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Alteration of Eupnea and Apneusis Reversal Induced by Constant Intravenous Morphine Sulfate Infusion in Decerebrate Cats

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ALTERATION OF EUPNEA AND APNEUSIS REVERSAL

INDUCED BY CONSTANT INTRAVENOUS MORPHINE SULFATE INFUSION

IN DECEREBRATE CATS

by

Andrea M. Zardetto-Smith

A Thesis Submitted to the Faculty

of the Graduate School

of Loyola University of Chicago

in Partial Fulfillment

of the Requirements for the Degree of

Master of Science

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1983

DEDICATION

This thesis is dedicated to Noni and Bob, for the love and caring you both have given me. I would also like to dedicate this thesis to Mr. Z., who opened up the world of biology for me and a hundred others through his unique style of teaching.

ACKNOWLEDGEMENTS

BIOGRAPHY

The authoress, Andrea Marie Zardetto-Smith, was born October 25, 1956, in Passaic, New Jersey.

Her elementary education was obtained in the public schools of Clifton, New Jersey, and secondary education at Clifton High School, Clifton, New Jersey, where she graduated in 1974.

In September 1974, she entered the College of St. Elizabeth, and in May 1978 received the degree of Bachelor of Science (biology), graduating Summa Cum Laude. While attending the College of St. Elizabeth, she was elected chapter vive-president of Beta Beta Beta National Biological Honor Society. In May 1978 she was elected to Who's Who Among Students in American Universities and Colleges, Kappa Gamma Pi (The National Women's Leadership and Scholastic Honor Society of Catholic Colleges), and selected as an Outstanding Young Woman of America.

In October 1978 she began studies in physiology at Loyola University. Currently she is employed as a Research Biologist at G. D. Searle Co., Skokie, Illinois.

Her husband, Robert L. Smith, whom she married in January 1978, is employed as a civil engineer.

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CHAPTER I

INTRODUCTION

Morphine has been characterized as possessing respiratory depressant qualities since the first studies of Gscheidlen in 1869 (see 7). Studies in the ensuing years have supported the finding that the action of morphine on the respiratory controller is depressive in nature, but upon close scrutiny, many of them prove to be ancedotal in nature. In almost all, morphine is administered via bolus injection, the disadvantage of which is the initial respiratory and circulatory depression that occurs which may be severe enough to compromise the physiological state of the preparation throughout the remainder of the experiment (6). The administration of anesthesia may affect respiratory related activity in several ways, a factor ignored in the majority of studies. Lastly, though a few studies have alluded to the ability of morphine to attenuate the abnormal ventilatory pattern of apneusis (4,31), the advantages of such a model in studying the respiratory effects of morphine have not been exploited. In light of the foregoing considerations, the present study was undertaken to study the alterations of two respiratory patterns, eupnea and apneusis, induced by constant intravenous infusion of morphine in the decerebrate cat.

CHAPTER II

LITERATURE REVIEW

A. Respiratory Effects of Morphine and Related Substances

Cushny (7), in his first studies of the action of various pharmacological agents on the respiratory center, cited Gscheidlen as being the first experimental investigator to describe the depressant action of morphine on respiration. Though Gscheidlen attributed the decrease in ventilation as being due to a decrease in the frequency of breathing, other researchers of the period ascribed the ventilatory depression to be a result of both decreased frequency and depth of respiration. Wood and Cerna (see 7) in the dog, injected very large doses of morphine intravenously which caused respiration to nearly cease, but renewed injections of morphine increased it in rate and depth, at the same time inducing convulsions. Filehne (see 7) previously noted the same phenomena in the rabbit, respiration being slowed at first and later becoming more frequent in the stage of increased reflex excitability (convulsions), though even during this stage ventilation was reduced from the control value. The mechanism of action by which morphine exerted these effects was thought to be a "lowered susceptibility of the respiratory center to a carbon dioxide stimulus."

Cushny's own studies on decerebrate rabbits, injected with 1-2 mg of morphine intravenously, showed morphine reduced the rate without lessening the depth to a corresponding extent. When challenged with

carbon dioxide (CO/2), he hypothesized the center could not respond with an increase in rate but could still increase the depth of respiration. Cushny likened this to the results of CO/2 challenge after vagotomy, in which an increase in the depth of respiration occurred but without significant acceleration. From this, he concluded the action of CO/2 on the respiratory center to be augmentation of depth, but not frequency, since acceleration could occur only as long as the center received impulses from the lungs through afferent pathways. Theoretically, then, because of the similarity in the responses to CO/2 challenge between the vagotomized and morphinized animal, it was possible that morphine might act on the respiratory center by ."blocking the passage through the synapse of afferent impulses to the respiratory center." This seemed an attractive hypothesis, for it would also explain the action of morphine in cough.

Experiments performed by Cushny on decerebrate, vagotomized rabbits, however, produced the same effects on respiration as those seen in rabbits with the vagi intact (although the fatal dose of morphine for the rabbits was much reduced when the vagi were cut). Cushny's conclusion was that morphine did not lessen the rate merely by preventing the access of impulses to the center from the lungs. The changes in respiration produced by morphine might be regarded as a secondary result of the action on the center and not the prime factor.

Cushny also reported on two experiments done with cats, in which he showed that 1-2 mg of morphine acted on the respiration of decerebrate cats in the same way as in decerebrated rabbits, and on

the whole appeared to depress it more strongly.

In his discussion, Cushny states that morphine (in addition to other drugs he examined) affected the rate of respiration more frequently than the depth, the change in frequency usually being opposite in direction to that in depth. Ruling out the possibilities that the alterations in respiration caused by morphine were entirely due to either a depressed sensitivity of the respiratory center to a $CO/2$ stimulus or by alteration of afferent impulses to the center, he concluded morphine to have a more fundamental action on the intrinsic respiratory rhythmic process on which both of those influences act.

Barbour (1) in 1914 mentions briefly the work of Filehne and his production of a periodic respiratory pattern in morphinized rabbits, and states that most researchers thought Filehne to be "mistaken not only in the interpretation of his results, but also in his conclusion that all periodic respiration had a basis in circulatory changes."

Periodic respiratory patterns were Barbour's main focus in his publication, but he produced the patterns by administering morphine to etherized cats, both vagotomized and with vagi intact. His first experiment, in which a 2 kg vagotomized cat was injected i.v. with 20 mg of morphine produced "a striking alteration both in respiration and blood pressure. The former shows at once a great decrease in rate which may or may not be accompanied by a compensatory increase in excursion. A periodic character (cluster type) may be assumed within 5 minutes, or at almost anytime thereafter; but even under repeated doses it may never appear at all." Similar results were obtained in two other experiments, though in one case the vagi were left intact

until the periodicity in respiration was well established. Neither the cutting of one nor of both vagi in this experiment affected appreciably the course of respiration. Lastly, in an experiment in which a 5 kg cat received 40 mg of morphine, a periodic respiration which persisted over a considerable period of time was produced.

Barbour's primary objective in these few experiments was not to examine the effects of morphine per se, but to examine different types of periodic respiration and advance theories of their etiology on the basis of circulatory changes. In retrospect, the value of these experiments lie not in his explanations of the role of the "respiratory hormone" but in his demonstration of the alteration of eupneic patterns by morphine.

Jackson (23) in 1914 was another early investigator who examined the effects of opiate drugs used on the lungs, particularly the bronchioles. His experimental preparation consisted of etherized dogs, which were subsequently pithed, and respired artificially by negative pressure. A glass window was placed directly over the heart to observe movement of both the heart and lung.

Two important findings resulted from Jackson's work. First, the discovery that larger doses of morphine caused bronchoconstriction in normal and atropinized dogs was confirmation of the work of Dixon and Brodie (10) who studied the action of morphine on bronchioles in the cat. They had found that large injections of morphine into cats caused a considerable constriction of the bronchiol muscle, regardless whether the vagi were intact or not. Thus, the oft-observed convulsions with larger doses of morphine could be caused by, or

increased through, an asphyxia resulting from bronchoconstriction. This would explain an earlier observation by Jackson that the injection of epinephrine often revived and saved animals with morphine poisoning, since epinephrine was a bronchodilator.

Secondly, in his dog model, Jackson corroborated the finding that periodic respiration is one of the most typical symptoms of morphine poisoning; its appearance is due to a direct action by morphine on the respiratory system.

A short time after Cushny published his first studies on the pharmacology of the respiratory system, Cushny and Lieb (8) presented a second series in which the effects of morphine were examined in more detail. Three of the experiments deserve mention. In the first, 3 mg of morphine were administered to a rabbit (weight unspecified) anesthetized with urethane. The frequency of respiration dropped from 68 breaths/minute to 35 breaths/minute within 6 minutes of the injection, while the CO_2 content of the blood remained nearly unchanged. Within 30 minutes the frequency had dropped to 19 breaths/minute and the CO_2 content increased to 65% of the control value. The authors concluded, "This increase in CO_2, which occurs only some time after the onset of slowing, indicates that the hypercapnia is the result of the decreased ventilation and not the cause of it."

Two other experiments were performed on decerebrate cats, in which an unspecified amount of morphine was administered, but in an amount sufficient to cause convulsions. Frequency and ventilation were both above normal, but with smaller doses, the rate and volume

were both decreased (as it was in the rabbit). Though the data from the two experiments was not published, the authors stated the changes in CO/2 content of the blood were much the same as in the rabbit.

Cushny and Lieb concluded that morphine decreases frequency without affecting depth, and the reaction to CO/2 inhalation under morphine was of the same type as normal: the respiration increased in both rate and depth (though of smaller magnitude than in the normal animal). The slowing of the respiration under morphine, they felt, could be ascribed to an action on the intrinsic rhythm of the respiratory center which is rendered slower. In death from morphine poisoning, the respiration failed because the intrinsic rhythm of the center was abolished.

Schmidt and Harer (39) were the first investigators to view the action of morphine and related narcotics not as an unselective effect on the respiratory center, but as exerting specific actions on either the inspiratory or expiratory mechanism. Though at that time they knew of "no convincing evidence to indicate either the existence of distinct centers controlling inspiration and expiration, or the possibility of a truly selective action by drugs on one or the other phase of respiration," they designed experiments to study the possibility of such a selective action.

In vagi intact decerebrate cats, they found expiration to become passive following intravenous or intramuscular injection of morphine. If the dose was sufficiently high (1-20 mg), inspiration was deeper than before. Thus, while morphine decreased the rate very definitely, the volume of each breath had slightly increased. It would be

possible, then, for the slower deeper breathing after morphine to be almost as efficient as the ventilation preceding the morphine. After cutting the vagi, expiration always became definitely active, and frequently respiration became a series of inspiratory pauses, broken by active expirations.

In giving successive doses of morphine (5-10 mg at a time) to a decerebrate cat, there usually occurred, instead of a progressive depression, a return of active expiration and acceleration in rate, regardless of whether the vagi were intact or cut. They observed a maximum reduction of rate, with completely passive expiration, to usually follow 20 mg if the vagi were intact, or 5 mg if they were cut. Following 60 mg, convulsions often appeared, accompanied by active expirations and an accelerated rate. Following 60 mg, tetanic convulsions often appeared, accompanied by active expirations and an accelerated rate. In one experiment, tetanic convulsions occurred after 270 mg, following a period of dyspnea. Schmidt and Harer felt these convulsions from large doses pointed to a stimulant action on the spinal cord, which apparently overcomes the central depression which followed smaller doses. They remarked: "The extremely slow rate, culminating in respiratory failure, which is so characteristic of advanced morphine poisoning in other animals, occurred only if a fairly large dose (30-60 mg) was injected rapidly, and was always accompanied by marked circulatory depression. As long as blood presure remained high, it was impossible to produce respiratory failure by morphine alone in the decerebrated cat."

On the basis of the results of their experiments, in which

morphine decreased selectively the rate without affecting the depth of breathing, Schmidt and Harer concluded morphine to have an apparently selective action on the central expiratory mechanism of the cat, and that whenever the rate was diminished, expiration was passive. In their experiments, the extremely slow rate, culminating in respiratory failure, seemed to be due to depression of circulation rather than of respiration directly; they did not regard it as being typical of morphine's action on respiration in the cat.

Dripps and Dumke (11) examined the effects of several different narcotics, morphine being one of them, on the balance between central and peripheral chemoreceptor control of respiration. Using decerebrate dogs and cats, morphine sulfate was injected intramuscularly in stepwise doses to a maximum of 16 mg/kg. Not only was there depression of the respiratory response to a CO_2 challenge, there was also a definite relationship between the degree of depression and depth of narcosis. In addition, minute volume of breathing was decreased, again correlating with the degree of narcosis. Peripheral chemoreceptor sensitivity of the decerebrate cats under morphine appeared to be increased, and morphine decreased the CO_2 response to a greater extent in the cat than in the dog.

These investigators believed exaggeration of chemoreflex functions by certain narcotics could be produced simply by depression of the center's ability to respond directly to CO_2 and did not necessarily require an actual increase in reflex excitability. They had no data bearing on the explanation for this decreased responsivity of the respiratory center to CO_2 (to the point of complete

disappearance) while peripheral chemoreceptor reflexes might be relatively increased (as was the case with morphine). In their opinion, this increase in sensitivity (as measured by the response to sodium cyanide) was probably due, at least to some extent, to the presence of anoxemia resulting from repiratory depression. Removal of anoxemia by inhalation of oxygen restored the sensitivity to near normal.

Dripps and Dumke concluded with these remarks: "From all of the data it is evident that no single pattern can be described for the effect of anesthetics (including morphine) on the control of respiration. This is a complex resultant of many forces."

The most complete study on the influence of morphine on respiratory patterns was done by Breckenridge and Hoff (4) in 1951. A number of different dog models were studied: unanesthetized dogs (n=10), decerebrate dogs with vagi intact (n=13) or cut (n=8), apneustic dogs produced by midpontine section followed by vagotomy (n=13), and dogs with the brainstem sectioned at the upper level of the medulla and the vagi cut (n=24). In all experiments morphine sulfate was administered intravenously as bolus injections.

In unanesthetized dogs, the most consistent respiratory effect was the production of a "sighing rhythm" after a short period of tachypnea, as well as a slight reduction in rate and amplitude of eupnea. Another phenomena observed was a post-sigh apnea, referred to as post-sigh inhibition. In decerebrate preparations in which the vagi were left intact, the results were almost identical with those in the normal animal except that the stimulation of the sighing type of

breathing, the post-sigh inhibition, and the consequent depression of eupnea were more pronounced. After sections within the pons and vagotomy, apneustic breathing developed, characterized by periods of inspiratory breath-holding alternating with expiratory apnea. When morphine was given to animals in this apneustic pattern, a characteristic increase in the rate of the slow rhythm of respiration occurred, expressed as an acceleration of apneustic breathing. Concomitantly, a diminution in this duration of the inspiratory breath-holding pattern occurred, though only rarely was it possible to completely abolish the apneusis even with larger doses of morphine.

Breckenridge and Hoff stated, "It cannot be concluded with certainty that this abolition of apneusis is a direct effect of morphine administration since after vagotomy in the mid-pontine preparation sensitivity to the cardiovascular effect of morphine is increased, and following all but minimal doses blood pressure falls precipitously and death may occur. Consequently, the disappearance of apneusis may be secondary to fall in blood pressure and anoxia." Their method of administration of large doses of morphine intravenously (causing the drops in blood pressure), instead of a constant intravenous infusion of the drug, would not allow a distinction between the true cause of the apneusis reversal.

In the medullary preparation, the influence of morphine on respiration was almost completely overshadowed by a much more striking effect on circulation. The usual early stimulating effect of morphine on respiration, the deep sighing and post-sigh inhibition, occurred as in the other groups, but the time span over which these phenomena

occurred was much compressed.

The most striking feature of all the experiments, they felt, was the facilitation of the deep sighing type of breathing both in the unanesthetized animal and after transection at all levels. This was suggestive of a disinhibition of lower brainstem activity by more rostral centers. Interpretation of their results in the intact animal showed morphine to produce a pattern identical to that produced by decerebration. The intact anesthetized animal showed almost exclusively eupneic breathing, but with rare deep breaths at random intervals. Decerebration or the injection of morphine instituted a regular rhythm of deep sighing respiration at variable rates and with varying amounts of post-sigh inhibition of eupnea. It was suggested that this type of breathing was facilitated "by pontine centers which were disinhibited, allowing a physiological decerebration by inactivating cortical and subcortical supressor mechanisms." According to this view, then, the respiratory depressive action of morphine was due to an increase in the intensity of post-sigh inhibition (release of the facilitory mechanism) rather than to a direct supressor action on eupnea itself. Left unexplained, however, was the primary rate-slowing action of morphine.

Ngai (31) in 1961 re-examined the action of morphine on central and reflex respiratory mechanisms, expressing the results in terms of the organization of the central respiratory controller popular at that time. Morphine was administered in doses of 1 to 2 mg/kg to vagotomized, midcollecular decerebrate cats, the maximal cumulative dose being 15 mg/kg. Though all animals showed a decrease in

respiratory frequency, the effect on tidal volume varied somewhat, either increasing or remaining unchanged. In some cats (n=4) the pontile penumotaxic center was stimulated both before and after morphine administration (same preparation as described above). The control response, consisting of respiratory acceleration, was reduced or abolished after 5-10 mg of morphine. Stimulation of the "inspiratory center" (5 cats) did not change after a dose of morphine from 2 to 12 mg/kg. In 3 cats, the effect of morphine on the response to stimulation of the medullary expiratory center was studied. With threshold stimuli, the response was hypernea, a response which after morphine required a less intense stimulus to occur. Also, the stimulus threshold for expiratory apnea appeared to be lower.

In the last group of experiments, morphine was administered to 5 cats in which the rostral pons had been transected to remove the pneumotaxic center. Four of the 5 animals received a total dose ranging from 2 to 8 mg/kg, and respiration was markedly slowed. In the fifth cat, the respiratory rate was not changed significantly even after a cumulative dose of 22 mg/kg. Following the morphine, bilateral vagotomy in 3 cats was followed by a further slowing in rate, and the tidal volume amplitude increased. The respiratory movements, however, were not gasping in character, and apneusis (usually observed in such preparations) did not occur. Levallorphan (an agent which reverses the effect of morphine), when administered to these animals, promptly initiated the typical apneustic pattern in these cats. In the other 2 cats, morphine did not prevent the onset of apneustic breathing following vagotomy, but levallorphan in these

cats caused the relative duration of the inspiratory phase to be increased.

From the data presented, Ngai concluded morphine to act mainly in affecting the respiratory rate; if a decrease in respiratory amplitude or apnea was produced, it was transient. Also, morphine altered the responses to stimulation of various components of the central respiratory mechanism and depressed the pontile apneustic center. The decrease in respiratory frequency following administration of the morphine indicated the neural mechanism for respiratory rhythmicity to be depressed, demonstrated by the loss of the acceleratory response to stimulation of the pneumotaxic center. The hyperpneic response to threshold stimulation of the medullary respiratory center also became reduced or absent. However, persistence of the inspiratory response to stimulation of the pneumotaxic center and to low frequency vagal stimulation (also demonstrated in this study) indicated that the inspiratory mechanisms were not depressed to the same extent as the mechanism controlling respiratory rhythm, a finding in agreement with the fact that morphine either increased or left unchanged the tidal volume.

In the pontile decerebrate animals, the expected apneustic pattern did not ensue upon vagotomy after the administration of morphine; it was either absent, or of relatively short duration. Arterial pressure monitored during these experiments failed to show any significant decrease following administration of the morphine, demonstrating depression of the apneustic center was not secondary to hypotension. Further evidence for this conclusion rested on the

success of levallorphan in restoring a typical apneustic pattern.

Ngai's explanation of his results were based on the assumption that apneusis is the manifestation of an unmodulated tonically active apneustic center, whose activity depends on certain driving forces, one of which is CO_2. In the case of morphine depression, Ngai hypothesized that the apneustic center was rendered unresponsive to CO_2, so that the prevailing driving force could not initiate or maintain the tonic activity of this center. Restoration of the responsiveness of this center, by antagonism of the morphine effects with levallorphan, was followed by typical apneustic breathing.

The analysis of the respiratory action of morphine by Ngai, indicating that all components of the respiratory rhythm generator were depressed, differed from the interpretation of Breckenridge and Hoff (4) quoted earlier. On the basis of their published records, Ngai felt the effect of morphine on their midcollicular decerebrate dogs was in many ways similar to his own results. Those results, which were incompatible with Ngai's own results, were attributed to species differences, or different experimental techniques.

Few studies of the respiratory actions of morphine are available for species smaller than the cat and dog, because of the experimental difficulties in measuring accurately the tidal volume and thus the minute volume of respiration. Conflicting studies with rats cite morphine as primarily depressing the minute volume, or depressing both rate and minute volume. A study by Kokka et al. (26) in which 5 mg of morphine was administered to rats, demonstrated a slight decrease in minute ventilation which lasted approximately 2 hours, and was due to

a fall in tidal volume since the rate was unchanged. Doses of 10 and 20 mg/kg produced the greatest depression in ventilation by decreasing both rate and tidal volume (though tidal volume was affected to a greater extent). In raising the dose to levels as high as 160 mg/kg, the ventilation was not depressed as much as it was after 10 to 20 mg/kg, and it was the decrease in respiratory frequency which became increasingly responsible for depression of minute volume. Interestingly enough, measurements of oxygen consumption showed the greatest change after 10 to 20 mg/kg, declining to approximately 70% of the control level. A reversal of depression similar to that observed for minute volume was noted in rats receiving doses larger than 20 mg/kg, with 160 mg/kg increasing oxygen consumption within 60 minutes.

From these observations, it appeared a separation of the respiratory responses to morphine was exhibited with increasing doses; the depression of ventilation predominated at the lower doses, but was minimized at higher doses. Kokka cites Tatum, who remarked: " . . the stimulant action of morphine serves as an antidote to the depressant action." Tidal volume was depressed before rate, but this was less marked at higher doses while depression of frequency continued. Thus, the lower doses of morphine depressed ventilation primarily by an effect on tidal volume while the higher dose primarily affected rate. Depression of oxygen consumption, occurring with doses up to 70 mg/kg, was interpreted by Kokka to mean that depression of respiratory rate, usually present even at high doses, was not merely a manifestation of decreased metabolism. The observation that metabolic depression was

apparently reversed at high doses of morphine could be a manifestation of a central nervous system stimulant effect of the morphine, since tremors and convulsions were observed in some animals. In summary, as the morphine dose was increased (40-160 mg/kg) the stimulant effects of morphine antagonized some of the depressant effects, but depression of respiratory rate persisted even at doses which produced tremors and convulsions.

The blood-brain barrier is known to impede the penetration of morphine into the central nervous system, yet in rats and dogs a significant amount of it is found in the cerebrospinal fluid (CSF). Florez et al. (17) addressed the question of whether the central effects are the same regardless of the route of entry into the central nervous system, or alternatively, whether different receptors are engagned depending on the route of entry. These investigators chose to study respiration after intraventricular injection of the drug, comparing those effects with that elicited by i.v. systemic administration. The study was an attempt to differentiate an action on the diencephalon from that on the lower brainstem by means of discrete injections of morphine into the third and fourth ventricles and the bulbar subarachnoid space.

Experiments were performed on 30 cats anesthetized with Dial-urethane, and screw-type cranial cannulae were inserted stereotaxically for access to the third and fourth ventricle, and subarachnoid space. Respiration was recorded plethysmographically, and effects of morphine evaluated by observing steady-state respiration at altered levels of alveolar carbon dioxide.

Responses to injections of 50 ug (in 50 ul) into the fourth ventricle and bulbar subarachnoid space were indistinguishable from each other, producing a maximum decrease from control values of approximately 50%. Latency of onset varied between injection into the fourth ventricle and subarachnoid space as compared to that for i.v. injection, but an equivalent decrease in the frequency of respiration was produced after i.v. injection.

In contrast to the reduction in frequency caused by morphine given systemically or by injection into the CSF in the lower brainstem, a similar dose injected into the third ventricle resulted in a prompt increase in frequency, between 125-150% over control values. Morphine did not decrease the tidal volume significantly when injected in all areas except in the third ventricle, which increased tidal volume substantially in the first 5 minutes, but then decreased below control and remained so throughout the rest of the experiment.

The time-course of changes in end-expiratory CO_2 were consistent with the observed changes in ventilation. Intravenous injection of morphine, which produced the fastest decrease in respiratory frequency was responsible for the most rapid decrease in ventilation, while the course of CO_2 accumulation after injections into the fourth ventricle and subarachnoid space paralleled the gradual depression of ventilation. The short initial stimulation in both frequency and tidal volume following injection of morphine into the third ventricle were reflected in the reduced end-expiratory CO_2, lasting about 5 minutes. A second phase, consisting of more shallow, but still rapid breathing, accounted for a sustained rise in CO_2 (consistent with the

decrease of tidal volume as stated above).

The results of Florez et al. showed that restriction of application of the morphine to the lower brainstem produced a form of respiratory depression qualitatively indistinguishable from that obtained after i.v. injection. Conversely, application of morphine to the third ventricle elicited a more complicated response in which an excitatory component was evident. In addition, despite the similarities of the responses to systemic, fourth ventricular and subarachnoid space injection, the time-courses of the ensuing respiratory depression differed markedly. Systemic i.v. injection of morphine rapidly reached the whole brainstem (where the respiratory centers are located), and while the blood-brain barrier for morphine is pronounced, a compensation might exist by having neurons·with a high susceptibility to its actions.

In agreement with previous studies, the results of Florez pointing to a depressed sensitivity of the central chemoreceptors to $CO/2$ "is best interpreted in terms of an elevation in the threshold of the respiratory chemostat or, in other words, an increase in $CO2$ setpoint." The respiratory response to morphine injected into the third ventricle however, consisted of two phases; the first, characterized by a brief stimulation of both frequency and tidal volume, suggested stimulation of receptors located in the wall of the third ventricle itself, or in the aqueduct. Since tremor, scratching, and vomiting were often observed during this time, the increased ventilation might be regarded as one component of a broad pattern of stimulation produced by actions there. The second phase, starting

about 10 minutes after injection and characterized by an increased frequency and a decreased tidal volume, had as its main consequence a progressive increase of end-expiratory CO_2. The reduction of tidal volume did not necessitate an increase in frequency, but seemed to be a direct effect of morphine acting either at the midbrain or brainstem level, where the drug was shown to reduce the tidal volume (possibly from the drug escaping from the third into the fourth ventricle subarachnoid space). These opposite effects on rate and depth would then be the result of the action on different sites of the central nervous system.

A 1972 study by Florez et al. (16) investigated the influence of drugs which modify brain amine content in the morphine depressed decerebrate cat. In his introduction, Florez states, "Respiration is one of the functions more consistently depressed by morphine. In decerebrate cats, respiratory depression has been shown to be the consequence of the disturbance in the regulatory mechanisms of the medullo-pontine respiratory center". These remarks are reminiscent of the earlier, very general conclusions reached by Cushny in 1912 (7). However, in this particular study Florez attempted to explain the actions of morphine through changes in local neurotransmitter concentration which would influence its respiratory center activity.

A control group of 10 decerebrate cats received 2 mg/kg of morphine i.v. with a similar injection following 60 minutes later. The injection of the first dose of morphine resulted in a prompt decrease of respiratory frequency, tidal volume, and minute volume. The maximum reduction was reached within a few minutes after

administration, followed by partial recovery of frequency and minute volume such that a steady level of respiratory depression was reached within 15 minutes. By the end of the first hour, the minute volume was still reduced to 70% of the control value. Response to CO_2 stimulus was quite diminished, accounted for by a reduction of both the frequency and tidal volume responses, a depression which lasted until the next injection. The second dose of morphine again reduced the resting respiratory variables, though the minimum values attained were no lower than those observed after the first dose.

The respiratory action of morphine was examined in several other groups of decerebrate cats, which had been pre-treated with paragyline, tranylcypromine, reserpine, PCPA, a-methyltyrosine, and 6-hydroxydopamine. Since PCPA, a selective inhibitor of serotonin synthesis, was the most consistent antagonist to the depressant action of morphine, Florez et al. concluded that serotonin in the brainstem was a synergistic factor with the action of morphine on the respiratory center.

The identification of specific receptors for morphine and its derivatives in the mammalian central nervous system (CNS) in 1973 (3,9,18), and the subsequent search for and discovery of endogenous opiatelike substances (known collectively as endorphins) in 1975 (38,41), cast an entirely new perspective on previous research describing the physiological actions of morphine. Prior to this, morphine's various physiological effects and mechanisms of action were of interest because of the use of morphine clinically (and abusively). Discovery of the endorphins and the localization of opiate receptors

to areas within the CNS associated with cardiovascular and respiratory control (20) created a fresh interest in mechanisms of action of opioid agents.

Florez et al. (18) in 1977 was one of the first investigators to follow one of the logically suggested courses of action: since depression of the respiratory center was one of the most characteristic actions of opiates, it was pertinent to investigate the respiratory actions of endorphins and other endogenous opioid peptides. In the study mentioned previously, Florez et al. determined the ventral surface of the brainstem to be the most sensitive site to the respiratory depressant action of morphine; therefore in this study, using cats lightly anesthetized with a pentobarbital-urethane mix (25 mg/kg and 500 mg/kg, respectively), met-enkephalin was injected at the ventral surface of the brainstem. Respiratory parameters were assessed by measuring spontaneous resting parameters (frequency, tidal volume, ventilation) as well as their responses to stimulation with 5% CO_2 in oxygen.

Met-enkephalin, at a dose of 1.6 umole, directly applied to the ventral surface of the brainstem, consistently depressed respiration through an immediate reduction of tidal volume; frequency was less affected. Though the depression of minute volume began almost immediately following injection, 3 minutes later the depression had been reduced substantially, and by 15 minutes the values were similar to control. However, the ventilatory response to CO_2 stimulation was still significantly lower than the control response. At maximal depression, frequency was minimally affected and thereafter slightly

increased; end-expiratory CO/2 values remained above control for about 15 minutes, with the low tidal volume accounting for the reduced ventilation. The short latency observed suggested met-enkephalin to be acting on ventral surface areas in the brainstem involved in respiratory regulation, rather than pontomedullary centers.

Denavit-Saubie (9) conducted a more specific study, by investigating the effects of microelectrophoretically applied opiate agonists and antagonists on extracellularly recorded neurons in cat pontine and bulbar respiratory centers of the cat. Acknowledging the evidence that respiratory depression from systemic administration of opiates is mediated through central mechanisms, the authors pointed out that the direct demonstration that neurons related to respiratory function were sensitive to opiate administration was still lacking.

Fifty cats were readied as "isolated respiratory center" preparations with bilateral vagotomy, spinal section (C7-T1), and immobilization with gallamine triethiodide (elimination of peripheral inputs to the respiratory centers). Central respiratory drive was monitored via a phrenic nerve recording, and respiratory neurons (inspiratory, expiratory, or phase-spanning) recorded extracellularly via multibarrelled micropipettes. The iontophoretically applied agonists (morphine, levorphanol, and met-enkephalin) depressed the spontaneous discharge of respiratory related units (RRU) by more than 50% and suppressed the discharge evoked by short applications of L-glutamate in the majority of RRU's tested. In 58 RRU's tested with morphine, 35 were depressed; 14 showed no effect, and 9 showed excitatory effects. The excitatory effects became prevalent when

applications were repetitive at relatively short intervals (less than 2 minutes), became more pronounced as dosages were increased, had a slower onset, and were generally longer lasting than the depressions.

An unspecified number of experiments were preformed in order to compare the effects of systemically administered opiates, which influence a number of neuronal systems, to actions seen after localized application. Administration of morphine (0.1-0.5 mg/kg i.v. bolus injection) to bilaterally vagotomized, paralyzed, artificially ventilated cats was followed, 50-100 seconds after the beginning of injection, by a depression of the phrenic nerve discharge, with the rate of rise of discharge slowing during inspiration. The duration of these bursts was increased 30-50% but the amplitude of the integrated inspiratory bursts decreased by 10-20%. The authors state, "Gasping or apneustic respiration was not seen even with rather high dosages," although the values these high dosages reached were never specified.

Lastly, naloxone at dose levels where it displayed no detectable effect on the discharge activity on spike generation, readily antagonized only the depressant effect of the opiate agonists without affecting the excitatory action evoked in those few neurons, thus indicating, in the author's interpretation, that only the depressant actions are mediated by specific opiate receptors.

The results demonstrated the depression of individual RRU's, "whose activity is probably driven by mutual re-excitatory connections and rhythmic inhibitory processes." The authors further explained, "It might be assumed that the slower rate of rise of phrenic nerve activity in vagotomized cats after intravenous morphine administration

is related to impairment of the excitatory inputs on RRU's. The triggering of the expiratory phase was delayed, as suggested by lengthening of inspiration. This might be a consequence of the reduced rise of activity during inspiration rather than an effect on the inhibitory processes."

Beubler (2) offered a hypothesis that the function of endogenous opiates would be to depress hyperactivity of biological systems whose functions were known to be inhibited by exogenously administered opiates. The endogenous opiates would form a "protective system," geared into activity by stimuli which were noxious at high intensity and which were consciously perceived; functions such as pain and respiration would be controlled by the protective system.

Since the respiratory response is a biological function inhibited by opiates, and in case of stimulation is enhanced by naloxone, Beubler investigated whether naloxone influenced basal respiratory minute volume and the CO_2-stimulated minute volume. In the urethane-anesthetized rabbit, he found the minute volume to be reduced by 60% with a 2 mg/kg i.v. bolus injection of morphine. This effect was reversed by naloxone, and in addition, transiently increased minute volume over control values (obtained prior to morphine). A 5 mg/kg i.v. dose of naloxone itself increased basal respiratory minute volume (an effect lasting about 5 minutes), and the minute volume stimulated by CO_2 also increased over that prior to the period of depression produced by morphine. Beubler's conclusion, therefore, was that endogenous opiates modulated respiration and, like exogenously administered opiates, reduced the sensitivity of the respiratory

controller to CO/2.

Zobrist et al. (46), in agreement with the hypothesis that endogenous opioid peptides might participate in or modulate normal regulation of respiration within the CNS, proposed to study in more detail the effect of met-enkephalin on respiratory function in the unanesthetized rat and to correlate those effects with those of morphine. Two days prior to drug administration, screw type cannulas were stereotaxically placed in the left ventricle of rats. This allowed respiratory variables to be determined in the unanesthetized rat using whole body plethysmography.

The onset of met-enkephalin induced respiratory depression was extremely rapid, with significant depression observed within 2 minutes following a 50 ug dose. Respiration continued to decrease, peaking at 20-25 minutes post-drug administration and continuing past 70 minutes; tidal volume was more sensitive to the depressant action than frequency. Naloxone administered subcutaneously reversed this respiratory depression and was accompanied by the "overshoot" phenomenon in which respiration increased above control levels, as noted by Beubler in his study (2).

The effects of met-enkephalin induced depression were seen to be diminished in a step-wise manner by increasing the CO/2 concentration inhaled by the rats from 100% 0/2 to 95% 0/2 - 5% CO/2 to 90% 0/2 - 10% CO/2. Minute volume progressively increased as the CO/2 concentration was increased. This was interpreted as met-enkephalin altering the sensitivity to CO/2, a desensitization of the central chemoreceptors to CO/2. Though similar experiments using morphine

were not published in this study, Zobrist et al. concluded the characteristics of met-enkephalin induced respiratory depression to parallel that of morphine. This suggested to them, "a common underlying mechanism existing between met-enkephalin and morphine-induced alterations in respiration."

Further support for the involvement of the opiate system in central respiratory chemosensitivity was presented by Pokorski et al. (32), who demonstrated fentanyl-depressed ventilation and naloxone-stimulated ventilation when the substances were applied to the intermediate "chemosensitive" areas of the ventrolateral medulla of the cat. Using 10 pentobarbital-anesthetized (30 mg/kg i.p. plus supplementary as required) spontaneously breathing cats, and monitoring the central respiratory output via a phrenic neurogram, pledgets of gelatin sponge soaked in the substances tested were topically applied to the chemosensitive caudal (L) and intermediate (S) areas, as well as the neighboring pyramids (non-chemosensitive). The remaining rostral (M) zones were not tested due to their far lateral localization.

Consistent and statistically significant respiratory responses to fentanyl and naloxone were evoked only from the intermediate (S) areas. Fentanyl depressed the amplitude of the integrated phrenic activity, with an increase in frequency sometimes observed; naloxone-induced stimulation of ventilatory output was achieved by an increase in both amplitude and frequency of integrated phrenic activity (this activity being proportional to tidal volume and respiratory frequency).

Though the application technique might be considered somewhat unspecific, Pokorski et al. felt the respiratory responses they observed to be related to the interaction of drugs with the neural structures beneath the S area, although non-specific effects of the drug naloxone could not be excluded entirely. Concluding the endogenous opiates to be involved in the chemical control of respiration at the central level, they nonetheless could not assess what the contribution of this mechanism to respiratory control would be.

Moss and Scarpelli (30) assessed the central depression of respiration (and circulation) using the endogenous opiate beta-endorphin, injected into the cerebrospinal fluid of pnetobarbital-anesthetized dogs. Their assessment of respiatory drive was by measurement of preinspiratory occlusion pressure (P500); the beta-endorphin effects on ventilation, its components, P500, and respiratory timing of unoccluded and occluded breaths were studied during CO2 rebreathing.

Tidal volume and P500 were depressed maximally within 15 minutes of an injection of 15 nmol (in 0.5 - 0.7 ml) of beta-endorphin into the CSF via the cisterna magna, but had recovered to control levels by 75 minutes. At this time, minute ventilation ($\dot{V}$) and frequency (f) were depressed maximally (though recovered to control by 135 minutes), leading to the suggestion that the decrease in f was the major contributor to the depression of ventilation. Analysis of the components of respiratory timing before and after beta-endorphin in both unoccluded breaths and preinspiratory occlusion showed the

duration of inspiration (Ti) to be shortened during the latter, with no significant effect on Ti in the former. In both unoccluded and preinspiratory occluded breaths, beta-endorphin increased the duration of expiration (Te) (as well as Ti) significantly, with the peak effect 75 minutes after beta-endorphin administration. Thus, the early hypoventilation was related to changes in both f and tidal volume (V/T), whereas the later, deeper, depression of ventilation was due primarily to the effects on f. The effects were evident both during spontaneous breathing, in which end-tidal PCO_2 increased, and during CO_2 rebreathing in which the CO_2 threshold increased and CO_2 sensitivity decreased.

Pointing to the rapidity inherent in the early hypoventilation, Moss and Scarpelli suggested anatomically superficial endorphin receptors to be involved, corresponding with the CO_2 receptors identified by Schlaefke close to or at the surface of the medulla. The most superficial respiratory neurons identified are inspiratory neurons rostral to the dorsal respiratory group, and Moss and Scarpelli (30) cite Denavit-Saubie et al. (9), whose data showed depression of inspiratory neurons there to be depressed by microelectrophoretic administration of endorphins.

The depression of f at 75 minutes, due to prolongation of Te, occurred as long as there was afferent vagal activity, i.e., during preinspiratory occlusion (at which time phasic vagal reflexes play a minor role, thus reflecting minimal activity), unoccluded breathing (normal vagal activity) and inflation occlusion (occlusion of the airway at end inspiration, increasing phasic vagal reflexes, measured

in three experiments). In 3 dogs bilaterally vagotomized before the beta-endorphin administration, insignificant changes of f and Te were produced. The authors therefore suggested the beta-endorphin effect on f to be mediated by facilitation of central vagal pathways known to be of major importance to respiratory timing and modulated by vagal afferents. This possibility was consistent with their finding of the significant shortening of Ti by beta-endorphin during preinspiratory occlusion, and the delay of peak depression of f following beta-endorphin, since central vagal pathways are located relatively deeply within the brainstem. Although endorphins are generally regarded as having inhibitory actions, in other areas of the brain they have been shown to excite neurons directly or to activate them by inhibition of inhibitory interneurons. Those studies were cited by Moss and Scarpelli to support that either one of those mechanisms would be in accord with facilitation of central vagal pathways as the mode of beta-endorphin effect on f, although other central-timing mechanisms may also be involved.

Following review of the foregoing evidence, which suggests the opiates to be influencing central chemoreception, a natural question to pursue would be possible specific effects on peripheral chemoreception. This line of questioning appears to be all the more obvious due to the discovery of enkephalin-like immunoreactivity in carotid body type I glomus cells, posing the possibility of an endogenous-opiate modulating carotid body chemoreception.

Accordingly, Pokorski and Lahiri (33) investigated the effects of naloxone on the responses of the carotid body chemoreceptors to

hypoxia and hypercapnia in 12 anesthetized cats, 5 of which were anesthetized with alpha-chloralose (60 mg/kg i.p.) and 7 with pentobarbital sodium (30 mg/kg i.p.). Cats anesthetized with alpha-chloralose breathed spontaneously and were used for both carotid chemoreceptor and ventilatory studies, whereas cats anesthetized with pentobarbital were paralyzed, artificially ventilated, and used only for carotid chemoreceptor studies. Single or few fiber activity was recorded from the left carotid sinus nerve, and the ventilatory parameters of tidal volume, end-tidal O_2 and CO_2 measured.

In the protocol, the experiment started with air breathing and proceeded to 3 successive levels of hypoxia and finally to hyperoxia, during which the effects of 3-4 levels of steady-state hypercapnia were studied. This was done before and after an i.v. injection of naloxone (0.4 mg/kg). In 4 cats in which only carotid sinus nerve activity was measured, the effect of close intra-arterial injections of met-enkephalin was studied, also before and after i.v. naloxone administration. In all cats studied, significant attention was paid to the acid-base status of the preparation.

Naloxone was seen to increase ventilation, thus lowering end-tidal CO_2; therefore, the inspired PCO_2 was raised to compensate. Without such adjustment, the arterial PCO_2 and H^+ decreased with the increase in ventilation, causing a decrease in the carotid chemoreceptor activity. This accounted for the finding that naloxone did not show a clear effect on carotid chemoreceptor activity. The investigators concluded from these results that naloxone caused a dominant ventilatory effect not mediated by the

peripheral chemoreceptors.

The effects of naloxone on chemoreceptor activity, measured at 5 different levels of arterial $PO/2$, showed chemoreceptor activity to increase hyperbolically, both before and after naloxone, with decreased $PO/2$. Ventilatory responses and arterial pH, also measured, showed ventilation to increase hyperbolically with the decrease of arterial $PO/2$ with and without naloxone, but arterial pH remained constant. The major effect of naloxone, which the authors found to be significant, was due to increases in V/T; the breath-frequency increase was small.

The effects of naloxone on ventilation and carotid chemoreceptor activity at 3 different levels of arterial $PCO/2$ during hyperoxia showed chemoreceptor discharge rates to increase with $PCO/2$. Naloxone showed no appreciable effect, but did increase ventilation at all 3 levels.

One example of the effects of close intra-arterial met-enkephalin on chemoreceptor activity before and after naloxone was illustrated. Before naloxone, an immediate inhibition of activity followed the injection, despite a decrease in systemic arterial pressure. Following naloxone, the chemoreceptor discharge was greater than control, and injection of met-enkephalin caused only a small transient inhibition.

Evaluation of the relative effects of naloxone on ventilation and chemoreceptor activity at a given arterial $PO/2$ was done by plotting ventilation against the corresponding chemoreceptor discharge rates. After naloxone, the curve describing this relationship shifted upward

with an increased slope, indicating a central effect on ventilation. This supported the investigators other results, indicating the major ventilatory effect of naloxone was mediated by mechanisms other than peripheral chemoreceptors.

Pokorski and Lahiri felt their findings to be consistent with other observations reported in the literature of naloxone facilitation of respiration. They felt their results indicated a central sensitization to a given carotid chemoreceptor input after naloxone. Also, the basic relationship between V/T and breath interval, though not specifically presented in the paper, was not changed by naloxone, indicating the pontomedullary neurons that determine respiratory timing mechanisms were not selectively affected.

The stimulatory effect of naloxone on carotid chemoreceptor activity, apparent during hypoxia, was felt by the authors to indicate inhibition of activity by endogenous enkephalin, an interpretation supported by the observation that naloxone blocked the effect of injected met-enkephalin. This effect during acute hypoxia suggested to the authors a possible significant role of endogenous opiates during chronic hypoxia. Thus, the authors concluded endogenous opiates to be potential modulators of respiratory regulation because of their effects on both central mechanisms and peripheral chemoreceptor activity.

B. Cardiovascular Effects of Morphine and Related Substances

Claude Bernard in 1864 was one of the first to note the hypotensive action of morphine, and his results were confirmed 4 years

later by Gscheidlen working on rabbits and cats (see 38). The search for a possible mechanism for this effect has continued , complicated by the conflicting results obtained by different investigators in different species. This review will therefore, for the most part, be confined to that work involving cats.

Gruber and Robinson (19), in experiments using isolated perfused cat hearts, noted dose-related effects of morphine when injected into the perfusate. In lower doses (10 mg) a marked dilation of the coronary vessels and a marked improvement in cardiac activity (increased force of contraction) was observed. A depressant action was seen with larger doses, "increasing the irritability of the heart" until fibrillation ensued.

Schmidt and Livingston (40) were the first investigators to thoroughly research the action of morphine on the mammalian circulation, and were the first to note that only the first i.v. injection of morphine in the cat and dog caused blood pressure to fall; upon repeated injections a steady recovery of pressure occurred. At this point, an i.v. injection of morphine in a dose far greater than that used to produce the initial hypotension would be without such effect, i.e., tachyphylaxis was apparent.

In their analysis of the mechanism of this phenomena, Schmidt and Livingston ruled out any participation of the central nervous system because decerebrated cats and dogs demonstrated the same effects. Their experiments showed that destruction of the medulla, or that portion of the spinal cord posterior to it, did not prevent or diminish the depressor effect (provided the blood pressure was not

already at shock levels). They also demonstrated the entire central nervous system could be eliminated, but if blood pressure was maintained (via a continuous infusion of epinephrine), it was reduced by morphine just as if the animal was intact.

Respiratory effects were also ruled out as an explanation. The authors observed respiration to be stimulated during the fall in blood pressure, and heart rate to be accelerated during the hypotensive period. Bradycardia appeared only during the recovery of blood pressure, and was maximal when pressure had returned to normal, so that too, was ruled out as being responsible for the acute depression. (This phenomenon referred to as "morphine bradycardia" is rarely referenced in the earlier literature). From this, they concluded morphine to have "a depressant action upon some part of the cardiovascular system, wholly apart from the central regulating mechanisms." Further experiments in the dog showed the "vasomotor center" to be depressed only slightly, so that its contribution to the effect was small.

Experiments utilizing isolated kidney and hindlimb preparations showed morphine to cause "a pure rise in perfusion pressure in the kidney experiments, a pure fall in that among the leg." Their conclusion was that morphine acted to constrict blood vessels of the abdominal viscera, whereas it dilated vessels in the skin and muscle. This was followed by bradycardia in the dog. The dilator action, then, was thought to be wholly peripheral, with the bradycardia being of central origin (larger doses of morphine producing a slight, minor depression of the vasomotor center).

Schmidt and Livingston summarized their findings: "We conclude, therefore, that tolerance of the circulation is rapidly brought about in the normal animal poisoned with large amounts of the drug; recovery from the shock-like effect of the first i.v. injection or injections in the face of additional quantities is due to the development of this tolerance. The term "acute tolerance" is suggested as descriptive of this condition." They went on to reject the notion that the depressor action might be due to liberation of a "chemical vasodilator agent" (such as histamine or acetylcholine), with repeated doses of morphine causing depletion of the tissue reserves of this agent.

Evans, Nasmyth, and Stewart re-investigated the phenomenon in 1952 (13). The work of Feldberg, published just prior to theirs, was cited by these authors for its direct demonstration of histamine released in the cat following morphine administration. Feldberg had concluded, however, that the fall in blood pressure caused by the i.v. injection of morphine could not be wholly accounted for by the release of histamine. The purpose of Evans et al. in this study was to determine what other factor(s) could be responsible.

Four different cat preparations were utilized: cats anesthetized with ether followed by 80 mg/kg chloralose; decerebrate cats; spinal cats; and anesthetized cats pre-treated with mepyramine, a histamine antagonist.

In the first preparation, the anesthetized cat, an i.v. injection of morphine caused a biphasic response; a transient rise in blood pressure occurred prior to a precipitous fall 15 to 20 seconds after completion of the injection. Heart rate, increased slightly during

the first 2 to 3 minutes after the injection, was subsequently slower than normal, even though the blood pressure remained low for a period of several hours. After a second or third dose of 4 mg/kg, tachyphylaxis was apparent. With an i.v. dose of 20 mg/kg morphine, the fall in blood pressure was more profound (20-30 mm Hg), but recovered to control levels within 2 hours. Repetition of this dose of morphine also produced transient and gradually diminishing responses. However, respiration was always seriously compromised with this large of a bolus injection.

Decerebrate cats showed similar responses to an i.v. injection of 4 mg/kg, though the fall in blood pressure was less precipitous and lasted only 10-15 minutes; tachyphylaxis was also exhibited on repeated injections. Evans et al. noted in these animals, the respiration never failed and was rarely more than very slightly depressed (though the cumulative dose attained was not stated).

A 4 mg/kg i.v. dose of morphine in spinal cats caused a large fall in blood pressure though of lesser magnitude than in the anesthetized cat, presumably because the control blood pressure was lower in these cats. The fall in blood pressure was quite transient; within 70 seconds it had returned to almost control level. The initial pressure level was usually regained within 15 minutes, and with repeated doses of morphine, the fall in blood pressure was no longer observed.

In the last group, the 4 mg/kg i.v. dose of morphine was preceded by a subcutaneous dose of 5 mg/kg mepyramine. In anesthetized cats, the morphine always produced a profound and prolonged fall of blood

pressure. In 2 decerebrate cats pre-treated with mepyramine, blood pressure fell but the magnitude of the response was less than half that normally attained in decerebrate cats. The effect in the spinal cat was always completely blocked by pre-treament with mepyramine.

Atropine or bilateral vagotomy did not modify the blood pressure or heart rate changes. Also, because the transient rise of blood pressure observed in the anesthetized (but otherwise untreated) cat suggested that there might be a considerable release of pressor substances from the adrenal medulla, experiments were preformed to assess epinephrine levels before and after an i.v. dose of morphine. The content of epinephrine in adrenal venous blood samples was shown to be increased 10 to 15 times after a dose of 4 mg/kg morphine, and to remain elevated for a minimum of 10 minutes.

Comparison of the duration of the depressor response showed it to be similar in spinal, and decerebrate cats, but prolonged in anesthetized cats. This suggested to the authors that the chloralose either potentiated the blood pressure lowering effect of the morphine or inhibited compensatory mechanisms.

Mepyramine pre-treatment did not alter the response in anesthetized cats, but reduced or completely abolished the response in spinal and decerebrate cats. The authors inferred that the phenomenon in the latter 2 groups was mediated by histamine released peripherally, and that any central actions of morphine affecting blood pressure were compensated by the increased adrenal secretion. The ineffectiveness of mepyramine in the anesthetized cat suggested morphine to have some central action which was potentiated either by

the chloralose or by vasomotor effects that were uncompensated in its presence. Since neither atropine nor bilateral vagotomy affected the response, it appeared morphine was affecting the "vasomotor" rather than "vagomotor" center.

Evans et al. concluded the hypotensive activity of intravenously injected morphine on the circulation of the cat was mediated in large part by an effect on the vasomotor center (i.e., removal of sympathetic tone) and by the peripheral release of histamine, with some compensation by increased secretion from the adrenal medulla.

Contreras and Huidobro in 1970 (6) confirmed the observation of tachyphylaxis produced by repeated doses of morphine in the spinal cat, and sought to explain its cause. During the period of hyposensitivity, they observed a concomitant decrease in the pressor response to norepinephrine. The diminution in the response was not due to progressive myocardial depression, because during repeated dosage with morphine, the characteristic signs of heart failure (concurrent venous hypertension and increasd left ventricular end-diastolic pressure) were not observed.

The scope of this investigation was wide; the effects of repeated doses of morphine on a number of preparations in the spinal cat (blood pressure, tibialis anterior, nicotating membrane, uterus, bladder, and small intestine) was examined. The dose of morphine involved ranged from 3-36 mg/kg, and was administered "as a series of relatively small, but gradually increasing doses repeated at about 5 minute intervals to avoid the intense hypotension, cardiac arrhythmias, and spinal convulsions which could be induced by single large doses." The

authors found a parallelism between morphine tachyphylaxis and decreased responses to epinephrine, norepinephrine, angiotensin, barium chloride, acetylcholine, and carbachol. The range of substances to which hyposensitivity was manifest indicated to them that the action was either non-specific at the receptor level or post-receptor processes. Moreover, the fact that the decreased responses to the drugs occurred in all the preparations indicated some basic underlying process which could be related to morphine hyposensitivity within the central nervous system. The authors did admit, however, that tolerance is not shown to all the central actions of morphine.

Contreras and Huidobro were concerned with the hypotension induced by morphine and the tachyphylaxis shown by this effect. Grundy in 1971 (20) pointed out the gradual development of tachyphylaxis to the pressor response as well, and attributed this to a continuous depletion of the adrenal medulla by the repeated administration of morphine, possibly in association with the hyposensitivity to the catecholamines Contreras and Huidobro (6) found.

Only two studies prior to those of Grundy really examined the effect of morphine on the heart itself. The studies of Gruber and Robinson (19) and Schmidt and Livingston (40), both cited earlier, determined the dominant effect of morphine to be depressive. Contreras and Huidobro (6) made no mention of changes in frequency or force of contraction; their concern was measuring variables that would indicate a progressive myocardial depression was developing).

Grundy (20), like Gruber and Robinson, studied the effects of morphine in the isolated, perfused heart; however, rather than cats, Grundy employed guinea pigs. Morphine sometimes produced a transient initial stimulation when first given (lower doses), but generally it acted as a weak myocardial depressant, results which agreed with those cited previously.

The discovery of the endogenous opiates had a tremendous impact on research dealing with the cardiovascular effects of morphine, just as it had for previous studies dealing with its respiratory effects. Interest in the cardiovascular effects of the endogenous opioid agonists generated several studies examining those effects when the substances were administered both centrally and peripherally.

Florez and Mediavilla (18), cited earlier, examined both the respiratory and cardiovascular effects of met-enkephalin when applied to the ventral surface of the brainstem. Coincident with the respiratory effects, met-enkephalin caused a reduction in blood pressure and heart rate, though the intensity of the depression was smaller than that induced on respiration. The time course of the cardiovascular responses also differed slightly; the peak effect appeared between 1 and 2 minutes after injection and the recovery was more gradual and delayed, so that at 5 minutes the depression showed a value corresponding to more than half the peak effect (refer to the previous citation for time course of the respiratory effects). Naloxone was able to completely antagonize both the cardiovascular and respiratory effects. Florez concluded the met-enkephalin to be acting on some of the ventral surface areas in the brainstem involved in

cardiovascular (and respiratory) regulation.

Feldberg and Wei (15) found morphine (400 ug) injected into the cerebral ventricles of chloralose-anesthetized cats produced tachycardia with a small, transient rise in blood pressure. The tachycardia still occurred after cutting the vagi, but not following removal of both stellate ganglia. This led to the conclusion that the increased cardiac rate resulted from augmented sympathetic discharge to the heart. The discharge was localized by the investigators to structures reached from the third ventricle because the strong tachycardia was also produced on injection of even smaller doses into the third ventricle (in such a way that little or no morphine would enter the lateral ventricles), and also when the aqueduct had been cannulated to prevent the morphine from reaching the aqueduct and fourth ventricle. Morphine injection into the fourth ventricle did not cause tachycardia, leading the authors to believe the drug was acting on structures in the walls of the third ventricle (probably on the hypothalamus) causing an increased sympathetic discharge to the heart.

Intracisternal injection of morphine (100-400 ug) produced a gradually developing, long-lasting fall in blood pressure and heart rate by acting on structures near the obex of the medulla, causing a reduction in sympathetic tone to blood vessels and heart and some increase in vagal tone to the heart. In bilaterally vagotomized cats, the fall in blood pressure and heart rate resulted entirely from a reduction in sympathetic tone. Injected subcutaneously, the main cardiovascular effects of morphine were found to be similar to that

obtained from intracisternal injections, inferring the effects to be of central origin.

Morphine thus produced opposite cardiovascular effects when injected intraventricularly and intracisternally. Experiments with met-enkephalin showed this substance to be nearly inactive; tachycardia was not produced with 100-400 ug injected intraventricularly, and a transient hypotension was only produced when 400 ug was administered intracisternally. Injection into the cerebral ventricles of 400-800 ug of beta-endorphin resulted in a tachycardia, but when administered intracisternally (200-400 ug) a long-lasting hypotension and bradycardia occurred.

The effect seems to be similar to that observed when opioid agonists are injected intracisternally in the dog and rat, though in these two species the long-lasting hypotension and bradycardia is preceded by a transient increase in blood pressure and heart rate (26). To examine more precisely the central site involved in these effects, Laubie et al. (28) injected (d-ala/2) met-enkephalinamide (a synthetic pentapeptide which is much more resistant to enzymatic inactivation than met-enkephalin) and fentanyl (a morphine-like substance) into the nucleus ambiguus (NA) and nucleus tractus solitarius (NTS) in chloralose (100 mg/kg i.v.) anesthetized dogs.

Microinjections of fentanyl (1 ug) unilaterally or bilaterally into the NTS did not change the blood pressure, heart rate, or splanchnic discharge. Unilateral microinjection of fentanyl (0.05 - 1 ug) into the right NA induced a dose-dependant decrease in heart rate without change in sympathetic discharge. The maximal effect was

reached after 2 minutes for 1 ug, was long-lasting, and was associated with a fall in blood pressure. Lower doses of fentanyl (0.05 - 0.2 ug) decreased the heart rate but did not change blood pressure. Unilateral microinjection of met-enkephalinamide (0.01 to 1 ug) into the NA also produced a dose-dependant decrease in heart rate, but splanchnic discharge was unchanged and the blood pressure reduced only when the heart rate was markedly decreased. Intravenous injection of naloxone (0.1 mg/kg) antagonized the effects of both substances in all experiments.

Previous work by Laubie (29) showed vagotomy to abolish the hypotension and bradycardia produced by morphinomimetic compounds, leading Laubie to conclude that such agents induced increases in vagal tone by acting on the medulla oblongata, confirming Feldberg and Wei 14,15). In addition, the NA was discovered to be richly innervated by terminals containing enkephalin-like immunoreactivity (21). Since microinjection of low doses of met-enkephalinamide or fentanyl into the NA of dogs induced a marked bradycardia without changing either blood pressure or sympathetic nerve activity, Laubie concluded this area to be "a highly sensitive site in the production of the vagal bradycardia induced by narcotic analgesic agents and morphinomimetic peptides."

Wallenstein (43) re-examined the biphasic cardiovascular action of morphine in cats in a 1979 study. A low dose of morphine (1 mg/kg) was administered intravenously to cats sedated with sodium pentobarbital (15-20 mg/kg i.p.), urethane (600-800 mg/kg i.p.) or nitrous oxide; all were artificially respired. A 1 mg/kg dose

produced a transient rise in blood pressure lasting less than 1 minute, followed by a long-lasting decrease; no effect on heart rate was seen.

In contrast, larger doses of morphine (8-12 mg/kg i.v.) produced an increase in blood pressure and heart rate, though in several cats Wallenstein noted a transient decrease to precede the increase. This was the result of a concurrent bradycardia, but it only occurred in cats receiving morphine at a rate faster than 2 mg/minute. The type of anesthetic used was found to have no independant effect.

Another group of cats, adrenalectomized and anesthetized with pentobarbital (10 mg/kg i.p.), were also tested. A 1 mg/kg dose of morphine produced a decrease in blood pressure, but was not preceded by a transient increase as observed in non-adrenalectomized cats. The adrenalectomized animals were demonstrated capable of producing such a transient increase in blood pressure by administration of 8 ug/kg epinephrine in another group. Heart rate decreased, an effect not seen in intact cats receiving the dose. The effect on pressure and heart rate of the larger doses of morphine (8-12 mg/kg i.v.) was inconsistent in the adrenalectomized cats, again in contrast to intact cats.

In agreement with the work of earlier investigators, the results of Wallenstein suggested low doses of morphine to induce an immediate, though brief, surge in catecholamine release from the adrenals which caused the pressor response; the following decrease in blood pressure and heart rate could be mediated by a decrease in sympathetic output, as suggested by Evans et al. (13). The fact that higher doses of

morphine did not produce a significant increase in the blood pressure or heart rate of the adrenalectomized cats suggested the adrenals to also have a role in the longer-lasting pressor effects usually seen with such doses.

The study of Moss and Scarpelli (30), referred to earlier, examined the effect of intracisternal injection of beta-endorphin (in pentobarbital-anesthetized dogs) on blood pressure and heart rate, in addition to the respiratory effects. In non-vagotomized dogs, both variables decreased progressively, reaching their lowest levels about 105 minutes after beta-endorphin administration (30 minutes after the peak depressant effect on ventilation). At 135 minutes, both heart rate and blood pressure had increased but remained significantly lower than control. In vagotomized dogs, beta-endorphin lowered pressure significantly but produced no significant change in heart rate. In the intact dogs, peak depression of arterial pressure and heart rate occurred later than peak respiratory depression, at a time when respiratory variables started to increase. Also, circulation was depressed progressively during moderate hypoventilation (which would be expected to facilitate cardiovascular reflexes). From these results, Moss and Scarpelli concluded that beta-endorphin affected the circulation and respiration by separate and discrete mechanisms. They went on to speculate that the delay in the cardiovascular, as compared with the respiratory, effect may stem from sites of action that were relatively less accessible to beta-endorphin. Since vagotomy sustained the drop in pressure but eliminated the heart rate response to beta-endorphin, it was evident that both central vasomotor

and vagal pathways were components of the circulatory response and that the opioid-induced bradycardia might involve facilitation of the vagal pathways.

CHAPTER III

METHODS AND TECHNIQUES

A.   Introduction

Twenty-five adult mongrel cats, of either sex, weighing 3.5 +/- 0.7 kg (mean +/- SEM), were studied in two different groups. The first group, hereafter designated as the "Eupneic group," consisted of cats with midcollicular decerebration only; the second group, hereafter designated as the "Apneustic group," consisted of midcollicular decerebrate cats in which apneusis was induced by bilateral vagotomy and bilateral pneumotaxic center lesion placements. Cats were randomly assigned to the Eupneic or Apneustic group using a computer generated list of random whole numbers between one and two. Of 25 experiments, 12 were judged successful, resulting in six cats in each group. Initial preparation, decerebration, and instrumentation as described in the following were identical for each group unless noted.

B.   Initial Preparation

Cats were placed in a restraining box with only the head protruding. By means of a cone placed over the face, anesthesia was effected with 4% halothane (Fluothane, Ayerst) using a closed delivery system (Drager Narkovet apparatus) with oxygen as the carrier gas. The anesthetized cat was then placed in the supine position. A tracheostomy was performed and a direct connection between the

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delivery system and tracheostomy tube established. At this time the halothane was reduced from its initial level of 4% to 2%.

To record pulsatile arterial pressure, the right femoral artery was cannulated with PE 90 polyethelene tubing connected to a Statham P23Db transducer. The right femoral vein was cannulated for administration of supportive agents but was not utilized in any of the 12 cats from which results are derived. The left femoral vein was cannulated for morphine sulfate infusion. In the Apneustic group, the right and left cervical vagus was isolated, and a loose moist cotton tie placed around each. Throughout the experiment, rectal temperature was monitored (Yellow Springs Instrument Co. Model 43TW Tele-thermometer) and maintained at 37-39 degrees C by external heating (Gormann-Rupp Industries Model K-1-3 Water Circulator).

C. Decerebration

The decerebration technique is similar to that reported by Kirsten (24). After the initial surgical preparation was completed, the external carotid arteries were exposed by extending the original midline excision (for insertion of the tracheal cannula) to visualize the genioglossus and styloglossus muscles. Lying between the two muscle bundles is the hypoglossal nerve, which was traced distally to the point where it crossed the external carotid artery (approximately 1 cm above the carotid sinus). Immediately below this crossing point the arteries were ligated. Since a significant portion of the arterial blood supply to the forebrain is derived from the external carotids (the internal carotid arteries of the adult cat usually are

not patent and hence make no contribution to the cerebral circulation), ligation of the external carotids significantly reduces hemorrhaging during the decerebration procedure (24). Furthermore, occlusion of these vessels rather than the common carotid arteries maintains both carotid chemoreceptor and baroreceptor function, and avoids the reflex pressor response that results from common carotid occlusion.

Cats were then placed in the prone position with the head firmly mounted in a stereotaxic frame. The head was shaved and muscle cleared from the left side of the skull. Once a clean exposure was obtained, the head was lowered below the thorax to reduce the probability of inducing air emboli through the cranial sinuses during the crainotomy (24). A trephine approximately 2 cm in diameter was used to make an opening in the left parietal skull (about the size of a nickel). The exposed bone edges were sealed with bone wax to further reduce probability of air emboli. An incision was made in the dura mater and the brain tissue rostral to the mid-colliculus removed by vacuum suction. The basilar artery was clipped with a McKenzie clip and a ball of oxidized cellulose (Surgicel, Johnson and Johnson) applied to the Circle of Willis to prevent seepage of blood. A small piece of Surgicel was also placed on the transected brainstem to minimize bleeding.

Following completion of the decerebration, cats remained on the halothane-oxygen mixture for another 30 minutes. Anesthesia as discontinued at this time, and the cats allowed an additional 30 to 60 minutes of stabilizing time. Twelve out of 25 decerebration attempts

were successful (48%) as judged by the presence of stable and unassisted eupnic breathing one hour after decerebration.

D.   Instrumentation

At termination of the stabilization period, instrumentation of each cat proceeded. Seven channels of cardiovascular and respiratory data were recorded using a Grass Model 7 polygraph. All channels were appropriately calibrated at the start and end of each experiment. Pulsatile arterial pressure (monitored since the time prior to decerebration) was recorded with a Statham P23Dc transducer. Intrapleural pressure was recorded using a Statham P23Dc transducer and a transthoracic needle probe inserted across the chest wall at about the fourth or fifth intercostal space. A heated Fleisch pneumotachograph and differential pressure transducer (Validyne MP451), connected in series with the tracheal cannula, was used to record inspiratory and expiratory airflows. The expiratory airflow signal was then integrated to give a tidal volume signal (Validyne FV156). Breath-to-breath oxygen (Beckman OM-11) and carbon dioxide (Beckman LB-1) were measured from a portion of the expired air constantly drawn (500 ml/minute) from a side port of the pneumotachograph through a heated sample inlet.

E.   Lesion Placement

After instrumentation, a second stabilization period ensued. Control cats received no further interventions following completion of stabilization until the infusion period began. Apneustic cats

underwent bilateral lesion of the pneumotaxic center located in the rostral dorsal pons. A calibrated electrode (Kopf K1388Z) was stereotaxically passed through the remaining brainstem to the Horsely-Clarke co-ordinates of P 3.5, H -4.0, L 4.5 mm (37), at an angle of 32 degrees to the anterior (37), bypassing the tentorium. The electrode position was functionally verified by electrical stimulation (W.P. Instruments Series 800 Digital Stimulator; 50 Hz, 1-2 msec, 0.5-1 mA). Lesioning was accomplished by heating the brain tissue to 70 degrees C for one minute with a radio frequency lesion generator (Radiotronics RFG 4). This procedure of stimulation/lesion placement was then repeated on the opposite side.

Once bilateral pneumotaxic center lesions were placed, the right and left cervical vagi were cut. In successful preparations, this resulted in prolonged inspiratory holds characteristic of apneusis.

F. Protocol

In both groups, morphine sulfate was continually infused through the femoral vein cannula (Harvard Apparatus Co. Inc., Model 940 Infusion/Withdrawal Pump) at the rate of 2 mg/kg/minute. This infusion rate was chosen on the basis of 8 preliminary experiments, 7 of which were performed in "Eupneic group" type cats and 1 in an "Apneustic group" type cat. An infusion rate of 0.08 mg/kg/minute (one cat) or 1 mg/kg/minute (one cat) resulted in prolonged experimental time courses lasting hours. Conversely, infusion rates of 3 mg/kg/minute (one cat) or 5 mg/kg/minute (two cats) resulted in an almost immediate severe respiratory depression, with death ensuing

shortly after the start of the infusion period. An infusion rate of 2 mg/kg/minute (three cats, two "Eupneic" type, one "Apneustic type) resulted in a reasonable time course that allowed for different respiratory phases to be distinguished. The data from the three cats in which an infusion rate of 2 mg/kg/minute was tested is included in the 12 experiments judged successful.

Morphine sulfate solutions were prepared just prior to each infusion by dissolving the calculated amount (in terms of salt, based on each particular cat weight) in 10 ml of 0.9% saline. Administration in the Eupneic group began a short time after the stabilization period ended, during which time baseline values for all recorded variables were measured. In the Apneustic group administration began once apneusis was seen to be definitely established. In both groups the morphine infusion continued until death of the cat.

In Apneustic cats, the brainstem was removed and stored in 10% formalin for histological processing. Brainstem sections were cut at 10 um, and every tenth section saved and contrasted with Kluver-Barrera stain to delineate lesion placement. Maps of lesion placement were constructed with the aid of a camera lucida attachment to the microscope at 12X magnification.

G. Data Analysis

The individual experimental record of each cat in the two groups was examined. On the basis of distinct changes in the ventilatory pattern throughout the period of morphine infusion, each cat was

identified as progressing through 4 phases. In both groups, these 4 phases, in addition to a control phase prior to the start of the morphine infusion, constituted the 5 phases named below. The letter identification indicated after each will hereafter be used for ease of referral.

Eupneic Group Phases:

    P - Control

    A - Depression

    B - Periodic Breathing

    C - Recovery from Periodicity

    D - Gasping

Apneustic Group Phases:

    P - Control

    A - Apneusis Attenuation

    B - Apneusis Recovery towards Eupnea

    C - Apneusis and Gasping

    D - Gasping

In each cat, a representative sample (2-4 minute) of each phase was quantitated. Variables quantitated were: frequency (f), tidal volume (V/T), heart rate (HR), and mean arterial pressure (MAP, estimated by adding one-third of the pulse pressure to the diastolic pressure). Ventilation was calculated by multiplying frequency by the tidal volume. Though end-tidal O_2 and CO_2 were measured, it proved impractical to quantitate either because: (a) calibrations were grossly exceeded; or (b) the gasping pattern superimposed on the "normal" ventilatory pattern, consisting of nearly instantaneous

inspirations and expirations (see following), did not allow for true estimations of the expired gases. Intrapleural pressure and airflow tracings were used as guides in the determination of respiratory cycles (frequency) and in delineating "normal" breaths from "gasps."

Upon visually inspecting the record of Eupneic cats, it was readily apparent that a gasping pattern gradually became interspersed among eupneic (hereafter referred to as normal) breaths, becoming more dominant as the final ventilatory phase was approached. The same was true of the Apneustic cats, the gasps interspersing among apneustic breaths (an abnormal ventilation, but "normal" for this group). In quantitating the phases in each group, therefore, total ventilation ($\dot{V}$/tot) was the sum of "normal" ventilation specific for that group ($\dot{V}$/n) and gasping ventilation ($\dot{V}$/g). $\dot{V}$/n was calculated by multiplying the frequency of "normal" breaths (f/n) by the average tidal volume of "normal" breaths (V/Tn) measured for a representative phase sample. Similarly, $\dot{V}$/g was calculated by multiplying gasping frequency (f/g) by the average gasping tidal volume for a representative phase sample. In many samples, V/Tn or V/Tg exceeded the channel limits, and so precise measurement was not always possible. The intermittant estimation of V/Tn and V/Tg thus introduces some error into the mean values of each for some phases in both groups.

Within each group, the control period was compared to the various phases specific for that group by use of Dunnett's method (see 44). A P value of 0.05 or less was accepted as indicating a significant difference between the control and the phase being compared with it.

CHAPTER IV

RESULTS

A. Total Cumulative Doses

     The average cumulative administered dose in the Eupneic group at

the end of phase D was 135 +/- 28 mg/kg (mean +/- SEM). In the

Apneustic group, the average cumulative administered dose was 166 +/-

23 mg/kg.   Both values exceed the cumulative doses reported in the

literature for bolus injection studies (see Table 2).

B. Alterations in the Eupneic Pattern

     Figure 1 illustrates the progression of respiratory changes in a

eupneically breathing cat during the course of the morphine infusion.

Following a stable control phase (P) the eupneic pattern becomes

depressed (A) almost immediately following the start of the morphine

infusion.   The appearance of a periodic pattern, resembling a

"cluster" (or Biot) type of periodic breathing, signals the beginning

of the next phase (B). The pattern during this phase is distinguished

by clusters of breaths, set apart from each other by long pauses,

which may be viewed as prolonged expiratory durations. The end of

this longer than normal expiration is the initiation of the next

inspiration, which then begins the next cluster of breaths. Recovery

from this marked pattern of periodicity ensues in the next phase (C),

during which the frequency increases to the point that distinct

clusters of breaths no longer exist. Some gasping is seen to occur

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Figure 1. Progression of phases in an eupneically breathing cat during constant morphine sulphate infusion (2 mg/kg/minute i.v.). Gasps are indicated by the sharp excursions in the intrapleural pressure and airflow tracings evident in the record; eupneic breaths, in contrast, produce more rounded tracings. Ppl = intrapleural pressure; $\dot{V}$ = tracheal airflow, inspiration downward; V/T = expired tidal volume. Phases: P = control; A = depression; B = periodic breathing; C = recovery from periodicity; D = gasping.

P   A   B   C   D

P_{pl}
cm H_2O
0
-12

$\dot{V}$
ml/sec
100
0
-100

V_T
ml
50
0

1   min
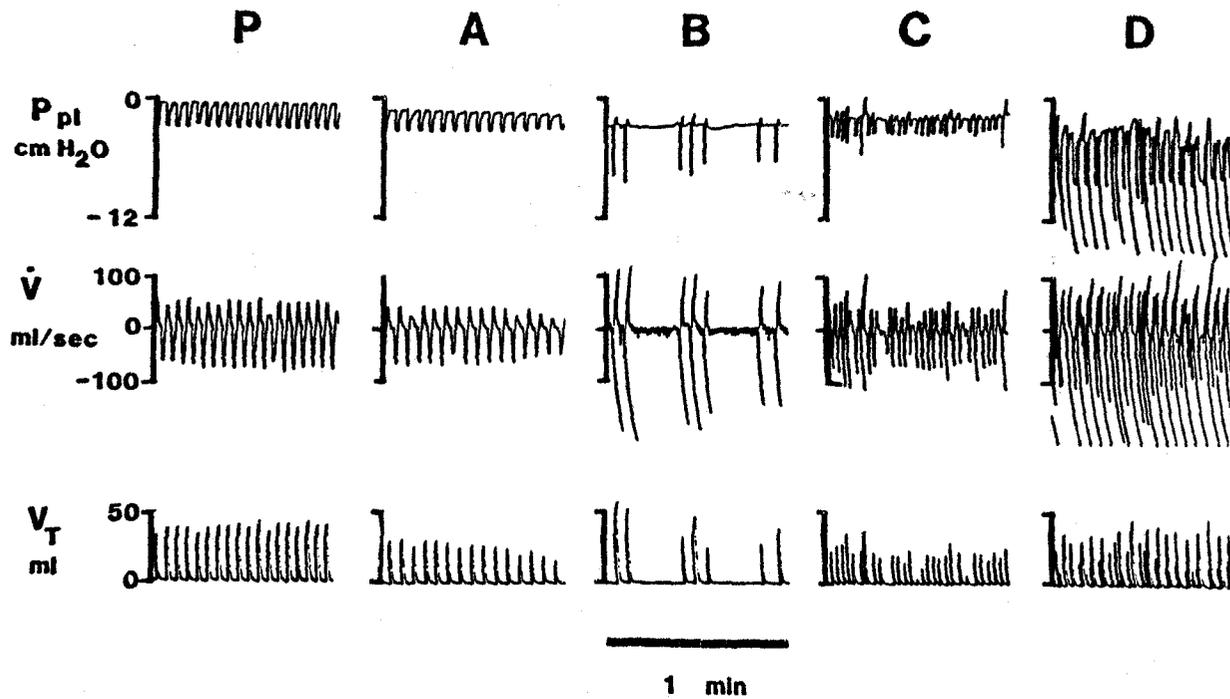
throughout this phase, increasing in intensity until it is obviously the dominant mode of respiration (D). Gasps are indicated by the sharp excursions in the intrapleural pressure and airflow tracings evident in the record; eupneic breaths, in contrast, produce a more rounded tracing.

Figures 2 and 3 graphically illustrate the changes in normal ventilation ($\dot{V}$/n) and gasping ventilation ($\dot{V}$/g), and their individual constituents, which occurred in the Eupneic group throughout the 5 phases. Means and standard errors are given in Appendix A.

In phases A and B, $\dot{V}$/n (Fig. 2) was significantly different from the control phase (P) (P<0.05); phase D was also significantly different from control (P<0.005). The significant decrease in $\dot{V}$/n (46%) during phase A from phase P was due to a nearly equal percentage decrease in f/n and V/Tn. The additional 9% decrease in $\dot{V}$/n during phase B resulted from the continuing decrease in f/n, even though an increase in V/Tn occurred. During the recovery from periodicity (phase C), though V/Tn was still reduced from control, the major increase in f/n over the preceding phase (and control value) caused an increase in $\dot{V}$/n. Thus, $\dot{V}$/n during phase C was not significantly different from control. In phase D, $\dot{V}$/n dropped 88% from the control value, again due to nearly equal decreases in f/n and V/Tn.

Gasping (Fig. 3) was present in only 1 cat during phase P, to a negligible extent (thus in phase P no standard error bar is present). Gasping ventilation ($\dot{V}$/g) was present to a minimal extent in all cats during phase A, and continues to increase in intensity throughout the next 3 phases. It was not until phase D, however, that $\dot{V}$/g and its

Figure 2.   Alterations in normal ventilation and its components during progression of phases in the Eupneic group. Values are mean + SEM (n=6). Phases: P = control; A = initial depression; B = periodic breathing; C = recovery from periodicity; D = gasping. * = P<0.05; *** = P <0.005 as compared to control (Dunnett's test).

Frequency
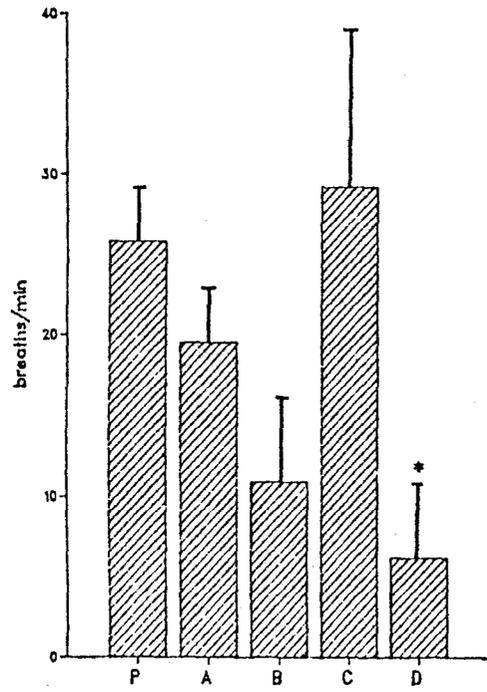
Tidal Volume
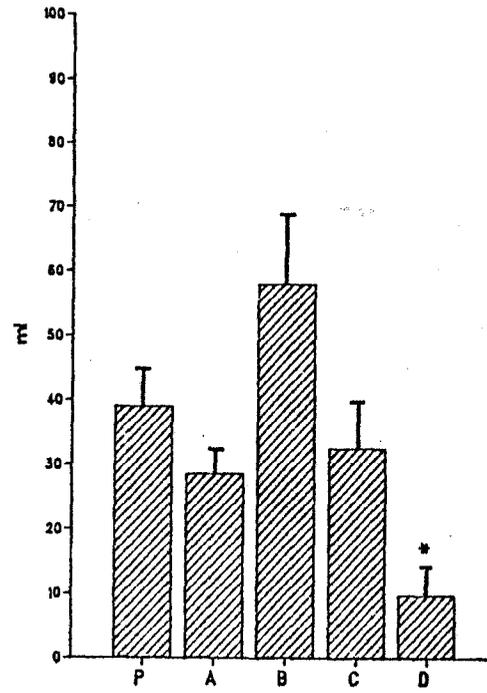
Ventilation
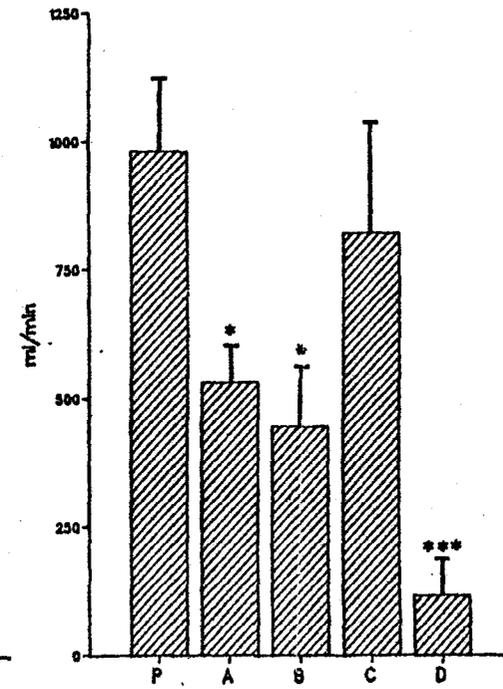
Figure 3.   Development of gasping during progression of phases in the
              Eupneic group. Values are mean + SEM (n=6). Gasping was
              present in only 1 cat during phase P to a negligible
              extent (thus no standard error bar is present). Phases:
              P = control; A = depression; B = periodic breathing; C =
              recovery from periodicity; D = gasping. *** = $P<0.005$ as
              compared to control (Dunnett's test).
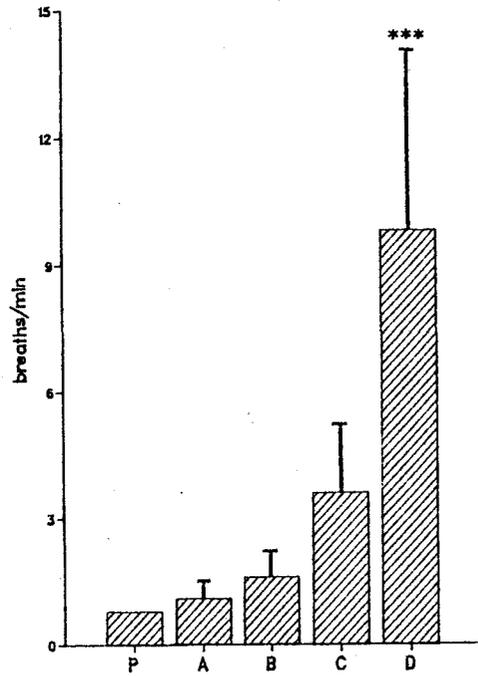
Gasping Frequency   Gasping Tidal Volume   Gasping Ventilation
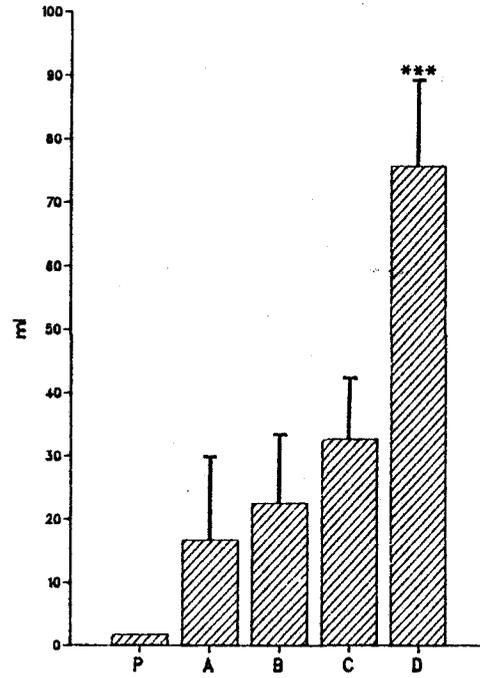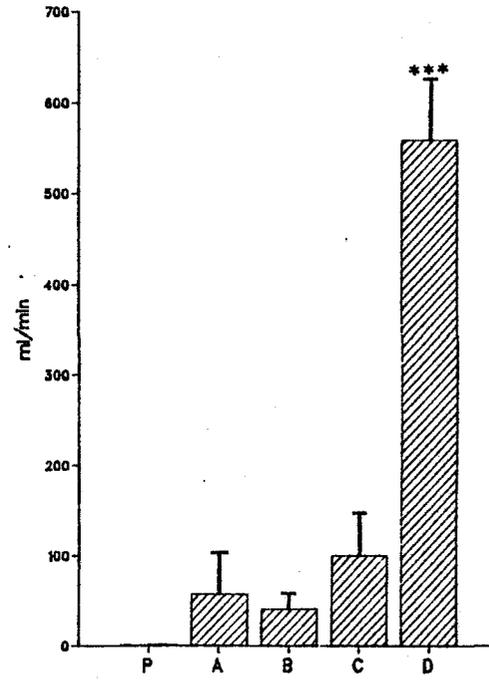
constituents V/Tg and f/g differ significantly from phase P values (P<0.005).

$\dot{V}$/tot was significantly different from control during phase B only (P<0.05) (see Fig. 10 and Appendix A). Though $\dot{V}$/n was depressed to almost half the value it was in A, $\dot{V}$/g was increased several thousand times; thus, $\dot{V}$/tot is decreased during phase A because of the large increase in $\dot{V}$/g. During phase B, $\dot{V}$/n decreases further; $\dot{V}$/g also decreases, but remains tremendously elevated above control levels.

During the recovery from periodicity (phase C), $\dot{V}$/tot increases towards the control value, due in part to the increase in $\dot{V}$/n. Though still depressed by 16% when compared to the control value, $\dot{V}$/n actually increases by 46% over its phase B value. $\dot{V}$/g begins a gradual rise during this phase, a rise which culminates during phase D when it was nearly one hundred thousand times the control value. $\dot{V}$/tot was decreased from the control value by only 30% during phase D, which was a moderate depression considering that during phase A it had been reduced by nearly half. This was due to the aforementioned increase in $\dot{V}$/g, since $\dot{V}$/n during this phase was decreased by 88% from its control value.

Figure 4 shows the variations in heart rate (HR) and mean arterial pressure (MAP) in the Eupneic group. HR decreases slightly during A, and changes throughout the rest of the phases were minimal. Thus, HR did not at any time differ significantly from the control value. MAP increased slightly during phase A, a change that was not significant. During phase B, MAP fell rapidly below control levels (a

Figure 4.   Variations in heart rate and mean arterial pressure during
            progression of phases in the Eupneic group. Values are
            mean + SEM (n=4 and n=6 respectively). The morphine
            tolerance effect noted by Schmidt and Livingston (40) and
            Evans et al. (13) was not apparent, since arterial
            pressure continued to decrease over the course of the
            infusion. Phases: P = control; A = depression; B =
            periodic breathing; C = recovery from periodicity; D =
            gasping.   * = P<0.05; ** = P<0.01; *** = P<0.005 as
            compared to controls (Dunnett's test).

Heart Rate
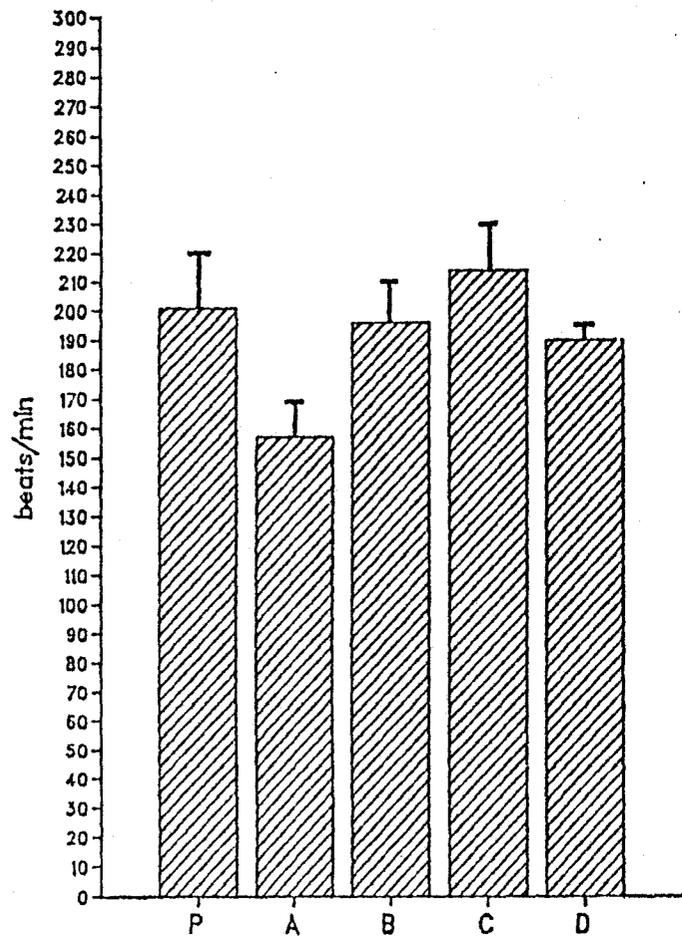
Mean Arterial Pressure
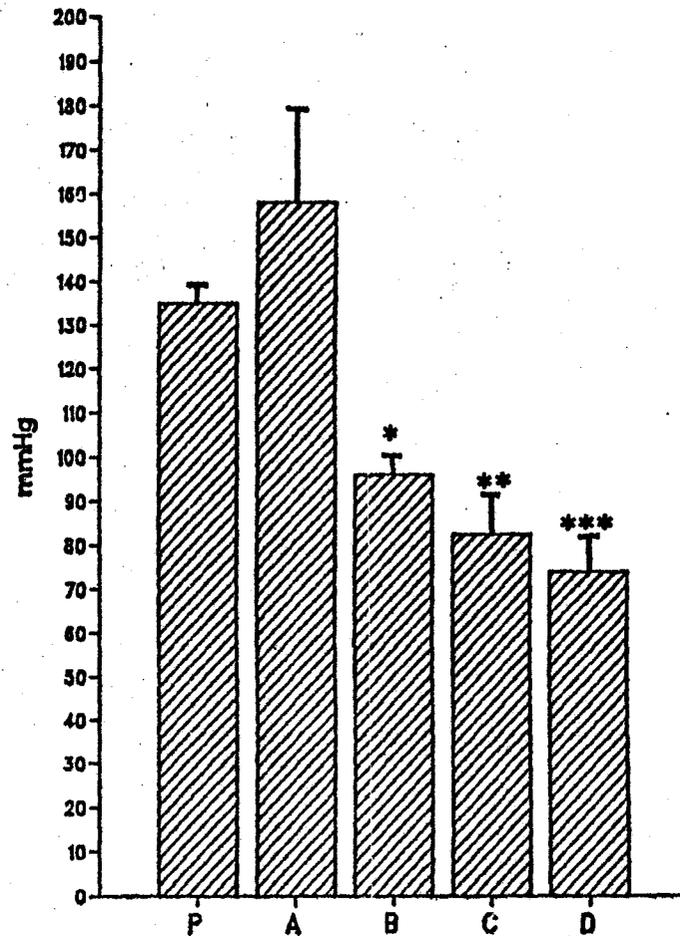
decrease of 29%), a difference that was significant when compared to control (P<0.05). MAP was also significantly different from control during phases C and D (P<0.01 and P<0.005, respectively), falling 39% from control during phase C and 45% during phase D.

C. Alterations in the Apneustic Pattern

The 6 cats comprising this group were all in a functional apneustic pattern prior to the start of the morphine infusion. Upon histological examination of the lesion placement, 2 cats were found to have unilateral, rather than bilateral, pneumotaxic center lesions. In both cats, however, apneusis resulted when the vagi were cut, and the data from these experiments was similar to the 4 cats with bilateral lesion placement. Therefore, these 2 cats were included in the final data analysis. Figure 5 shows a typical brainstem demonstrating histologically verifiable bilateral pneumotaxic center lesions.

Figure 6 illustrates the progression of respiratory changes in an apneustically breathing cat during the course of the morphine infusion. Prior to the start of the infusion, severely prolonged inspiratory holds that are definitive of apneusis are clearly present (phase P). At the start of the infusion, an immediate effect observed was a shortening of the apneustic hold such that the apneustic rhythm was accelerated, i.e., frequency increased (more apneustic breaths, of shorter duration, per minute). Thus, the apneusis was attenuated (phase A). In 2 unsuccessful experiments, where apneusis was allowed to continue for some time before beginning the infusion, the cats

Figure 5.   Destruction   borders   of   lesion   placements   in   the
pneumotaxic   center   of   the rostral pons. The stereotaxic
.coordinates   were: posterior (P) 3.5 mm, vertical (H) -4.0
mm,   and   lateral   (L) 4.5 mm.   This frontal plane section
was   drawn with a camera lucida attachment to a microscope
at   12x   magnification.   Bilateral   lesion   sites   are
indicated   by   the   shaded   areas.   NPBM = nucleus
parabrachiolis medials;   KF = Kolliker-Fuse nucleus; BC =
brachium conjunctivum; BP = brachium pontis.

PNEUMOTAXIC CENTER LESION PLACEMENT

BP

K
F
BC

BC
K
F

BP

NPBM

NPBM

P   3.5

H   -4.0

L   4.5

1 mm
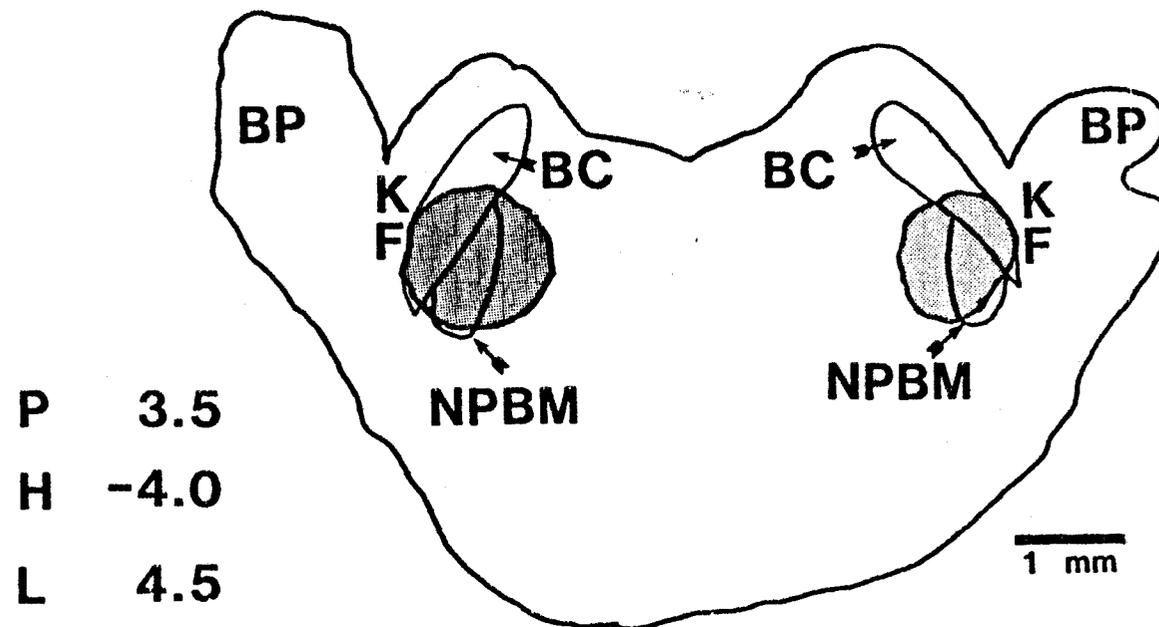
Figure 6.   Progression of phases in an apneustically breathing cat

during constant morphine sulphate infusion (2 mg/kg/minute

i.v.). The start of the inspiratory phase begins with the

downstroke of the pen, and terminates at the upstroke.   In

panel P, therefore, only one complete inspiratory phase is

shown, which is definitive of the prolonged holds of

apneusis.   Ppl = intrapleural pressure; $\dot{V}$ = tracheal

airflow, inspiratin downward; V/T = expired tidal volume.

Phases: P = control (apneusis); A = apneusis attenuation;

B = apneusis recovery towards eupnea; C = apneusis and

gasping; D = gasping.
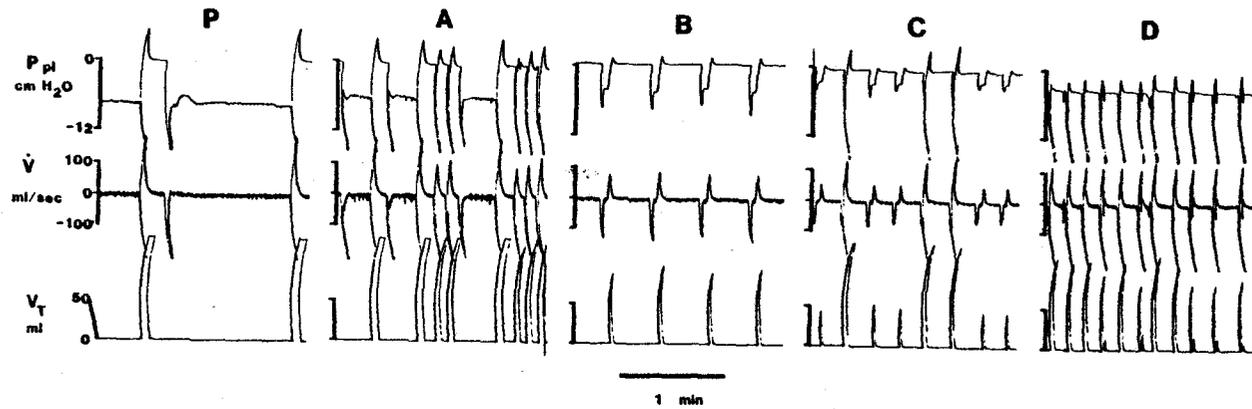
died. Consequently, in 4 out of the 6 successful experiments the infusion was begun almost immediately after apneusis was seen to be established.

Through phase B, the apneusis was diminished. Though inspiratory holds are still present, they are of shortened duration and intensity. The apneustic tendancy continues through phase C, but gasping noticeably appears and is intermixed among the apneustic breaths. During phase D, the apneustic pattern was no longer dominant; instead, as in the Eupneic group, gasping has become quite prominant, and can be viewed as being superimposed on the "normal" apneustic rhythm in these cats.

Figure 7 illustrates graphically the changes in f/n, V/Tn, and $\dot{V}$/n for the Apneutic group. The "normal" pattern referred to in these cats is the apneustic pattern seen before the start of the infusion. During phase P, f/n was minimal, or, in other words, the inspiratory hold was so prolonged that there are nearly zero complete respiratory cycles per minute. V/Tn during phase P was extremely high; this, together with the decreased frequency, constituted a characteristic apneustic pattern. During apneusis attenuation (phase A) f/n was increased, though not significantly, thus accounting for the increase in $\dot{V}$/n seen at this time. V/Tn was minimally affected in this phase.

By phase B, f/n increased significantly over the control value (a 300% increase, $P<0.05$), while V/Tn decreased slightly but not significantly. $\dot{V}$/n was significantly increased over the control phase ($P<0.005$) because of the increase in f/n, reaching its maximum value this phase. V/Tn continued to decrease through the next phase, a

Figure 7. Alterations in normal ventilation and its components during progression of phases in the Apneustic group. Values are mean + SEM (n=6). The normal pattern referred to in these cats is the apneustic pattern seen before the start of the infusion. During phase P, the inspiratory holds are so prolonged that there are nearly zero complete respiratory cycles per minute. Phases: P = control (apneusis); A = apneusis attenuation; B = apneusis rrecovery towards eupnea; C = apneusis and gasping; D = gasping. * = P<0.05; ** = P<0.01; *** = P<0.005 as compared to control (Dunnett's test).

Frequency
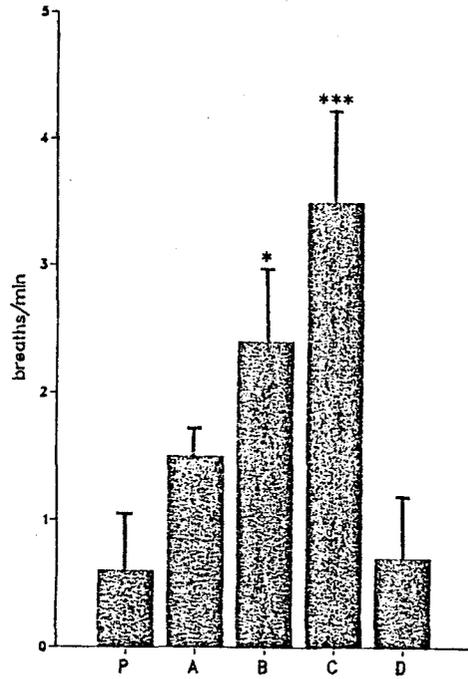
Tidal Volume
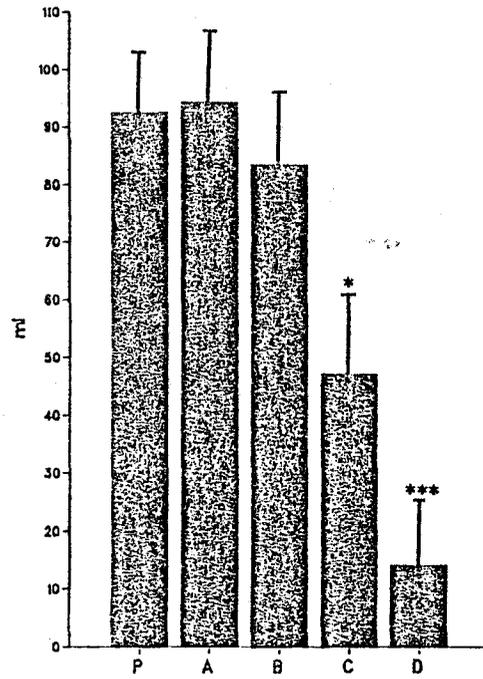
Ventilation
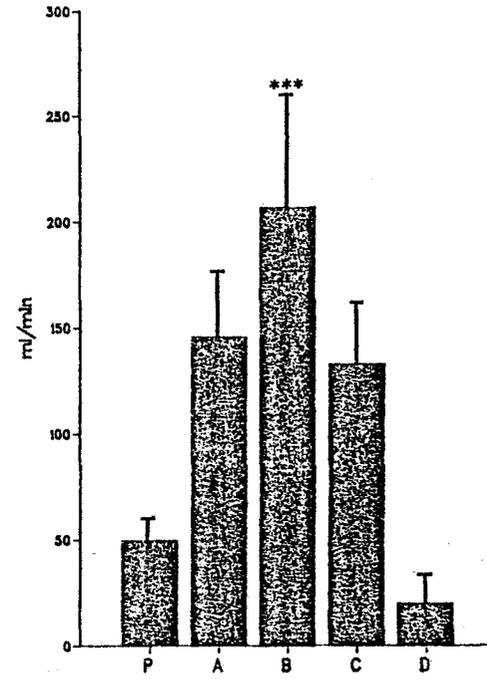
significant drop ($P<0.05$) of 49% from control. However, f/n continued to increase significantly over control ($P<0.005$). The large decrease in V/Tn during this phase is the primary factor determining the decrease in $\dot{V}/n$, such that it was still elevated from its control level but lower than phase B. During the gasping phase D, $\dot{V}/n$ was lower than it was during phase P by 60%; f/n was nearly returned to control level, and V/Tn was reduced significantly ($P<0.005$) even more from the initially elevated value.

Figure 8 presents the changes in f/g, V/Tg, and $\dot{V}/g$ throughout the phases. Gasping gradually increases as the infusion progresses, reaching its peak during phase D. The increase in f/g and V/Tg over control becomes significant during phase C ($P<0.05$), but $\dot{V}/g$ becomes significantly different from control ($P<0.005$) during phase D. By the final phase, f/g increased 279% over control while V/Tg had increased 650%; the increase in V/Tg, thus, accounted for the rise in $\dot{V}/g$ this phase.

$\dot{V}/tot$ differs from control during all phases. The increase in $\dot{V}/tot$ during phase A is primarily due to the 500% increase in $\dot{V}/g$ over control. In contrast, the increase in $\dot{V}/n$ accounts for the increase in $\dot{V}/tot$ during phase B. The continuing increase in $\dot{V}/g$ predominates during phase C, causing $\dot{V}/tot$ to rise further. $\dot{V}/tot$ peaks in the last phase, coinciding with the large increase in $\dot{V}/g$ over control levels which overcomes the large decrease in $\dot{V}/n$.

Figure 9 shows that heart rate was unchanged throughout the majority of the experiment (as in the Eupneic group), though by phase D it had decreased significantly from the control value ($P<0.01$).

Figure 8.   Development of gasping during progression of phases in the

Apneustic   group.   Values are mean + SEM (n=6). Gasping

gradually increases as the infusion progresses, reaching

its   peak during phase D.   Phases: P = control (apneusis);

A = apneusis attenuation; B = apneusis recovery toward

eupnea; C = apneusis and gasping; D = gasping. * =

P<0.05; *** = P<0.005 as compared to control (Dunnett's

test).
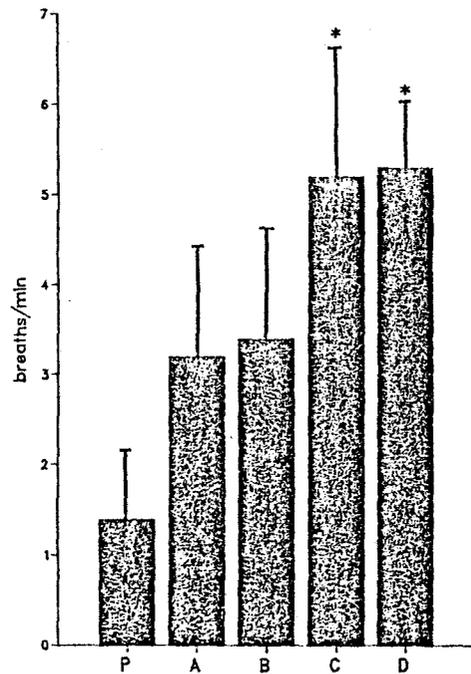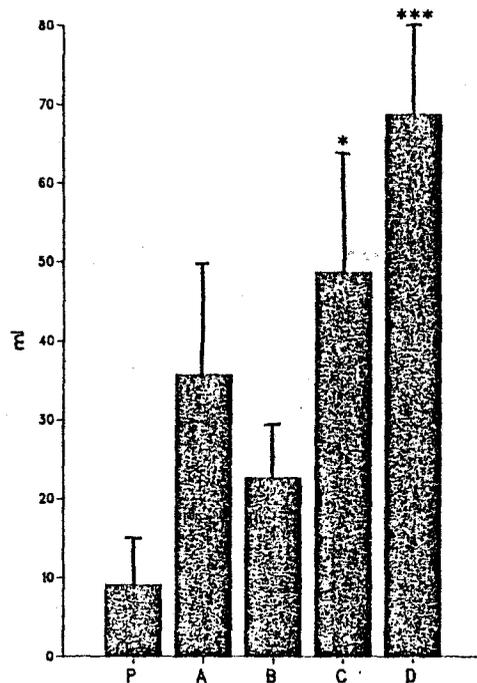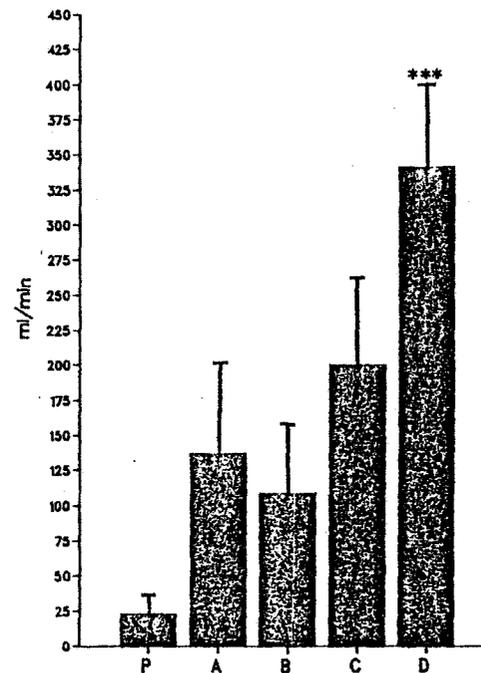
Figure 9. Variations in heart rate and mean arterial pressure during

progression of phases in the Apneustic group. Values are

mean + SEM (n=3 and n=6 respectively). Heart rate was

unchanged throughout the majority of the experiment,

though by phase D it had decreased significantly from the

control value (p<0.01). The high rates probably reflect

the hypoventilation (relative to a normal ventilation)

that existed throughout the course of the infusion. The

decrease in rate is in agreement with the literature and

may be a reflection of a direct depressant effect of

morphine. Mean arterial pressure, which was quite

elevated during phase P, decreased steadily (to a

significant extent) throughout the remaining 4 phases. As

in the Eupneic group, the morphine tolerance effect was

not apparent. Phases: P = control (apneusis); A =

apneusis attenuation; B = apneusis recovery towards

eupnea; C = apneusis and gasping; D = gasping. ** =

P<0.01; *** = P<0.005 as compared to control (Dunnett's

test).

Heart Rate
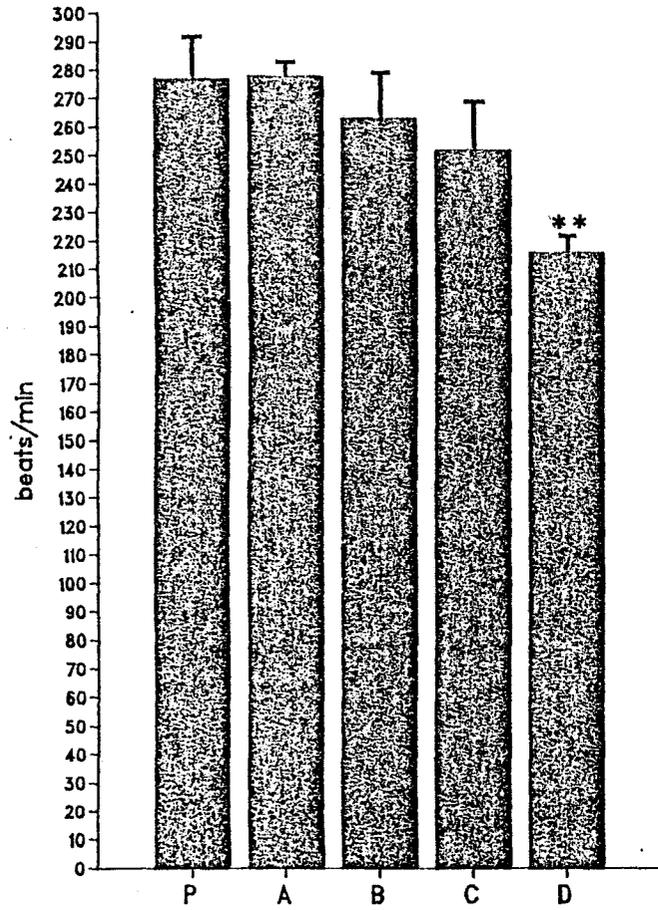
## Mean Arterial Pressure
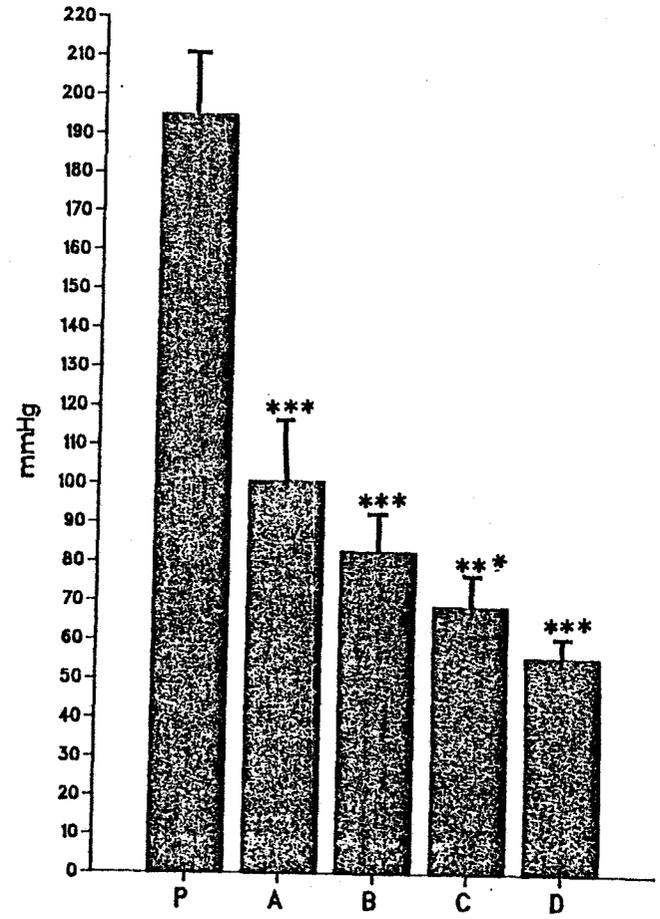
Mean arterial pressure, quite elevated during the control phase, decreased steadily (to a significant extent) throughout the remaining 4 phases.

D. $\dot{V}/g$ as an Increasing Component of $\dot{V}/n$

Table I details the per cent contribution of $\dot{V}/n$ and $\dot{V}/g$ to $\dot{V}/tot$ as the phases progress in both the Eupneic and Apneustic group. It is evident in both groups that as the cumulative dose of morphine increases, the contribution of $\dot{V}/g$ to $\dot{V}/tot$ increases, while that of $\dot{V}/n$ decreases.

Figure 10 is a graphic summary of these changes in non-gasping ventilation $(\dot{V}/n)$, gasping ventilation $(\dot{V}/g)$, and total ventilation $(\dot{V}/tot)$. Before the start of the morphine infusion, only 1 cat among those respiring eupneically had any gasping present. In contrast, gasping was present in 3 out of 6 cats in the Apneustic group. Since total ventilation at this time was minimal in these cats, a probable cause of the gasping was hypoxia. Gasping gradually became superimposed on the normal ventilatory pattern, eupnea or apneusis, as each became depressed. The depression of the normal pattern was quite obvious for the Eupneic group, but depression of the normal pattern in the Apneustic group (that is, apneusis) was more subtle. It was reflected as an increase in non-gasping ventilation, since as apneusis was decreased, the pattern resembled a more eupneic-like type of respiration. In phases C and D, however, the non-gasping ventilation was decreased, reflecting a return of apneusis. Figure 11 shows experimental records from cats in both groups. As shown in Figure 10,

Table 1

Percent contribution of normal breathing and gasping to total ventilation in the Eupneic and Apneustic groups.

		Phase P	Phase A	Phase B	Phase C	Phase D
	Group					
$\dot{V}/n$	Eupneic	100.0%	90.2%	91.6%	89.1%	17.2%
	Apneustic	68.2%	51.5%	65.5%	39.9%	5.5%
$\dot{V}/g$	Eupneic	Neglig.	9.8%	8.4%	10.9%	83.2%
	Apneustic	31.8	48.5%	34.5%	60.1%	94.5%

The normal $(\dot{V}/n)$ and gasping $(\dot{V}/g)$ ventilation values are normalized from mean values given in Appendix A. The contribution of gasping to total ventilation is maximal for both groups during phase D, when the cumulative dose of morphine sulfate is also maximal. See text for definition of phases (page 54).

Figure 10. Comparison of total ventilation, normal ventilation, and gasping ventilation during the progression of phases in both the Eupneic and Apneustic groups. Values are mean + SEM (n=6 for each group). See text for discussion. Phases for the Eupneic group as in Figure 1, and for the Apneustic group as in Figure 6. Graphs of normal and gasping ventilation are composites of Figures 2, 3, 7, and 8. Statistical comparisons were made only within groups, not between groups. * = P<0.05; ** = P<0.01; *** = P<0.005. (Dunnett's test).

Ventilation
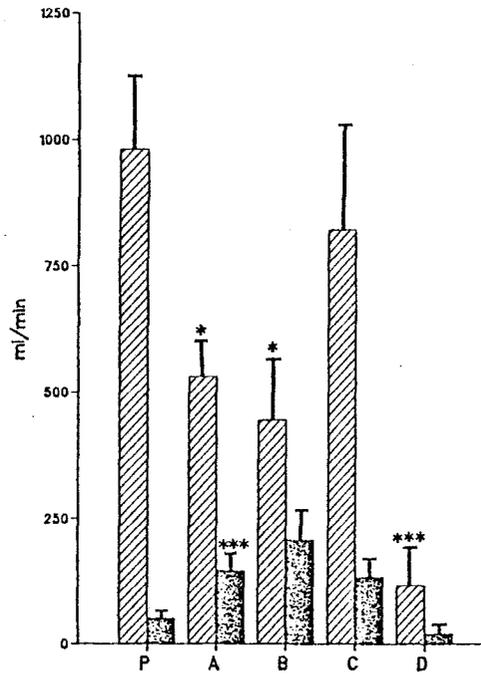
Gasping Ventilation

Total Ventilation

Legend
Eupneic
Apneustic
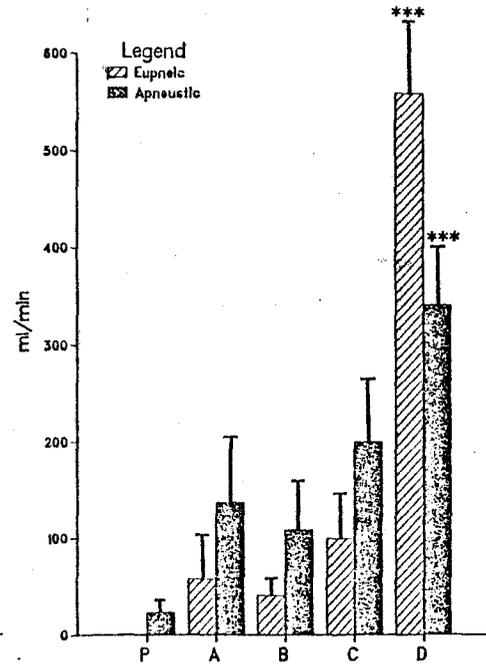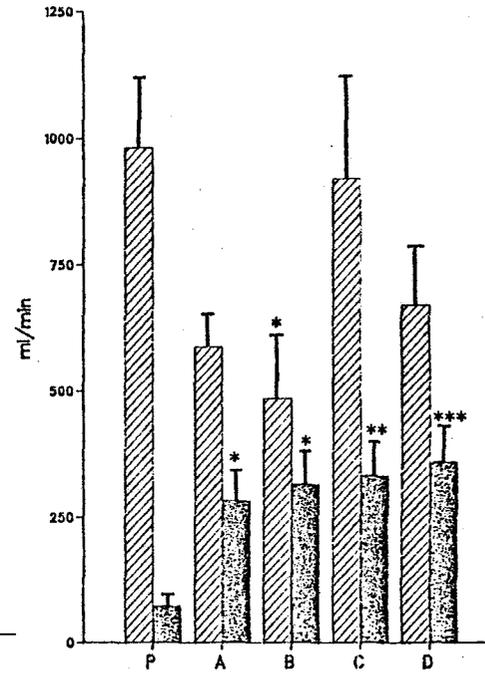
Figure 11. Superimposition of gasps on eupneic or apneustic breaths. The experimental tracings illustrate the combination of eupnea and gasping (left), and apneusis and gasping (right), occurring during phase C for both groups. Ppl = intrapleural pressure; $\dot{V}$ = tracheal airflow, inspiration downward; V/T = expired tidal volume.

EUPNEIC APNEUSTIC

P_{pl}
$cm H_2O$

0

−12

$\dot{V}$

ml/sec

100

0

−100

V_T
ml

50

0

1 min
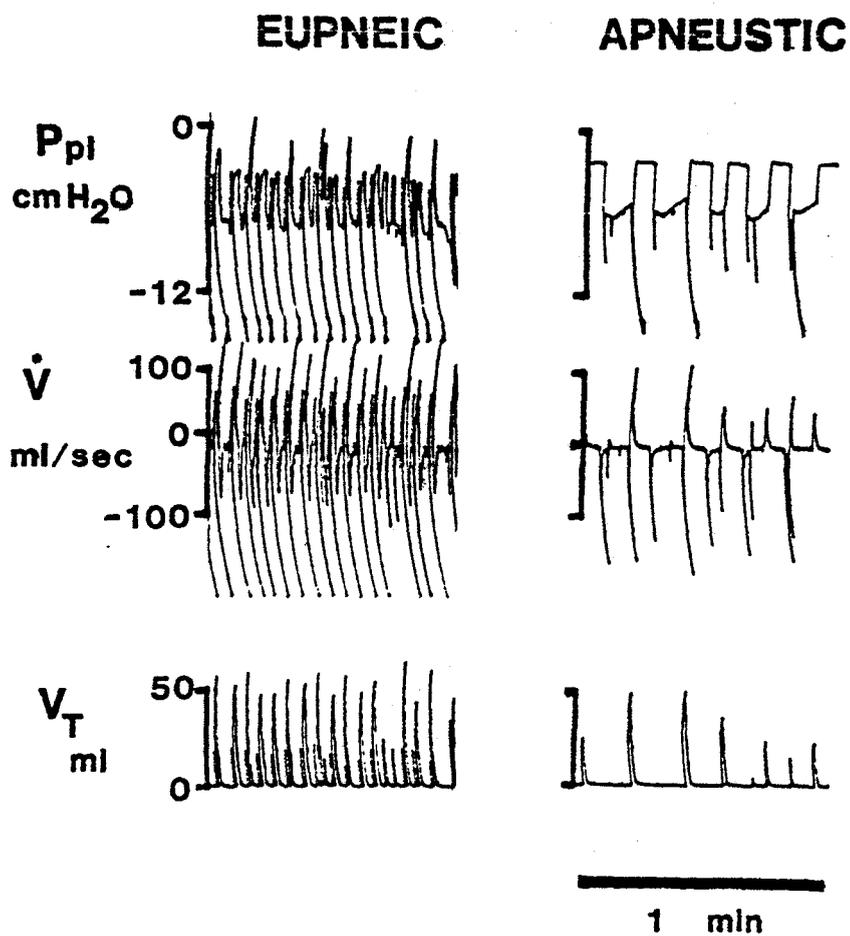
the increase in gasping ventilation as the phases progress was similar for both groups. Likewise, as seen in Figure 11, imposition of gasping upon the normal pattern was similar in both groups, although their normal patterns are so dissimilar. The gasps occurring during the apneustic holds are especially intriguing, because the two types of ventilation are at opposite ends of the spectrum as regards respiratory timing. Gasps, regarded as having almost instantaneous inspiratory durations (Ti times), were superimposed on inspiratory holds of much longer duration. Thus, two distinctly separate types of pattern generation were occurring simultaneously.

CHAPTER V

DISCUSSION

In a recent study, Hug et al. (22) concluded it is not possible to relate any specific concentration of morphine in the plasma or cerebrospinal fluid to a given intensity of ventilatory depression. Their studies demonstrated changes in morphine levels in plasma do not indicate proportional changes in its concentration in the central nervous system, the site of morphine's respiratory actions. For this reason, measurement of plasma levels of morphine was not performed in this study. Consequently, no attempt is made to correlate a specific dose level with an observed effect.

A. Literature Summary

1. Respiratory Effects of Morphine and Related Substances

Earlier studies dealing with morphine and its effects on ventilation are difficult to assess largely due to their ancedotal nature. Often, the number of animals and morphine dosages (per body weight) were not indicated (7,39). The type of anesthetic (and dose) used for a particular experiment reported was not always clear. Measurements of ventilation, tidal volume and frequency were crude, and averaging of results and statistical comparisons not done.

Beginning with the study of Dripps and Dumke (11), the papers reviewed start to become somewhat more quantitative than observational. The number of animals used for each procedure was

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noted, dosages were expressed in terms of body weight, and the possible influence of the anesthetic used considered. The data presented was normalized and, at times ancedotal in nature, and the overall presentation had a "semi-qualitative" approach. This was evident in the work of Breckenridge and Hoff (4) and Ngai (31).

Of the papers cited, Kokka in 1965 (26) represented the first quantitative presentation of the type which has come to be expected in the literature today. Measurements of ventilatory parameters were more sophisticated, and data were averaged and statistically evaluated. However, with the emergence of more technically intricate experiments (such as the microinjection of opioid agonists into discrete areas of the brain, and microelectrophoretic application of agonists to extracelluarly recorded neurons), the presentation of data again, at times, bordered on the ancedotal. Though technically sophisticated, upon reflection, experiments of this type are inherently crude, for the investigator is unable to determine accurately the concentration of the compound at the neuronal surface. The question then arises as to whether or not physiological doses of agonists - or antagonists - are being examined.

Though the major weakness of the earlier studies was the presentation of data in a descriptive manner, that is also their major strength. Ending with Ngai's study (31) in 1960, most of the papers reviewed present pages of actual experimental records, and detailed observations made during the course of the experiments. The concept of the organization of the respiratory controller used in interpretation of the results is presently different from that in

earlier studies. However, the presence of these original records allows the earlier studies to remain instructive.

One problem common to all studies is the superposition of the depressor effects of opiate agonists upon anesthesia. Unanesthetized freely-moving animals, however, present a whole different set of problems, especially since the opiates are known to be influential in a variety of behavioral activities. Though not without its own problems, the use of the decerebrated animal largely avoids the problems associated with anesthesia. The complicating effects of anesthetics are seen in a study by Urca and Liebeskind (see 25). Morphine had an inhibitory effect on periaqueductal neurons in anesthetized animals, but an excitatory effect in unanesthetized animals.

A possible complication in many of the whole animal studies dealing with opioids is the common method of administration: bolus injection. Agents administered in this way can cause an initial respiratory and circulatory depression so severe (depending on the dose), that the physiological state of the preparation may be compromised throughout the remainder of the experiment. The same dose given in repetitive, small bolus injections may have different effects than when administered as one bolus (6).

Lastly, in assessing the respiratory effects of morphine and the endogenous opiates, few of the recent papers presented recognize the fact that a number of different kinds of receptors for these substances exist. Two classes of opiate receptors shown to exist are: (a) the "mu" receptors, or morphine receptors, which preferentially

bind morphine and the endorphins; and (b) the sigma receptors, or enkephalin receptors, which preferentially bind enkephalins (41). Thus, if enkephalins (or their analogs) are used in one experiment, it may not be possible to extend the conclusions from such a study to encompass the effects of all opioid substances. The hope that Oorphine itself could give insight into the role of all endogenous opiates in respiratory control may, therefore, not be entirely fulfilled. Also, multiple forms of opiate receptors require different concentrations of naloxone to antagonize effects mediated by these receptors. Many recent investigators assume the effects of naloxone are due to blockade of the receptor, but there have been reports which indicate that naloxone may have pharmacological actions unrelated to opiate receptor blockade. This observation emphasizes that antagonism by naloxone is a necessary, but not sufficient, criterion for invoking the mediation of a response by an endogenous opiate (a fact too often ignored by investigators in drawing conclusions from studies utilizing naloxone) (38).

Since the publication of Krueger et al. in 1941 (27) and Reynolds and Randall in 1957 (34), it has been accepted in the literature that the effects of morphine on respiration are very much dependant upon the species studied and the experimental conditions under which the study is conducted. A summary of the literature reviewed in the literature section, part A, is presented in Table 2. In general, the predominant effect in a given species depends upon the dose and the resistance of respiratory (and cardiovascular) centers to depression. Upon closer examination, however, similarities do exist.

Table 2

Summary of the literature reviewed dealing with the effects of morphine and related substances on the respiratory system. The following abbreviations are used in this table:

BE	beta-endorphin	MI	microinjection
C	cat	MPK	mg/kg
D	dog	NE	no effect
E	ether	RT	rat
F	fentanyl	S	species
f	frequency	SAS	subarachnoid space
IC	intracisternal	SC	subcutaneous
IM	intramuscular	SMLD	small dose
IV	intravenous	Ti	inspiratory duration
IVT	intraventricular	Te	expiratory duration
LRGD	large dose	U	urethane
MCD	maximum cumulative dose	$\dot{V}$	ventilation
		V/T	tidal volume

Investigator	S	Anesthesia	Drug	Route of Admin.	Effects on f	Effects on V_T	Overall Effect on $\dot{V}$	Effect on Response to CO_2
Wood & Cerna 1892	D	?	M LRGD	IV	?	?	near cessation	
		?	M cum. dose	IV	↑	↑	↑ convulsions	
Filehne 1879	R	?	M	IV	slowed first ↑-convulsion	?	slowed, then ↑ during convulsions	
Cushny 1913	R	DC	M 1-2 MPK	IV	↓	↓ but not to same extent as f	reduced	depressed
		DC vagot.	M 1-2 MPK	IV	↓	↓ but not to same extent as f	reduced	depressed
	C	DC	M 1-2 MPK	IV	↓	↓ but not to same extent as f	reduced, more strongly than in rabbit	
Barbour 1914	C	E vagot.	M 10 MPK	IV	↓	may or may not ↑	periodic character which may not appear w/repeated doses	
Jackson 1914	D	E, then pithed	M LRGD	IV	?	?	constriction of bronchiolar muscle; periodic respir. w/repeated doses	

Investigator	S	Anesthesia	Drug	Route of Admin.	Effects on f	Effects on V_T	Overall Effect on $\dot{V}$	Effect on Response to CO_2
Cushny & Lieb 1914	R	U	M 3 MPK	IV	↓	?	↓, but blood CO_2 unchanged until f very depressed	
	C	DC	M LRGD	IV	↑	?	↑; convulsions present	
			M SMLD	IV	↓	?	↓	
Schmidt & Harer 1922	C	DC	M 1-5 MPK	IV or IM	↓	↑	↓	depressed
		DC vagot.	M 1-5 MPK	IV or IM	apneustic tendancy	?	Expiration active rather than passive	depressed
		DC vagot.& non-vagot.	M suc. doses 5-10 MPK	IV or IM	↑	?	Expiration active w/LRGD's; convulsions	After 60 mg f ↑ in response to CO_2
Dripps & Dumke 1943	D C	DC	M MCD 16 MPK	IM	?	?	depressed	depressed but to a > extent in C than D
Breckenridge & Hoff 1952	D	Unanesth.	M	IV	slight, ↓	slight ↓	tachypnea prior to depression; post-sigh inhibition	

Investigator	S	Anesthesia	Drug	Route of Admin.	Effects on f	Effects on V_T	Overall Effect on $\dot{V}$	Effect pm Response to CO_2
Breckenridge & Hoff 1952 (cont'd.)	D	DC non-vagot.	M	IV	↓	↓	post-sigh inhibition; depression stronger than in unanesthetized dog	
		DC (apneustic)	M	IV	↑ (↓ in T_I)	?	acceleration of apneustic rhythm	
		DC upper med. sec.; vagot.	M	IV	↓	↓	short period of tachypnea, followed by sighing rhythm	
Ngai 1961	C	DC vagot.	M 1-2 MPK	IV	↓	↑ or NE	↓	
		DC rost. pons sec.; vagot.	(15 MPK MCD)	IV	↓	↑	apneusis and gasping not observed	
Kokka et al. 1965	R T	Unanesth.	M 5 MPK	IV	NE	↓	↓	
			M 10MPK 20MPK	IV	↓	↓ but to > extent than f	↓	
			M 720 MPK	IV	↓–as dose ↑, this caused ↓ $\dot{V}$	↓, but < marked than @ lower dose	not as depressed as with 10-20 MPK	
Florez et al. 1968	C	Dial-U 0.7 ml/kg, IP	M 50µg1 50 µl	IVT-4th ventricle; SAS	↓	NE	↓	depressed

Investigator	S	Anesthesia	Drug	Route of Admin.	Effects on f	Effects on V_T	Overall Effect on $\dot{V}$	Effect on Response to CO_2
Florez et al. 1968 (cont'd.)	C	Dial-U 0.7 ml/kg IP	M 50µg1 50 µl	IV	↓= to injec. into 4th vent.or SAS	NE	↓ = ↓ from 4th vent. and sub-arch. space	depressed
				IVT 3rd	↑	↑ 1st 5 min, then ↓ below control	↓	improved f response
Florez et al. 1972	C	DC	M 2 MPK	IV	↓	↓	↓	depressed
			M 2 MPK 4 MPK MCD	IV	↓ but minimum values not < than aft.1st dose	↓ but minimum values not < than aft.1st dose	↓	depressed
Florez & Mediavilla 1977	C	PB-U (25 MPK- 500 MPK)	ME 1.6 µmole	MI vent. surface brainstem	less affected than V_T	↓	↓ for very short duration	depressed & remained so $\dot{V}$ ↑
Denavit- Saubie' et al. 1978	C	isolated resp.ctr. prep.	M	phoretical-ly applied to RRU's	–	–	majority of RRU's depressed; some excited	
		isolated resp.ctr. prep; paralyzed	M 0.1- 0.5 MPK	IV	↓ (measured by phrenic nerve dis-charge)	↑ (measured by phrenic nerve dis-charge)	Gasping or apneu-sis not observed	
Beubler 1980	R	U 1.4 g/kg IP	M 2 MPK	IV	↓	↑	↓	depressed

Investigator	S	Anesthesia	Drug	Route of Admin.	Effects on f	Effects on V_T	Overall Effect on $\dot{V}$	Effect on Response to CO_2
Zobrist et al. 1981	R T	Unanesth.	ME 50µg	IVT	↓	↓ but to > extent than f	↓	
Pokorski et al. 1981	C	PB 30 IP	F	topical application to L&S areas	S – NE L – NE	S – ↓ L – NE	–	
Moss & Scarpelli 1981	D	PB 25 MPK	BE 15 nmol.	IC	max. ↓ @ 75 min. ↑ in T_E	↓ max. w/in 15 min; recovered by 75 min.	max. depression @ 75 min (↓ in f major contributor)	depressed
		PB 25 MPK vagot.	BE 15 nmol.	IC	insignif. change	↓	↓	

Almost unequivically, the respiratory response to $CO/2$ is reduced. Also, smaller doses of morphine produce different effects than larger doses. In both the dog and cat, there is evidence that morphine can alter apneusis to some extent. The acceptance of morphine as a respiratory depressant masks the complexity of the morphine action pattern that is evident in the literature.

2. Cardiovascular Effects of Morphine and Related Substances

Much of the foregoing discussion concerning the effects of previous studies dealing with the respiratory effects of opiates apply when assessing studies dealing with cardiovascular effects, and will therefore not be reiterated here. However, an additional factor not yet considered is the degree of interaction between the respiratory and cardiovascular systems, that is, to what degree will the response of one system to an opiate affect the response of the second system.

Concern about system interactions have been minimal. The separation of both literature and discussion into respiratory and cardiovascular effects of morphine and related substances is admittedly artificial, but it is a division which has persisted in the literature. For example, of the literature reviewed in this thesis, few investigators (18,30) examined both respiratory as well as cardiovascular effects of the opioids in any detail. Partly to blame for this existing separation is the relative lack of quantitative knowledge concerning the amounts of interaction occurring between the two systems, thus allowing only for a qualitative estimation.

In this study, significant detail has been paid to both the respiratory and cardiovascular changes occurring over the course of

the constant i.v. infusion of morphine, though the primary focus has been on alterations of respiratory patterns. Some of these alterations that were observed in this study, however, may be the direct result of morphine's action(s) on the cardiovascular system (see following discussion). Therefore, although this interaction between the two systems has yet to be quantitated, it may be a key consideration when explaining the actions of morphine on the cardiopulmonary system as a whole.

Table 3 is a summary of the related cardiovascular literature reviewed in Literature, part B. Generally, morphine and related substances appear to cause some degree of hypotension (sometimes in the cat preceded by a transient rise in blood pressure). The effects on heart rate have not been as well characterized, and it appears that they may depend on the route of administration. In contrast to the microinjection studies dealing with the respiratory effects of centrally administered opiates, interest has begun to focus on more discrete areas in the brainstem that may be mediating the cardiovascular effects of these substances. It may be that localization of these areas will precede that of the areas mediating respiratory effects, because of the diffuse nature of the respiratory controller network.

B. Alterations of Respiratory and Cardiovascular Variables in Eupneically Breathing Cats

1. Morphine effects on breathing frequency and tidal volume

The specific effects on morphine on f and V/T are not clear,

Table 3

Summary of the literature reviewed dealing with the effects of morphine and related substances on the cardiovascular system. The following abbreviations are used in this table:

BE	beta-endorphin	MI	microinjection
BP	blood pressure	ME	met-enkephalin
C	cat	MEA	met-enkephalinamide
CL	chloralose	MPK	mg/kg
D	dog	NE	no effect
DA	direct application	PB	pentobarbital
DC	decerebrate	PIP	perfusate-isolated preparation
DD	dose-dependant	RI	results inconsistent
F	fentanyl	S	species
GP	guinea pig	SC	subcutaneous
IC	intracisternal	SP	spinal
IV	intravenous	trans.	transient
IVT	intraventricular	U	urethane

Investigator	S	Anesthesia	Drug	Route of Admin.	Effects on the Heart	Effects on BP	Other Observations
Gruber & Robinson 1929	C	—	M low & high dose	PIP	low dose – ↑ force of contraction high – ↓	· –	
Schmidt & Livingston 1933	D C	DC, SP, U ether phenobarb.	M 0.5 & 1-2 MPK	IV	?	↓ both doses	
Evans, Nasmyth, & Stewart 1952	C	CL-80 MPK atropine vagoto-mized	M 4 MPK	IV	↑ rate w/in 2-3 min, then ↓ lasting several hours	trans ↑, then ↓ lasting several hours	
		DC	M 4 MPK	IV	?	↓	Fall in BP, lasting 10-15 min
		SP	M 4 MPK	IV	?	↓	↓ of lesser magnitude and trans.
		CL-80 MPK mepyramine	M 4 MPK	IV	?	↓	↓ in BP profound & prolonged
		DC	M 4 MPK	IV	?	↓	↓ in BP < than ½ normally attained
		SP & mepyramine	M 4 MPK	IV	?	NE	

Investigator	S	Anesthesia	Drug	Route of Admin.	Effects on the Heart	Effects on BP	Other Observations
Grundy 1971	GP	–	M	PIP	trans. initial stim	–	
Florez 1979	C	PB 25 MPK U 500 MPK	ME 1.6 µmole	DA, ventral surface of brainstem	↓	↓	
Laubie 1979	D	CL 100 MPK	MEA 0.01–1 µg	MI into NA	↓ – DD	↓ when HR is markedly ↓	unilateral injection
			F 1 µg	MI into NTS	NE	NE	unilateral & bi-lateral injection
			F 0.05–1 µg	MI into NA	↓ – DD	↓	unilateral injection; no change in symp discharge
			F 0.05–0.2 µg	MI into NA	↓	NE	
Wallenstein 1979	C	PB 15–20 MPK;u 600–800 MPK; nitrous oxide	M 1 MPK	IV	NE	long-lasting ↓	↓ before trans.↑
			M 8–12 µg/kg	IV	↑	↑	↓ HR, ↓ BP before ↑
		PB 10 MPK adrenalect.	M–1 µg/kg	IV	↓	↓	no trans.↑ preceding↓

Investigator	S	Anesthesia	Drug	Route of Admin.	Effects on the Heart	Effects on BP	Other Observations
Wallenstein 1979	C	PB 10 MPK adrenalect.	M-1 µg/kg	IV	RI	RI	
Feldberg & Wei 1981	C	CL	M 400 µg	IVT	tachycardia	small, trans. ↑	
			M 100-400 µg	IC	↓	↓	Both effects long-lasting
			M	SC	↓	↓	
			ME 1.6 µM	IVT	NE	trans. ↓	
			BE 400-800 µg	IVT	tachycardia	NE	
			BE 200-400 µg	IC	↓	↓	Both effects long-lasting
		CL-vagot-omized	M 400 µg	IVT	tachycardia	?	
		CL-stellate removed	M 400 µg	IVT	NE	?	
Moss & Scarpelli 1981	D	PB as MPK non-vagot.	BE 15 nM	IC	↓	↓	
		PB as MPK vagot.	BE 15 nM	IC	NE	↓	

although in most studies, f decreases (see Table 2). Convulsions, resulting in an improved ventilation due to increases in f, were noted by early investigators such as Wood and Cerna (see 7), Filehne (see 7), Cushny and Lieb (8), and Schmidt and Harer (39) when the cumulative morphine dose was large. The effects of V/T are less certain; the depression of V/T has been described as being both greater than or less than the depression of frequency. Interest in which of these two parameters' was more affected by morphine has continued throughout the years, for it was hoped that the overall depression of ventilation by morphine could be explained on the basis of a simple depression of f and/or V/T.

The results of the current study may help to shed a new light on the discrepancies in the literature as regards the effects on f and V/T. Maximum cumulative doses of morphine in most bolus injection studies appear not to have exceeded 20 mg/kg, in contrast to the totals reached by constant infusion in this study (Eupneic group: 135 +/- 28 mg/kg; Apneustic group: 166 +/- 23 mg/kg). An arbitrary division of previous studies can be made, such that those studies in which effects are produced by small, cumulative doses of morphine (<15 mg/kg) are viewed independently of those studies dealing with larger cumulative doses of morphine (>15 mg/kg) (studies dealing with microinjection/iontophoresis will be examined later). A spectrum of ranges of bolus injections has thus been constructed, with two general divisions: "low" and "high".

In the bolus injection studies in which low doses of morphine were administered, f and V/T were reported to be decreased, f

apparently being the more affected of the two variables. Those studies in which higher doses of morphine were employed, reported f to be increased, and convulsions (along with gasping) to be present. These observations correlate well with phases A and D, respectively, seen in the Eupneic group in this study. During phase A, when the cumulative levels of morphine were still low, both f and V/T were depressed to a nearly equal extent. Through phases B and C, however, it would appear that f indeed was the variable affected more by the increasing morphine level. The stimulation of ventilation by higher doses of morphine reported in the literature parallels phase D, during which the $\dot{V}$/tot is not significantly different from control due to the large increase in $\dot{V}$g. Convulsions were often observed during this terminal phase, in agreement with other studies (7,8,39).

The transition between the effects of low and high doses of morphine might be the effects noted during phases B and C. The assumption of a periodic character in phase B agrees with the observations of Barbour (1) and Jackson (23); the disappearance of the pattern as the cumulative doses of morphine rises (as phase C begins) correlates with a similar observation also made by Barbour (1). The decrease in f/n and the increase in V/Tn noted in phase B (which led to a much reduced $\dot{V}$/n) may correspond to the later observation of Schmidt and Harer of a decreased f and increased V/T (though they made no mention of the nature of the pattern at the time the measurement was made; morphine dose was 1-5 mg/kg).

Phase C apparently has no correlates in the literature, possibly due to the inherent differences between the bolus injection and

constant infusion technique. Obvious parallels have been drawn between the current study and previous studies, regarding the effects observed at low and high doses of morphine. However, because during bolus injection studies the transition from low to high doses was nearly instantaneous, rather than gradual as in this infusion study, those effects marking the transition may have been lost (perhaps due to other factors, such as the marked depression of circulation that can occur with larger bolus injections). The constant infusion technique stretches out the spectrum of effects, allowing phases of different ventilatory patterns to be distinguished.

Studies employing discrete injections of morphine or related substances have failed to provide more than a gradual description of the possible sites of action mediating respiratory effects of the opioids. Apparently, however, met-enkephalin and beta-endorphin (in the amounts tested) both cause depression of f and V/T (V/T being the more affected of the two variables) when injected at the ventral surface of the brainstem (18), intraventricularly (17), or intracisternally (30). Only one microinjection study employed morphine (17); when injected into the fourth ventricle and subarachnoid space, a decrease in f with no effect on V/T was noted. Allowing for differences between the studies, it is apparent that the opioid substances (in the doses tested) exert a similar effect on f.

The depression of f and V/T in phase A of this study possibly correlates with the effects of the endogenous opiates cited above; indeed, Florez et al. (17) found an intraventricular injection of morphine to exert a similar effect as that which was injected

intravenously. The similar effects may be due to the substances acting at similar sites in the lower brainstem; a more exact localization of such sites will have to await further studies.

Alterations in the eupneic pattern by morphine as seen in this study do not lend themselves to speculation regarding possible mechanisms in terms of the inspiratory off-switch model of the respiratory controller because changes in the duration of inspiration were not quantitated. A qualitative evaluation of such changes, as will be made in the discussion concerning the effects of morphine on the apneustic pattern, is not as easily accomplished in eupneically respiring cats. Some general remarks, however, contrasting eupnea with gasping in terms of the off-switch model will be made in a following section.

The actions of morphine on f and V/T in eupnea are more complex than previously recognized by most investigators. Kokka's remark, "it appeared a separation of the respiratory response to morphine was exhibited with increasing doses; the depression of ventilation predominated at the lower doses, but was minimized at higher doses" (26) is apropos in light of the present results. In this study, the depression of ventilation apparent in the earlier phases was overcome by the gradual increase in gasping ventilation. The two types of respiratory activity that occurred simultaneously in phase C (eupnea, gasping) would appear to be the result of two distinct and separate kinds of activity. As such, the first type of inspiratory activity would determine eupneic respiratory cycles. A second type of inspiratory activity, apparently activated as the cumulative levels of

morphine increased, would determine the gasping ventilation. By the final phase D, it would appear as though the first type of activity had disappeared, since gasping predominated during this phase. Discussion concerning the second type of activity will be deferred to a later section.

2. Morphine effects on blood pressure and heart rate

During the initial phase of the morphine infusion (as when the cumulative level of morphine was low), a non-significant increase in MAP over the control level was seen; this was followed by a significant decrease in MAP, lasting the remaining three phases. The evidence in the literature of morphine-induced catecholamine release from the adrenal medulla (13,43) would account for this immediate, transient pressor response. The gradual hypotension that developed over the course of the infusion agrees with the observations of Wallenstein, with the exception that he observed this prolonged decrease in MAP to occur at low levels of morphine (43). Also in agreement with the present results are the studies of Feldberg and Wei (14,15), in which both intracisternal and intravenous injections of morphine produced a gradually developing fall in MAP. The "morphine tolerance" effect noted by Schmidt and Livingston (40) and Evans et al. (13) was not apparent, since MAP continued to decrease (rather than recover to control levels) over the course of the infusion.

The slight decrease in HR noted during phase A need not have been an opioid-induced bradycardia; HR may have been slowed reflexly due to the slight increase in MAP. Over the next three phases, though MAP was declining by a significant amount, HR did not increase above

control levels, probably because rates were quite elevated already. The minimal changes in HR are more difficult to reconcile with the results of previous studies; only Wallenstein (43) reported a transient decrease in HR followed by an increase in rate, an effect observed with larger doses of morphine. Feldberg and Wei (14,15), whose studies showed changes in MAP similar to those seen in the present study, observed a long-lasting fall in HR, though a tachycardia (due to increased sympathetic discharge to the heart) could occur if morphine and beta-endorphin were injected intraventricularly. Moss and Scarpelli (30), in non-vagotomized dogs, had observed a decrease in HR (in addition to the decrease in MAP) upon intracisternal administration of morphine (an effect abolished by vagotomy).

Two suggestions have been put forth previously as to possible mechanisms for these effects. The first hypothesis is a decrease in sympathetic outflow to the heart and circulation (13,14,15,43). Thus, MAP declines in spite of an increasing $PACO_2$ and decreasing PAO_2 level, which would be expected to increase sympathetic outflow to certain circulatory beds as a consequence of chemoreceptor activation. The slowing of HR, due to : (a) direct depressant effects of morphine on the heart itself; (b) chemoreceptor activation (in the absence of an increased ventilation); (c) a combination of (a) and (b), would therefore be reinforced by a decrease in sympathetic tone to the heart. A second hypothesis is that central vagal pathways are facilitated alone (28,29) or in combination with a decrease in sympathetic outflow (thus decreasing MAP and HR; 30) or an increased

sympathetic discharge (transient pressor response which is centrally and peripherally mediated; 14,15,43).

The changes in MAP seen in the Eupneic group are consistent with either of the two proposed mechanisms. The minimal changes in HR, however, are more difficult to assess. During phases B and C, as V/tot is increasing toward control levels, HR is also increasing. The progressive increase in V/tot (which is not necessarily a consequence of chemoreceptor activation, discussed subsequently) may be sufficient to induce small increases in HR; a reduction in sympathetic outflow, with or without a facilitation of vagal pathways, would then counterbalance the increase. The net result, then, would be an insignificant change in HR during phases B and C. A small decrease in HR during phase D, concurrent with the decrease in V/tot this phase, may reflect the absence of the reflex increase, thus leaving whatever retarding influences there were to predominate.

To what extent the changes in cardiovascular variables are due to changes in arterial gases as opposed to direct effects of opiates is not clear in the literature. The view of Moss and Scarpelli (30) does not account for the interaction of primary effects (produced in each system through discrete and separate pathways) to produce secondary effects. In the present study, the hypercapnic hypoxia gradually developing over the course of the infusion may activate either chemoreceptors (if still functional) or the "gasping" respiratory controller (see subsequent discussion); either could induce reflex circulatory changes. This does not exclude, however, the possibility of direct effects of morphine on the cardiovascular

system by either central mechanisms (effect on brainstem cardiovascular centers) or peripheral mechanisms (direct depressant effect of morphine on the heart or release of catecholamines form the adrenal medulla).

C. Alterations in Respiratory and Cardiovascular Variables in Apneustically Breathing Cats

1. Alteration of the apneustic pattern

The apneustic pattern is characterized by inspiratory activity which starts normally, but does not decline abruptly when it reaches its maximum level. Instead, it continues at a plateau level for several seconds to minutes (5,45). Thus, an imbalance exists between inspiratory and expiratory phase durations (Ti and Te, respectively) such that Ti is greatly prolonged. In contrast, in the eupneic pattern timings of Ti and Te are of the same order of magnitude, the Ti/Te ratio being 0.5-0.7 (5).

The crucial region in the dorsolateral pons whose activity must be disrupted to induce apneusis in vagotomized cats is the nucleus parabrachialis medialis (NPBM). Apneusis can be abolished or markedly diminished by additional transections at the border of the pons and medulla, intermediate pontine levels (partial reversal) or limited midline lesions at the pontomedullary border. Such results, as well as electrophysiological studies (5), have suggested the existence of a pontine "apneustic center" that can promote apneusis if released from rostral and vagal afferent influences.

Alternatively, apneusis can be modeled in terms of the

"inspiratory off-switch mechanism" (5,45). Briefly, a centrally generated inspiratory activity (CIA) (originating at an undefined site within the brainstem respiratory controller) is received by inspiratory cells in the ventrolateral nucleus of the solitary tract, which also receive input from pulmonary stretch receptors via vagal afferents. CIA is also received by respiratory motorneurons of the spinal cord. Efferent activity from this pool of summated inspiratory and vagal activity is conveyed to a "switch neuron pool"; when the threshold ("off-switch threshold") of these neurons is reached, the inspiratory activity generation is inhibited and inspiration terminated (5,45). The off-switch threshold appears to be independent of vagal input, so that the continuously increasing CIA signal alone will eventually cause the inspiratory cells to reach off-switch threshold. At any time earlier than this, a sufficient lung inflation signal can sum with the CIA and cause the cells to reach off-switch threshold early.

Cells in the pneumotaxic center fire tonically during normal (vagus-intact) breathing, and this output feeds into the inspiratory off-switch. When the NPBM is lesioned, inspirations are prolonged; thus, it appears the off-switch threshold is raised. The NPBM output, then, seems to directly sum with the CIA and vagal afferent activity to achieve threshold. Eliminating both the NPBM and vagal inputs to the switch neuron pool would cause inspiration to be prolonged (apneusis) because threshold would take even much longer to attain.

The occurrance of apneusis can be influenced by several peripheral and central inputs. For example, increases in body

temperature (carefully controlled in the present study) have been shown to produce substantial reductions in respiratory duration, converting an apneustic to a eupneic pattern (5). The influence of elevating PCO_2 however, is not as clear. It is generally agreed that the CO_2 stimulus exerts two different actions which, in the vagotomized cat, are roughly proportional: 1) an excitatory effect on CIA generation and 2) an inhibitory, threshold-increasing action on the off-switch mechanism. Thus, no change in the duration of inspiration occurs. Studies by von Euler et al. (12) in the apneustic cat failed to show major or consistent changes in the duration of apneustic inspiration in response to changes in PCO_2 (under normoxic conditions). These investigators suggested that this proposed dual effect of CO_2 persists in apneustic breathing (12). St. John (35), however, found that under normoxic or hyperoxic conditions, elevations of PCO_2 typically resulted in prolongation of Ti, Te, and the total respiratory cycle duration (Ttot). In addition, he observed an augmentation of the apneustic depth (i.e. increased inspiratory activity) and an increased inspiratory duration (increased off-switch threshold).

Hypoxia-induced changes in the apneustic pattern have been less thoroughly investigated. The studies of Tang (42) and von Euler (12) had suggested hypoxia to diminish the inspiratory duration, though neither considered the possible site of mediation (carotid chemoreceptors or central nervous system) responsible for the change. Other studies, however, showed direct pharmacological stimulation of carotid chemoreceptors by cyanide could cause both a premature

development of an apneustic inspiratory spasm and/or a premature termination of the inspiratory phase (35).

In the same study examining the effects of hypercapnia on the apneustic pattern, St. John found the effect of decreasing PO_2 to hypoxic levels (at isocapnia) resulted in significant diminutions of the apneustic inspiratory and expiratory durations, and the total apneustic breathing cycle (35). Moreover, an increase in depth was observed in most cases following such a PO_2 change. Elevations of PCO_2 under hypoxic conditions caused no systematic change in Ti, Te, or Ttot, but following bilateral carotid sinus nerve section, normoxic hypercapnia again caused significant increases in Ti, Te, and TTOT. The responses to hypoxia were markedly different in cats without peripheral chemoreceptors. Thus, the results obtained subsequent to PO_2 decreases implied hypoxia to be causing an increase in inspiratory activity and a reduction in the inspiratory and expiratory off-switch threshold. St. John concluded his data to be consistent with the notion that hypoxia-induced alterations of apneusis were the net result of carotid chemoreceptor stimulation and brainstem hypoxia.

The foregoing considerations of various influences that affect the apneustic pattern are important in light of the results of the present study. The anecdotal observations of Breckenridge and Hoff (4), and Ngai (31) that morphine attenuates apneusis have been confirmed and extended by the present study. Though not specifically examined in this study, the possible mechanisms through which this effect was mediated merits speculation.

In terms of the model based on that of the inspiratory off-switch

model, cats in this study were deprived of input from the NPBM and vagal afferents. Thus, for CIA to reach threshold sooner, either the inspiratory activity was augmented or the switch threshold lowered. As mentioned in the results section, the morphine infusion began shortly after apneusis was induced in 4 of 6 cats. In the 2 cats in which apneusis was allowed to continue for five minutes or so after induction, no sign of decreasing inspiratory duration was evident. Though some hypoxia was undoubtedly present, as evidenced by the higher frequency of gasping during the control phase (P) in the apneustic group, it did not appear to be influencing inspiratory duration. When the morphine infusion began, however, all cats demonstrated a marked and nearly immediate change in inspiratory duration, decreasing it in such a way that although apneusis was still present, the duration and intensity of the apneustic holds were reduced. This effect of morphine appears to be similar to the direct stimulation of peripheral chemoreceptors by cyanide mentioned previously, a stimulation which increases inspiratory activity. The augmentation of inspiratory activity would account for the increase in ventilation through phase B (apneusis attenuation).

During phase C, however, the apneustic pattern recurs, accompanied by increased gasping (which had decreased during phase B). MAP during this phase is quite reduced, and gasping ventilation largely increased. A hypercapnic hypoxia could reasonably be assumed to be present because of the hypoventilation existing during phases A and B, though that of phase A was of relatively short duration in most cats. The increase in gasping ventilation would support this

contention.

It is here that speculation becomes complex because of the two separate, simultaneous types of ventilation. It would appear that two respiratory off-switch mechanisms are in operation. First, there is the off-switch mechanism that determined the duration of the apneustic inspirations; in this mechanism, inspiratory activity appears to have been augmented in the early phases. The second off-switch mechanism, apparently activated as the cumulative level of morphine increased, was responsible for the gasping ventilation. Use is made of the term "off-switch mechanism" because it encompasses the possibility that either inspiratory activity or threshold level may have changed. This secondary ventilatory activity will be considered separately in the following section.

From the work of St. John (35), hypoxia appears to increase the CIA and reduce the off-switch threshold; hypercapnia, however, increases CIA as well as the threshold. Though the literature generally agrees that CO_2 responsivity is decreased after morphine, it may be that this is only true at lower doses. Also, lesioning of the pneumotaxic center does not necessarily entail a decrease in CO_2 responsivity (12). Thus, if a hypercapnic hypoxia exists by phase C, it may be that the effects of hypercapnia prevail at this time. CIA would thus remain augmented but a net increase in off-switch threshold would increase inspiratory duration. The apneustic holds which reappear during phase C are of shorter duration and intensity than those seen during phase P (speaking qualitatively). However, frequency remains significantly elevated over control during phase C;

it is the significant decrease in V/T which accounts for the change in ventilation during this phase. This decrease in depth indicates a decreased inspiratory activity. Possibly, the augmentation to CIA which existed during phases A and B was decreased, but the switch threshold is lowered due to hypoxia. Mismatches between changes in CIA and those of the inspiratory off-switch have been postulated to exist (35), so such a suggestion is in the realm of possibility. Hypercapnia, then, may not have any effects, as postulated by von Euler et al. (12).

Apneusis is nearly absent during the final phase D. It would appear the first type of respiratory activity "drops out" as the second type predominates during this phase. If mismatches in the level of CIA and the off-switch threshold are generated by the higher levels of morphine, it may be that the relationship between the two are so disrupted by the final phase that the first type of respiratory activity is no longer functional.

Morphine, thus, has fundamental actions on the neurons of the respiratory controller, with secondary effects on the off-switch threshold occurring as a result of the hypercapnia and hypoxia generated as a result of that fundamental action, the nature of which still remains to be elucidated.

2. , Effects of morphine on MAP and HR

The gradual decrease in MAP during the course of the infusion generally resembled those seen in the Eupneic group with the followoing exceptions. First, the MAP during phase P was tremendously elevated, most probably due to the apneusis existing at this time.

Secondly, a pressor effect was not evident during phase A as it seemed to be in the Eupneic group. Lastly, MAP had fallen to much lower levels by phase D, when compared to the level of the Eupneic group during its phase D. The morphine tolerance effect was not apparent in this group, a similar finding to that seen in the Eupneic group.

The gradual decrease in heart rate which occurred over the course of the infusion does not become significant until the final phase. The rate is extremely elevated throughout the course of the infusion, most probably due to the hypoventilation (relative to a normal ventilation) that existed. The decrease in rate is in agreement with the literature and may be a reflection of a direct depressant effect of morphine on the heart, or a decrease in sympathetic tone; facilitation of vagal pathways is not a consideration in this group, since the animals were vagotomized.

D. Induction of Gasping in the Eupneic and Apneustic Groups

Gasping has been shown to represent a respiratory pattern that differs fundamentally from both eupnea and apneusis. Not only does the pattern of phrenic nerve activity during gasping differ markedly from that during eupnea or apneusis, but also (in contrast to eupnea and apneusis) gasping is not systematically altered by stimuli acting on the peripheral or central chemoreceptors (36). The observation that diminutions of PAO/2 cause gasping in both carotid chemoreceptor intact and denervated cats leads to the conclusion that peripheral chemoreceptor mechanisms are not responsible for hypoxia-induced changes in gasping. As St. John et al. (36) noted, the augmentations

of gasping frequency with reductions of PAO/2 imply that the controller for gasping may be directly sensitive to the partial pressures of oxygen within the medulla, a concept which is not new.

In the present study, cats breathing eupneically or apneustically demonstrated an increased frequency of gasping as the cumulative levels of morphine increased, an observation agreeing with the literature. A gradual decrease in MAP is seen to occur in both groups, falling to or below the lower limit of the autoregulatory range of the cerebral circulation; this, along with the hypoventilation that occurred in each, undoubtedly contributed to a brainstem hypoxia, which has been shown to release gasping mechanisms (36).

In phase C, for both groups, gasping appeared superimposed on either eupnea or apneusis. Evaluation of the appearance of the ventilatory pattern during this phase has led to the conclusion that two kinds of respiratory activity are present, each of which can be discussed in terms of the off-switch model. In the Eupneic group, the normal off-switch operating would determine eupneic respiratory cycles; in the Apneustic group, an altered mechanism (elimination of vagal afferent and pneumotaxic center activity) would determine apneustic respiratory cycles. In both groups, brainstem hypoxia would release a gasping mechanism. Inspiratory activity would reach a maximum within a few hundred milliseconds of its onset and cut off sharply, rather than augmenting gradually (45). In other words, the inspiratory threshold is reached nearly instantaneously. The strongest evidence in this study that two mechanisms are operating

CHAPTER VI

CONCLUSIONS

The results of the current study may help to shed a new light on discrepancies existing in the literature as regarding the effects of morphine on frequency and tidal volume during eupneic breathing. In previous bolus injection studies in which low doses of morphine were administered, frequency and tidal volume were reported to be decreased (frequency apparently being the more affected of the two variables). Those bolus injection studies employing higher doses of morphine reported frequency to be increased, and convulsions (accompanied by gasping) to be present. These observations correlate well with phases A and D, respectively, seen in the Eupneic group in this study. The transition between the effects of low and high doses of morphine may be those effects occurring during phases B and C. The assumption of a periodic character in phase B agrees with the observations of Barbour (1) who also noted the pattern to disappear as the cumulative dose of morphine increased. Phase C apparently has no correlates in the literature (as to the effects on frequency and tidal volume), possible due to the inherent differences between the bolus injection and constant infusion technique. The constant infusion technique thus stretches out into a broader spectrum the effects of morphine, allowing phases of different ventilatory patterns to be distinguished.

Previous studies observing morphine to attenuate apneusis (4,31) have been confirmed and extended by the present study. The results

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indicate morphine to have a fundamental action on the respiratory controller, effecting changes in the off-switch mechanism controlling the duration of inspiration in such a way as to attenuate the duration of apneustic inspiratory holds.

Lastly, the gasping that appeared superimposed on either eupnea or apneusis has led to the conclusion that two kinds of respiratory activity are present. In addition to the normal (eupneic) or altered (apneustic) off-switch mechanism operating, a second mechanism in which the inspiratory threshold is reached nearly instantaneously (gasping) would also be active (possible triggered by a brainstem hypoxia due to the developed hypoventilation/hypotension over the course of the infusion). The strongest evidence in this study that two off-switch mechanisms are operating simultaneously lies in the records of the apneustic group. Gasps, with extremely shortened durations of inspiration are seen superimposed on apneustic breaths with prolonged inspirations. The hypoxia possibly responsible for the gasping ventilation could be considered a secondary effect of the fundamental action of increasing morphine levels on respiratory controller neurons (to which the disappearance of both eupnea and apneusis during phase D, leaving gasping to predominate, may be related). The present results thus appear to reflect a combination of primary and secondary effects of morphine on respiratory rhythm generation.

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APPENDIX A

APPENDIX A

Table of respiratory and cardiovascular variables quantitatively measured during the progression of phases in the Eupneic and Apneustic groups. Eupneic (E) and Apneustic (A) group values are for measured (f/n, V/Tn, f/g, V/Tg, HR, MAP) and calculated ($\dot{V}$/n, $\dot{V}$/g, $\dot{V}$/tot) variables. Values represent mean and standard error of 6 cats in each group, with the exception of heart rate in the Eupneic group (where n=4) and the Apneustic group (n=3). Abbreviations: f/n = frequency, normal pattern; V/Tn = tidal volume, normal pattern; $\dot{V}$/n = ventilation, normal pattern; f/g = frequency, gasping pattern; V/Tg = tidal volume, gasping pattern; $\dot{V}$/g = ventilation, gasping pattern; $\dot{V}$/tot = total ventilation (sum of $\dot{V}$/n and $\dot{V}$/g); HR = heart rate, ·MAP = mean arterial pressure. Phase abbreviations for the Eupneic group: P = control; A = initial depression; B = periodic breathing; C = recovery from periodicity; D = gasping. Phase abbreviations for the Apneustic group: P = control; A = apneusis attenuation; B = apneusis reversal towards eupnea; C = apneustic gasping; D = gasping.

Appendix

Variables	Group	P	A	B	C	D
f_n	E	25.8± 3.0	19.5± 3.0	10.9± 5.1	29.2± 9.4	6.2± 4.3
breaths/min	A	0.6± 0.1	1.5± 0.2	2.4± 0.6	3.5± 0.7	0.7± 0.5
V_{T_n}	E	39.0± 5.6	28.6± 4.1	58.0± 11.2	32.5± 7.4	9.7± 4.8
ml	A	92.5± 10.9	94.3±12.1	83.5± 12.2	47.1± 13.6	14.1± 11.8
$\dot{V}_n$	E	981.0±141.5	531.3±72.9	444.4±122.6	820.8±217.8	115.6± 74.9
ml/min	A	49.7± 9.7	145.6±32.8	207.1± 54.4	132.8± 29.5	19.8± 13.6
f_g	E	0.8	1.1± 0.4	1.6± 0.6	3.6± 1.6	9.8± 4.2
breaths/min	A	1.4± 0.7	3.2± 1.2	3.4± 1.2	5.2± 1.4	5.3± 0.7
V_{T_g}	E	1.7	16.7±13.6	22.6± 11.3	32.7± 10.7	75.7± 14.0
ml	A	9.1± 6.0	35.7±14.3	22.6± 6.8	48.7± 15.3	68.8± 11.8
$\dot{V}_g$	E	0.8	57.8±48.9	41.0± 17.2	99.9± 50.1	558.8± 76.6
ml/min	A	23.2± 13.4	137.3±68.5	108.9± 51.0	200.1± 65.6	341.2± 60.7
$\dot{V}_{Tot}$	E	981.8±141.4	589.1±67.2	485.4±127.4	920.7±208.1	671.4±119.2
ml/min	A	72.9± 18.2	282.8±54.5	316 ± 62.0	332.9± 64.6	360.9± 69.7
HR	E	201 ± 22	157 ±133	196 ± 14	214 ± 24	190 ± 46
beats/min	A	277 ± 16	278 ± 4	263 ± 16	252 ± 18	216 ± 5
MAP	E	135 ± 4.4	158 ±21.9	96 ± 5.0	82.5± 8.8	74 ± 7.5
mmHg	A	195 ± 15	101 ±14	83 ± 9	69 ± 7	56 ± 4

The thesis submitted by Andrea M. Zardetto-Smith has been read and approved by the following committee:

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The final copies have been examined by the Director of the thesis and the signature which appears below certifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

December 6 1982
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