOPAMINE	DOPAC	HVA
CORPUS STRIATUM			
EXPERIMENTAL	7.81 ± 1.06	1.85 ± 0.24	0.89 ± 0.07 *
CONTROL	7.65 ± 0.74	1.78 ± 0.10	0.43 ± 0.10
HYPOTHALAMUS			
EXPERIMENTAL	2.66 ± 0.09 *	0.56 ± 0.14 **	0.28 ± 0.04
CONTROL	2.08 ± 0.23	0.34 ± 0.08	0.30 ± 0.02

** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to controls.

Rats 140-165g; 42 days of age. n=4 per group

Measured by HPLC.

5. The effect of 1-carboxy-THP (50 mg/kg i.p.) on the catecholamine and acid metabolite levels.

A 50 mg/kg i.p. dose of 1-carboxy-THP had no effect upon the striatal dopamine or DOPAC levels after one hour. However striatal HVA levels were significantly elevated by acute 1-carboxy-THP treatment. Hypothalamic DA, DOPAC, and HVA levels for the experimental group was not statistically different from controls (Table 33).

Table 33

TISSUE DOPAMINE AND ACID METABOLITES FOLLOWING ACUTE

1-CARBOXY-THP (50 MG/KG I.P.) UG/G ± S.E.M.

TISSUE	DOPAMINE	DOPAC	HVA
CORPUS STRIATUM			
EXPERIMENTAL	8.18 ± 0.76	1.98 ± 0.05	0.93 ± 0.13 *
CONTROL	7.65 ± 0.75	1.78 ± 0.10	0.43 ± 0.01
HYPOTHALAMUS			
EXPERIMENTAL	2.21 ± 0.21	0.48 ± 0.10	0.24 ± 0.04
CONTROL	2.08 ± 0.23	0.34 ± 0.08	0.30 ± 0.02

* denotes $p < 0.05$ as compared to controls.

Rats 140-165g; 42 days of age. n=4 per group

Measured by HPLC.

CHAPTER V

DISCUSSION

A. Lack of evidence for in vivo decarboxylation of 3-CSAL to SAL or O-methyl-SALs.

Animals given a 100 mg/kg dose of 3-CSAL had relatively greater THIQ levels at 30 minutes than at the 15 minute time point. Non-oxidative decarboxylation of 3-CSAL or of its O-methyl metabolites could have produced SAL, 6-M-SAL, or 7-M-SAL. Despite the fact that large (ug/g) levels of 3-CSAL were present in the blood and brain, neither SAL, 7-M-SAL, nor 6-M-SAL was seen. Based on the electrochemical responses (and corresponding limits of sensitivity), SAL had to constitute <0.05% of the 3-CSAL (10.17 nMoles/g) found in the striatum, or <0.02% of the injected THIQ (20.77 nMoles/g) present in the hypothalamus if it were to be present at analysis. The blood contained 5.48 nMoles/ml 3-CSAL. Neither SAL nor O-methylated SALs were seen in the blood. Based upon the 5.48 nMole/ml level of 3-CSAL, <0.09% of the 3-CSAL was decarboxylated peripherally in animals given a 100 mg/kg i.p. dose of 3-CSAL for 30 minutes. SAL was not apparent in heart or liver tissues analyzed even though these tissues are rich in decarboxylases.

Collins and Weiner (30) previously noted that 3-CSAL was not an inhibitor of DDC *in vitro* further indicating that 3-CSAL is a poor substrate for this enzyme in rats. Relatively high levels of 6-M-3-CSAL and 7-M-3-CSAL were seen in the brain tissue but no 6-M-SAL or 7-M-SAL were detected there above the limits of sensitivity described above. It is probable then that the O-methylated-3-CSALs are also poor substrates for decarboxylases. Similiar to the 3-CSALs, alpha-methyl-para-tyrosine is not decarboxylated *in vivo*. In contrast, l-dopa analogues such as alpha-MD and AMMT are readily decarboxylated non-oxidatively *in vivo* (40,41,42,43). Also 3-CSAL was not decarboxylated by the enzyme which decarboxylates pipecolic acid to piperidine (44,45).

THIQs carboxylated at the 1-position can be oxidatively decarboxylated *in vitro* to their corresponding 3,4-dihydroisoquinolines by anodic oxidation at low electrochemical potentials (82), prolonged treatment in neutral aqueous media (83), and by the plant enzymes laccase and peroxidase (84). Origitano found <57 pMoles of 1,2-dihydro-SAL but large amounts of 1-CSAL present in the brains of rats chronically treated with 1-CSAL (91). Rapid O-methylation and conjugation of the carboxylated THIQs might be expected to block the oxidative decarboxylation.

B. Is it possible that 3-CSAL is metabolized beyond O-methylation?

O-methylation and decarboxylation were the two major routes of metabolism of the carboxylated THIQs which were addressed in this dissertation. It is possible that other routes of metabolism exist for the carboxylated THIQs. Trimetoquinol is a catechol THIQ with known bronchodilation activity(85). Only 11.5% of trimetaquinol is excreted as the free drug; 27.5% is excreted as O-methylated trimetaquinol; and 61% is excreted as the glucuronide conjugate. Sulfate conjugation of biogenic amines and their metabolites is also an important pathway of metabolism in brain.

N-oxidation is another metabolic alternative. Various drugs such as pentobarbital and ethanol are known to induce hepatic microsomal oxidative systems. Dajani and Saheb (16) found that varying metabolic conditions could favor oxidation of beta-carbolines both in vitro and in vivo. The products were N-oxides. It is possible therefore that an alternate pathway of THIQ metabolism could be the formation of N-oxides and fully aromatic compounds. Hamilton and Gause (86) have suggested the formation of N-oxides from SAL and DesmethylSAL after incubation in mild alkaline conditions.

C. Lack of in vivo stereospecificity for O-methylation

of 3-CSAL by COMT.

Endogenous catecholamines have been shown to be stereospecific substrates for COMT. Catecholic THIQs have been shown to be inhibitors of COMT *in vitro* (87) and *in vivo* and themselves are O-methylated (88,89,90). Studies with purified rat liver COMT have monitored the stereospecific O-methylation of DesmethylSAL and its N-methyl analog (89), and THP (32). Consistently, the compounds were predominantly O-methylated at the 7-position. Bail et al (89) found that SAL and NorSAL, given centrally to rats, were predominantly O-methylated at the 7-position. Origitano et al (91) found that SAL and 1-CSAL (both DA derived catecholic THIQs) were stereo-specifically O-methylated at the 7- (para) position. This is in contrast to DA which is specifically O-methylated meta-to the side chain.

L-dopa, the parent amine of 3-CSAL is stereospecifically O-methylated at the hydroxyl group meta- to the side chain *in vivo* (87). 3-CSAL is however non-specifically O-methylated. Equal amounts of 6-M- and 7-M-3-CSAL were found in the serum of animals given a dose of 3-CSAL. Origitano deduced that a hydrophobic region may be present in the active site of COMT which prevents random binding of substrates (91). Charged groups on substrate molecules are directed away from the hydrophobic centers giving a stereos-

pecific orientation. The presence of the carboxylic acid moiety at the 3-position in 3-CSAL provides an equal opportunity for O-methylation at either the hydroxyl group meta- or para- to the side chain.

D. The levels of 6-M-3-CSAL were consistently higher than 7-M-3-CSAL in tissue samples.

The levels of the two O-methylated isomers of 3-CSAL were essentially the same in the serum samples analyzed. When a single dose of 3-CSAL was followed over a 24 hour period of time, there was no difference in content of the two isomers at the 30 minute time point. However, significantly more 6-M-3-CSAL was present in the brain than 7-M-3-CSAL at both the 1 and 3 hour time points ($p<0.01$). At the 30 minute time point the liver 6-M-3-CSAL content was more than twice the 7-M-3-CSAL content ($p<0.001$). The one hour 6-M-3-CSAL content was non-significantly greater than the 7-M-3-CSAL content. At the 3 hour time point the 6-M-3-CSAL levels were non-statistically greater than the 7-M-isomer. The 8 and 24 hour liver levels of 6-M-isomer were significantly greater than the 7-M-isomer. In heart tissue, the 30 minute, 3, 8, and 24 hour levels of the two isomers were not statistically different. However at 1 hour,

there was 73% more 6-M-3-CSAL than 7-M-3-CSAL ($p<0.01$). One hour after a 50 mg/kg i.p. dose of 3-CSAL hypothalamic 6-M-3-CSAL was 24% greater than the 7-M-3-CSAL content.

There are several possibilities for these results. One is that the 6-M-isomer is formed more easily than the 7-M-isomer. A second explanation could be significantly better storage of 6-M-3-CSAL than the 7-isomer in nerve terminals. A related third explanation is that either conjugation, N-oxidation and/or exodus of the 7-isomer is much faster in these tissues.

E. The effects of the THIQs: neurotransmitter content and turnover in the tissues analyzed.

The presence of the THIQs in the different brain regions, and the neurochemical effects seen must be viewed in light of the type of innervation that the tissue receives, and the relative turnover of the respective biogenic amine neurotransmitters. Smith et al. (92) ranked seven brain regions with respect to neurotransmitter content and turnover. The seven brain regions examined were the cerebral cortex, diencephalon (which contains the hypothalamus), hippocampus, mesencephalon, pons-medulla, striatum, and telencephalon. A summary of their work is provided in

Table 34. With a rank of 1 being highest and 7 being lowest, we can use the ranking system of Smith et al. to rate the tissues analyzed in this dissertation.

The striatum has the highest DA content and the fastest DA turnover; although the striatum ranked sixth in 5-HT content, the turnover of 5-HT was ranked second. The hypothalamus ranked fourth in both DA content and turnover; hypothalamic 5-HT ranked second in content, but turnover of 5-HT was ranked seventh (slowest). The hippocampus was ranked seventh in content and sixth in turnover for both DA and 5-HT. However, the effect of 3-CSAL does not seem to be attributable to increased synthesis since 5-HTP did not accumulate above control levels when its decarboxylation was inhibited.

Table 34

A SUMMARY OF RANK OF SEVEN BRAIN TISSUES BY BOTH NEUROTRANSMITTER CONTENT AND TURNOVER

TISSUE	SEROTONIN CONTENT	SEROTONIN TURNOVER	DOPAMINE CONTENT	DOPAMINE TURNOVER
CEREBRAL-CORTEX	5	5	3	3
DIENCEPHALON	2	7	4	4
HIPPOCAMPUS	7	6	7	6(a)
MESENCEPHALON	3	1	6	6(a)
PONS-MEDULLA	1	4	5	5
STRIATUM	6	2(a)	1	1
TELENCEPHALON	4	2(a)	2	2

Rank of 1=Highest; Rank of 7=Lowest

(a)=tie between the turnover rates for these two tissues.

Adapted from Smith et al. Prog. Neuro-Psychopharm. 2: 359-367 (1978).

F. Acute 3-CSAL elevates striatal 5-HT without consistently lowering 5-HIAA.

Acute experiments with 3-CSAL have demonstrated a rather specific effect upon the serotonin content of the corpus striatum. Serotonin and 5-HIAA levels in the hypothalamus and hippocampus were unaffected at the one hour time point despite the fact that 3-CSAL and its O-methylated metabolites were present in these tissues. The effects of a single 50 mg/kg dose of 3-CSAL was assessed at the time points of 30 minutes, 1, 3, 8, and 24 hours. Fig 22 shows the percent relative concentrations of 5-HT and 5-HIAA at these time points. At the 30 minute time point, 5-HT was significantly elevated whereas 5-HIAA was unaffected. This would be consonant with increased synthesis of 5-HT. At the one hour time point, both 5-HT and 5-HIAA were significantly elevated, but synthesis exceeded catabolism, as one might see in the case of increased turnover. At three hours after the dose was administered, 5-HT was further elevated but 5-HIAA was not different from the control levels. By 24 hours of time, the levels of both 5-HT and 5-HIAA were returned to the normal range.

The eight hour maximal effect upon striatal 5-HT does not correlate with the levels of the 3-CSAL, which is nearly undetectable at this time. This fact raises the possibility

that unanalyzed metabolites of 3-CSAL were present and affected the 5-HT system.

Quipazine is a drug which increases whole brain 5-HT. Medon et al (93) found that quipazine was a potent blocker of 5-HT uptake. Jacoby et al (94) saw that quipazine increased whole brain 5-HT levels while significantly decreasing 5-HIAA by 35% one hour post a 10 mg/kg injection. Regionally, the striatum was the least sensitive to quipazine effects whereas the greatest activity was seen in the hypothalamus. This is the converse of 3-CSAL's regional effects. Fuller et al (95) documented the effects of quipazine on serotonin metabolism in rat brain. Quipazine decreased 5-hydroxytryptophan accumulation when the total aromatic amino acid decarboxylase inhibitor NSD-1015 was given. Thus the increase in 5-HT was not due to MAO inhibition but rather by the fact that quipazine acts as a serotonin receptor agonist. The results of the synthesis experiment suggests that 3-CSAL acts in the same way.

Ho et al (96) investigated the mechanism of serotonin elevation by 6-methoxy-THBC. This compound exerted no effect upon mouse brain tryptophan hydroxylase. 6-methoxy-THBC did, however, produce a significant facilitation of labeled 5-HTP uptake into mouse brain. In another publication, Ho et al (97) stated that 6-methoxy-THBC did not affect norepineph-

rine levels but did alter 5-HT levels. Since NE was unchanged, they speculated that the 5-HT increase was not due to the inhibition of MAO activity. Subcellular fractionation studies in mouse brain showed a significant increase in the particulate fraction of "bound" 5-HT. The 6-methoxy-THBC slowed the disappearance of intracisternally injected labeled 5-HT, but had no effect upon the levels of 5-HIAA. Increased binding of 5-HT was suggested as a mechanism for the effect of 6-methoxy-THBC's "protection" of brain 5-HT. Shields and Eccleston (98) have offered evidence for the synthesis and storage of 5-HT in two separate pools in the brain. In their experiment, animals were given labeled tryptophan followed by LSD, which decreases the firing rate of 5-HT neurons. They found that the conversion of labeled tryptophan to labeled 5-HT was changed, but the overall rate of 5-HT formation was not changed. 5-HT formed from tryptophan was called the functional pool of 5-HT, whereas the other 5-HT formed was called the reserve pool of 5-HT. When DDC was totally inhibited, 3-CSAL still elevated 5-HT. Thus if increased synthesis was the source of the elevated 5-HT, the 5-HT must be coming from the "reserve pool". It is speculated that perhaps 3-CSAL increases the bound fraction of 5-HT, protecting the neurotransmitter from metabolism. However, further studies would be necessary to answer this question.

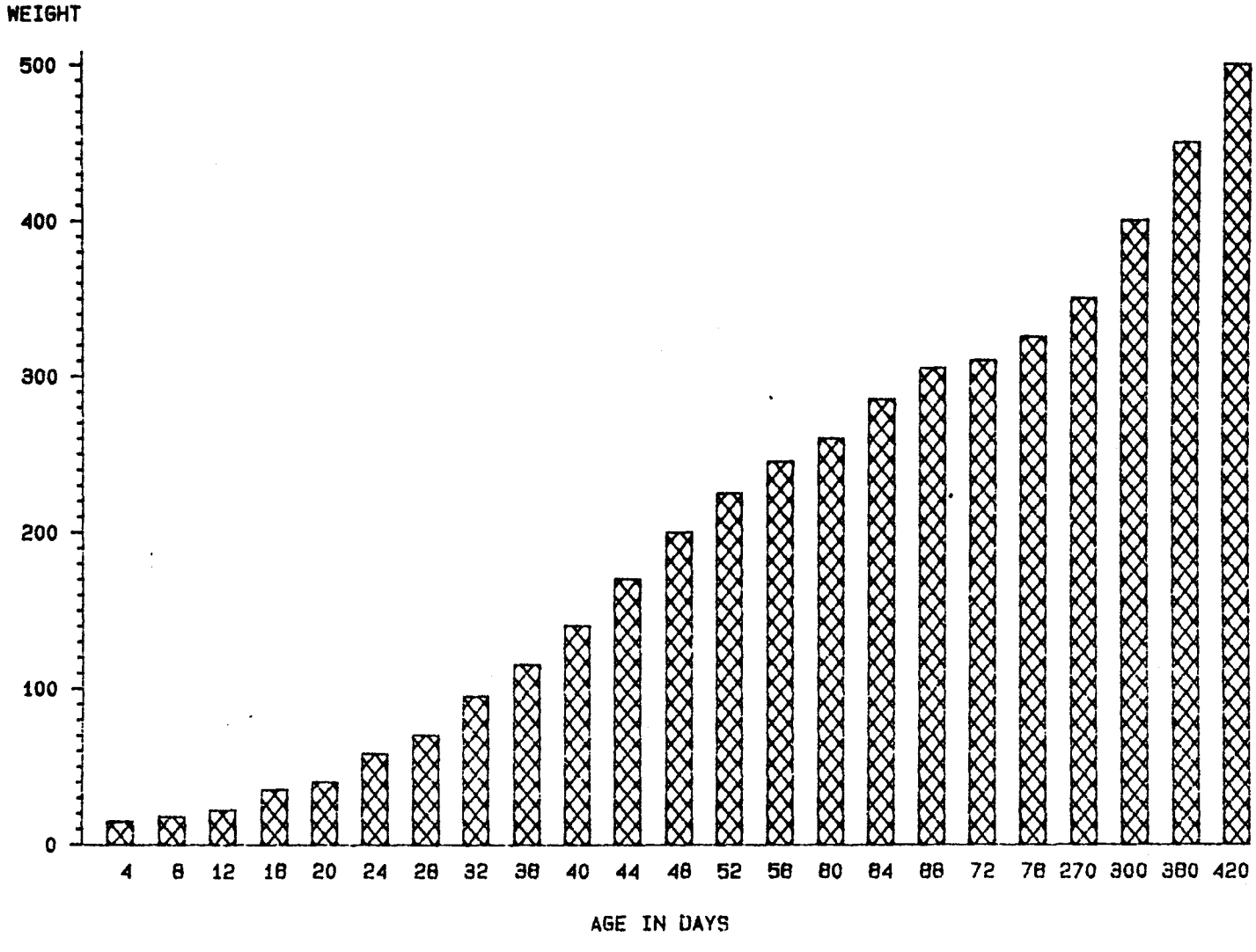
G. The relationship of age to the effect of 3-CSAL on 5-HT.

The weight gain with respect to age for Sprague-Dawley rats on normal rations is shown in Figure 23. By 35 days of age the rats are fertile. At King Animal Laboratories (Madison, Wisc.) breeding begins at 42 days of age (6 weeks). The typical weights for these animals are 125-150g for the females and 150-175g for the males. More success in breeding is seen in 63 day old rats (9 weeks). Typically, these females weigh 175-200g and the males weigh 225-250g.

Proven breeders are animals which have raised two consecutive litters to the age of weaning. However if an animal fails to continue to produce litters it becomes a retired breeder; typically females are retired by King by 6-8 months of age; males are retired at 8-10 months of age. By 9 months of age the estrous cycle in the female becomes prolonged and with further aging the animal becomes anestrous. Considering the fact that the life expectancy of a rat can be up to 36 months, retired breeders have only lived 25% of their life expectancy and yet they have clearly aged. The CNS of the rat does not achieve developmental maturation until 2 months of age (99). The levels of dopamine, dopamine sensitive adenyl cyclase, synaptosomal dopamine uptake and tyrosine hydroxylase are stable between 2-12 months of age.

Figure 23: The relationship of age to gram weight in male Sprague-Dawley rats.

THE RELATIONSHIP OF AGE TO GRAM WEIGHT IN MALE SPRAGUE-DAWLEY RATS



After this time dopamine levels, receptors, tyrosine hydroxylase levels, and dopamine sensitive adenyl cyclase levels decline.

Vasko et al. (100) have measured steady state levels of brain acetylcholine in young (28 days), adult (90 days), and old (24 months) male Holtzman rats. Despite the fact that there was no difference in steady state brain control levels of acetylcholine, Vasko found marked changes in brain acetylcholine in response to d-amphetamine in young and old rats, but not in the adults. Timiras (101) found that choline acetyltransferase activity does not change markedly from 2-12 months in rat cerebellum and cerebral hemispheres. But choline acetyltransferase activity in the spinal cord decreased significantly with age.

Both young and mature male Sprague-Dawley rats showed a significant increase in striatal 5-HT 8 hours after 3-CSAL was administered. However this effect was not seen in older rats. It is possible to speculate that the differences between groups could be related either to a loss of neurons due to aging, a reduced regional blood flow, an age related altering of metabolic pathways, or to altered 5-HT turnover. Marquis et al (102) have demonstrated dopamine receptor alterations in aging mouse and rat striatum. Cubells and Joseph (103) also have shown an age related DA receptor loss

in rats. Samorajski (104) has demonstrated decreased striatal DA synthesis in older rats. Synaptosomal DA uptake is reduced in hypothalamic and striatal synaptosomes of aged mice (105). Striatal adenyl cyclase activity is significantly lower in aged mice (106,107) and rabbits (108). Also dopamine content of the human striatum is significantly decreased in older subjects (109,110). It is not clear whether the DA neurons per se or their metabolic abilities are required for 3-CSAL's effect upon striatal 5-HT.

Ohata et al (111) have shown that there is no significant difference in striatal or hypothalamic blood flow in rats at the ages investigated. Therefore the difference in 5-HT effect seen is not related to perfusion. It is possible that relative competency of peripheral drug metabolism by the liver could account for the change in effect with respect to age. For example, Macklon et al (112) have shown that older patients are not able to metabolize diazepam as quickly as younger adults. Also, the lean mass or relative percent body fat could be different between groups. With more body fat, a fat soluble drug may not realize the same brain drug levels as a lean animal would. The exact reason for the lack of an 8 hour effect of acute 3-CSAL is not readily clear, but it is reproducible.

H. Chronic 3-CSAL reduces 5-HT in both the striatum and the hypothalamus.

The acute studies consistently demonstrated an increase in striatal 5-HT levels. However, chronic 3-CSAL administration significantly depleted striatal 5-HT to 48% of the control values. Tryptophan, the precursor amino acid for 5-HT, was significantly elevated in the striatal tissue of the experimental animals. However, striatal 5-HIAA levels were not significantly different between the groups.

Chronic 3-CSAL also produced a significant depletion of 5-HT in the hypothalamus. Hypothalamic tryptophan was not significantly different between groups but 5-HIAA was significantly depleted. The fact that a hypothalamic effect was seen chronically but not at the one hour time point could well be related to the fact that 5-HT turnover is very slow in the hypothalamus.

The mechanism of the 5-HT depletion by chronic 3-CSAL is unclear as are the effects of other THIQs upon the serotonergic system which have been reported in the literature. Livrea (113) saw a paradoxical change in the serotonergic system's response to THP when acutely or chronically administered. When THP was given acutely at a dose of 60 mg/kg, 2 hours post injection 5-HIAA was significantly elevated. This elevation was short lived in that 5-HIAA levels returned to

normal at 4 hours post injection and remained so when further checked at 6 and 12 hours post injection. Chronic THP (8 mg/kg) significantly lowered 5-HIAA levels. Livrea concluded that this was a result of decreased turnover. Another explanation however is that this decrease could have resulted from a toxic effect upon serotonergic neurons. Similar to the neurotoxicity of 3-CSAL is the neurotoxicity of halogenated phenethylamines and amphetamines. Chronic doses of these drugs cause marked depletion of 5-HT(114-121).

It is unknown whether or not 3-CSAL is taken up into serotonin nerve endings. It is known however that the depletion of 5-HT by l-Dopa is independent of uptake into 5-HT neurons by the serotonin pump (122). One approach to answer this question is to lesion the 5-HT system of rats with 5,7-dihydroxytryptamine followed by 3-CSAL administration. The resultant levels and disappearance of 3-CSAL and its mono-O-methyl metabolites could be measured in all 7 of the brain regions described by Smith et al and compared to control levels. A low accumulation of 3-CSAL in 5,7-dihydroxytryptamine lesioned animals may indicate the necessity of uptake of the THIQs into the serotonergic nerve endings for the effects upon the serotonergic system to be seen. When followed by experiments which block uptake into the serotonergic system by the serotonin pump, it would be

possible to define if 3-CSAL uptake into serotonergic neurons is necessary for the increase of 5-HT to be seen.

I. 3-CSAL after pretreatment with decarboxylase inhibitors elevates 5-HT and 5-HIAA is unchanged or lowered.

When a peripheral DDC inhibitor was given prior to 3-CSAL, striatal 5-HT was elevated 3 fold compared to controls whereas 5-HIAA levels were unchanged. For both the hippocampus and hypothalamus neither 5-HT nor 5-HIAA levels were altered despite the fact that 3-CSAL and its mono-O-methyl metabolites were present in these tissues. It is not yet known if 5-HT content in these brain regions would be affected at other time points. It is known however that of the tissues analyzed, 5-HT turnover is fastest in the caudate.

Also, animals pretreated with the total decarboxylase inhibitor followed by 3-CSAL had significantly elevated 5-HT levels and lower 5-HIAA levels in the striatum. Hypothalamic 5-HT was elevated with this treatment, but 5-HIAA levels were decreased. Neither 5-HT nor 5-HIAA levels were altered in the hippocampus. In none of the experiments has there been a hippocampal 5-HT effect although 3-CSAL levels are

detected in this tissue. The increases in 5-HT by 3-CSAL seen when decarboxylase inhibitors were given could be due to MAO inhibition, activity as a serotonin agonist, or facilitated 5-HT turnover and uptake. The increases in serotonin seen were similiar to the MAO inhibition by beta-carbolines described by Ho et al (97). Meyerson et al (123) demonstrated that MAO A, the 5-HT specific oxidase, was more sensitive to inhibition by isoquinolines than was MAO B. The hypothalamic changes seen with the total decarboxylase inhibitor resemble MAO inhibition. However, other experiments indicate that 3-CSAL does not inhibit MAO in vivo (Dr. Collins; personal communication). Thus there is a synergistic action of NSD-1015 and 3-CSAL which resembles MAO inhibition.

J. Other carboxylated THIQs studied also alter brain serotonin content.

Acute 1-CSAL i.p. significantly elevated both 5-HT and 5-HIAA in the striatum. In another brain area, the hypothalamus, however, 1-CSAL appeared to increase 5-HT turnover at one hour, as suggested by the elevated 5-HIAA levels with no change in 5-HT levels. Origitano (91) found similiar results in the hypothalamus when 1-CSAL was given intraventricularly.

It is of interest to note that the 5-HT and 5-HIAA levels were increased in the striatum for both of the geometric isomers of carboxylated-SAL. This could indicate a similiar mechanism of action in the striatum. The 3-carboxy isomer however achieved significantly higher brain levels than did the 1-carboxy isomer. The hypothalamic effect of 1-CSAL was dissimiliar to the 3-carboxy isomer. In none of the experiments with 3-CSAL as the sole drug given was 5-HIAA elevated in the hypothalamus.

There was no effect of 1-CSAL upon hippocampal 5-HT or 5-HIAA levels at the time point examined despite the fact that tissue levels of 1-CSAL were seen. In contrast, when the 1-CSAL was given intraventricularly, (Origitano (91)), an increase in hippocampal 5-HIAA was seen. The differences in these results may lie in the dose and route of administration. Another explanation for the increased hippocampal 5-HIAA could be due to displacement of 5-HT stores. Uptake and release of THIQs have been described by Cohen et al (124), but they did not examine carboxylated THIQs.

Peripherally administered 1-carboxy-THP significantly elevated 5-HT and 5-HIAA in the striatum. The hippocampal and hypothalamic 5-HT and 5-HIAA content were unaffected. The levels of 1-carboxy-THP were the highest in the hypothalamus and yet there was no serotonergic effect in that tissue at this one hour time point.

K. 3-CSAL does not alter DA levels.

Acute 3-CSAL treatment had no effect upon striatal DA or hypothalamic DA or NE content. Increasing the dosage of 3-CSAL also had no effect upon striatal DA content, furthermore chronic exposure to 3-CSAL (5 days) had no effect upon striatal DA or hypothalamic NE or DA.

When the acid metabolites of DA (HVA and DOPAC) were analyzed, it could be seen that DA turnover was probably increased by acutely administered 3-CSAL. The data from the single-dose/time course experiment revealed that the HVA levels were significantly lowered at the 30 minute time point. This was probably due to the fact that 3-CSAL was competing with DOPAC or DA for the enzyme COMT. At the one and three hour time points HVA levels had returned to normal, suggesting either that 3-CSAL was mostly O-methylated by this time or that some compensatory mechanism was in effect. At the eight hour time point, the HVA levels were significantly elevated over the control levels. Remembering that DA levels were not different than those for the controls, one can conclude that DA turnover has been accelerated. HVA levels were further elevated at the 24 hour time point, which suggests that dopaminergic activity was still elevated despite the fact that neither 3-CSAL nor its O-methylated metabolites were detected in the tissues at the 24

hour time point. Again, the possibility that other metabolites of 3-CSAL were active at these time points must be considered.

The striatal DOPAC levels were not significantly different for the controls at the 30 minute time point. Non-statistical increases in DOPAC levels were seen at the one and three hour time points. A statistically significant elevation in DOPAC levels were seen at the 8 and 24 hour time points. These results concur with the theory of increased dopaminergic activity in the corpus striatum. More precise information could be gained using radiolabeled l-dopa to follow the turnover of DA when 3-CSAL is given.

L. 3-CSAL and DDC inhibitors lower DA levels in rats.

Striatal DA was significantly depleted in 3-CSAL treated animals pretreated with RO4-4602 such that peripheral DDC was inhibited. In this experiment striatal DOPAC levels were significantly decreased when compared to controls. However HVA levels were no different between groups. These results suggest that DA turnover was not increased by 3-CSAL.

When 3-CSAL was preceded by total decarboxylase inhibiton by NSD-1015, DA levels also were significantly

depleted. This drug combination produced striatal DOPAC levels which were significantly lower compared to the controls.

Thus 3-CSAL produced lower DOPAC levels when DDC was either peripherally or totally inhibited. The decarboxylase inhibitors prevented the replenishment of DA and subsequently its acid metabolites.

No 3-CSAL hypothalamic effect upon the dopaminergic system was seen in animals pretreated with peripheral DDC inhibitor, but central DDC inhibition in tandem with 3-CSAL did produce a hypothalamic dopaminergic effect; there was no decrease in dopamine levels, but DOPAC levels were significantly elevated for the experimental group. These findings suggest an increased DA turnover as a real effect of 3-CSAL. However, the possibility of increased turnover requires verification by an alternate method.

M. Other carboxylated THIQs and DA levels.

1-CSAL affected the striatal dopaminergic system, as seen by statistically increased HVA levels in the experimental group. There was, however, no difference in striatal DA and DOPAC levels between the experimental and control

groups. Thus in the striatum either turnover was increased or 1-CSAL competed with HVA for egress from the system. Origitano (91) found that 1-CSAL intraventricularly had increased striatal HVA and DOPAC but had no effect on DA. The lack of agreement between these results must be viewed in light of the differences in dose and route of administration.

The hypothalamic dopaminergic system was affected by 1-CSAL in a different way. Hypothalamic dopamine as well as DOPAC was significantly elevated when compared to controls. HVA levels however were not altered. Thus it appeared that peripheral 1-CSAL increased the synthesis and turnover of DA. Central 1-CSAL (91) had no effect on DA or HVA and significantly decreased hypothalamic DOPAC content. Again, it is interesting to note the differences in results from the central versus peripheral administration

In the striatum DA levels were unaffected by acute 1-carboxy-THP; DOPAC levels also were unchanged; only striatal HVA was elevated, Again, this was either due to an increased DA turnover or a decreased egression of HVA. Whereas 1-CSAL had an effect upon the hypothalamic dopaminergic system, 1-carboxy-THP had no effect upon any of the components of the hypothalamic dopaminergic system assayed.

The effects of the carboxylated THIQs on DA systems can be compared to the effects of non-carboxylated THIQs. When Livrea et al (113) administered THP acutely, levels of HVA in rat brain first increased at 2 hours post dose, decreased by 4 hours, and returned to normal at 6 to 12 hours. The acute changes were explained as a DA displacement and HVA elevation followed by DA and HVA depletion. This was subsequently followed by recovery. When THP was given chronically, the Livrea group saw that HVA levels 12 hours after the final dose were no different than that for the control groups. Awazi and Guldberg (125) reported that striatal DA levels were unaffected 3 hours after a centrally administered 10 ug dose of THP. Paradoxically, THP doses of 70 to 250 ug resulted in lower DA levels.

In separate experiments SAL and THP have been given intraventricularly to rats in "physiological doses" acutely (126). Low doses of SAL (40-200 ng) produced no significant change in catecholamine levels in the pons or striatum.

Awazi and Guldberg (125) found that after a 250 ug dose of SAL striatal DA was elevated 5 to 6 hours later, and diencephalic NE content was reduced significantly. Haloperidol, the DA receptor antagonist blocked the SAL induced changes in the DA contents. Thus this THIQ and others may well interact with DA receptors.

It appears that all of the THIQs investigated affect the turnover of DA. It would be of great interest to see in future experiments if haloperidol or other DA receptor antagonists block the effects of the carboxylated THIQs reported in this dissertation.

N. Central interactions between dopaminergic and serotonergic systems.

Since 3-CSAL has been demonstrated to affect both dopamine turnover and 5-HT levels in the corpus striatum, it is fitting to speculate that these facts may be related. Researchers have demonstrated interactions between the dopaminergic and serotonergic systems of the central nervous system. For example, 5-HT is involved in the l-dopa induced cortical synchronization in the rabbit (127). Unilateral nigrostriatal lesions in rats followed by apomorphine produces contralateral turning responses and stereo-typic gnawing (128). The rate of contralateral turning was significantly decreased by pretreatment with 5-HTP. The effectiveness of apomorphine induced turning was enhanced by depletion of 5-HT by p-chlorophenylalanine or by blockade of 5-HT receptors by methysergide.

Serotonin appears to diminish many aspects of behavioral arousal. When 5-HT is depleted by p-chloro-phenylalanine locomotion in response to l-dopa is increased (129). Green (130) has seen an enhancement of amphetamine action after interruption of ascending serotonergic pathways by electrolytic lesions. The inhibitory function of 5-HT is reinforced by the fact that the threshold dosage of amphetamine required to elicit stereotypic behavior in the guinea pig are decreased by methysergide and increased by 5-HTP. Also, 5-HTP reverses abnormal jaw, mouth and other movements produced by l-dopa in monkeys with tegmental lesions (132). Amphetamine induced locomotion and stereotypy are significantly elevated after p-chlorophenylalanine pretreatment of rats (133). The l-dopa induced emission of seminal fluid has been shown to be mediated through 5-HT mechanisms (134).

O. Interactions between DDC inhibitors and 3-CSAL

Both peripheral and total decarboxylase inhibition followed by 3-CSAL resulted in significantly elevated striatal 5-HT and significantly depleted striatal DA. The common factor between NSD-1015 and RO4-4602 is that the both inhibit peripheral decarboxylation. This leads to elevated serum levels of l-dopa and tryptophan. If peripheral decar-

boxylase inhibition results in decreased striatal levels of tyrosine then a mild inhibitor of tyrosine hydroxylase could significantly reduce dopamine levels.

P. Behavioral effects of 3-CSAL.

Known behavioral effects of 3-CSAL are analgesia (38) and potentiation of ethanol induced narcosis (36). It is a distinct possibility that these effects are mediated through 3-CSAL's effect upon 5-HT. Fibiger et al. (135) have shown that animals pretreated with the tryptophan hydroxylase inhibitor p-chloro-phenylalanine were more reactive to electro-shock. This sensitivity could be reversed by injecting the animals with 5-HTP. Fluoxitine, which inhibits the serotonin-amine pump, has been shown to produce analgesia in rats receiving electro-shock (136). Fluoxitine also reverses the p-chloro-phenylalanine induced hyperalgia. Quipazine, which is a serotonin receptor agonist produces analgesia in rats tested by the hot plate method (137).

The dorsal raphe nucleus sends 5-HT projections to the pontine recticular formation. Jouvet (138,139) has provided evidence that the serotonergic system is involved in the sleep process. Elevating 5-HT in the pontine recticular formation prolongs deep sleep. The work of this dissertation

has shown 5-HT to be elevated in the striatum, one could speculate then that the potentiation of ethanol induced narcosis by 3-CSAL could be through elevated pontine recticular 5-HT.

CHAPTER VI

SUMMARY

3-CSAL was used as a test alkaloid to see if carboxylated THIQs are decarboxylated *in vivo* in a rat model. When given intraperitoneally, large amounts of 3-CSAL and its mono-O-methyl metabolites were taken up into the brain. In contrast, SAL itself when given i.p. does not enter the brain to any appreciable extent due to the blood brain barrier(39). No appreciable decarboxylation of 3-CSAL or its mono-O-methyl metabolites to THIQs was seen in any of the tissues analyzed (<1%). The oxidative decarboxylation of 3-CSAL to DHIQs was also found to be negligable (<1%).

In contrast to other THIQs such as SAL, 1-CSAL, and THP which are stereospecifically O-methylated, 3-CSAL was mono-O-methylated to an equivalent extent on both available hydroxyls of the ring. Despite the fact that serum levels of the two O-methylated isomers of 3-CSAL were essentially the same, tissue levels of the 6-M-isomer were consistently higher than the 7-M-isomer. It is speculated that the 7-M-isomer has a meta- hydroxyl available for conjugation or sulfation which would lead to its more rapid clearance. The 6-M-isomer has the 7-hydroxyl remaining, but this site may not be preferred for these reactions.

Levels of THIQs were highest when no inhibitor of DDC were given. It is possible that l-dopa and 5-HTP which accumulate with the decarboxylase pretreatment compete with 3-CSAL and its mono-O-methylated metabolites for uptake into the brain. Levels of the THIQs were consistently highest in the hypothalamus which has the greatest serotonergic innervation of any of the tissues analyzed. It is suggested that carboxylated THIQs may be preferentially taken up into serotonergic neurons.

Summaries of the effects of 3-CSAL upon the dopaminergic system and serotonergic systems are provided in Tables 35 and 36 respectively. These two tables state the changes in the levels of the particular neurotransmitters and their acid metabolite(s) with respect to the various experimental conditions examined. Increases are depicted by the upward arrow; decreases by the downward arrow; and no significant difference by the horizontal arrow.

Levels of DA were relatively unaffected by carboxylated-THIQs unless animals were pretreated with decarboxylase inhibitors. Turnover of DA appears to be increased by carboxylated-THIQs in certain brain regions, but the significance of the mild dopaminergic effect is not understood at this time.

Table 35

A SUMMARY OF CARBOXYLATED-THIQs EFFECTS UPON THE STRIATAL DOPAMINERGIC SYSTEM

CONDITION	DA	DOPAC	HVA
ACUTE 3-CSAL			
30 Min.	→	→	↓
1 Hr.	→	→	→
3 Hr.	→	→	→
8 Hr.	→	↑	↑
24 Hr.	→	↑	↑
RO4-4602 and Acute 3-CSAL	↓	↓	→
NSD-1015 and Acute 3-CSAL	↓	↓	↓
Chronic 3-CSAL	→		
1-CSAL	→	→	↑
1-Carboxy-THP	→	→	↑

Table 36

A SUMMARY OF CARBOXYLATED-THIQs EFFECTS

UPON STRIATAL 5-HT and 5-HIAA

CONDITION	5-HT	5-HIAA
ACUTE 3-CSAL		
30 Min.	↑	→
1 Hr.	↑	↑
3 Hr.	↑	→
8 Hr.	↑	↑
24 Hr.	→	↑
RO4-4602 and Acute 3-CSAL	↑	→
NSD-1015 and Acute 3-CSAL	↑	↓
Chronic 3-CSAL	↓	→
1-CSAL	↑	↑
1-Carboxy-THP	↑	↑

Despite the fact that the alkaloids are structurally related to dopamine the carboxylated THIQs examined here have their most prominent effects on the serotonergic system. The precise mechanisms underlying the elevations are not certain. 3-CSAL is not a good inhibitor of MAO A but it could be acting as a serotonin agonist or could be causing increases in the amount of "bound" 5-HT.

Nevertheless, chronic 3-CSAL depletes the 5-HT content of both the hypothalamic and striatal tissues of rats. The nature of this deficit is unknown. It is possible that this is a neurotoxic effect. In support of this theory, Druse-Manteuffel et at (140) have found that repeated injections of DNLCA (the 1-carboxy-THIQ condensation product of DA and phenylpyruvic acid), significantly reduced the striatal 5-HT content of rat pups. More experiments need to be designed to ascertain the mechanism of the 5-HT depletion and to determine if there is indeed a toxic effect of carboxylated-THIQs on the serotonergic system.

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