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Monoamine Oxidase as a Target Enzyme in Pineal and Testicular Tissue: Adrenal Cortical Steroids and Arginine Vasotocin

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MONOAMINE OXIDASE AS A TARGET ENZYME IN PINEAL AND TESTICULAR TISSUE: ADRENAL CORTICAL STEROIDS AND ARGININE VASOTOCIN

by

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Randall James Merrill

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ABSTRACT

Monoamine Oxidase as a Target Enzyme in Pineal and Testicular Tissue: Adrenal Cortical Steroids and Arginine Vasotocin

by

Randall J. Merrill, Master of Science

Utah State University, 1975

Major Professor: Dr. LeGrande C. Ellis
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The purpose of the research was twofold: (a) To ascertain what effects the adrenal glucocorticoids and catecholamines have on pineal N-acetyltransferase and monoamine oxidase activity, and (b) to observe what effect arginine vasotocin (a pineal polypeptide) has on testicular monoamine oxidase activity.

Rat pineal N-acetyltransferase activity was numerically increased when rats were injected with cortisol. It was increased significantly when the animals were injected with cortisol plus norepinephrine. Adrenal demedullation significantly increased N-acetyltransferase activity in starved rats when compared with intact control animals. Moreover, bovine pineal monoamine oxidase activity was markedly inhibited in a dose-dependent manner when incubated in vitro with cortisol. The results strongly implicate the adrenal gland as one factor controlling melatonin synthesis by the pineal gland, especially with food deprivation.

Arginine vasotocin, a polypeptide consisting of eight amino acids, was incubated with rat, hamster and rabbit testicular monoamine oxidase. The polypeptide significantly inhibited rat and hamster enzyme preparations, but had no effect on the rabbit preparation. These data suggest that the pineal may act peripherally to decrease reproductive function partially through a serotonergic mechanism involving the enzyme monoamine oxidase.

(86 pages)

INTRODUCTION

The pineal gland is a small, yet very active organ arising from an evagination of the neuroepithelial cells of the diencephalic roof (41). Observations of the unique structural and biochemical features of the pineal have generated considerable interest in the quest to determine how the physiological actions of this organ integrate with other mammalian systems. During the past decade, significant strides have been made in clarifying the biochemical and physiological activities of the pineal. An abundance of evidence now indicates that certain substances (indoleamines and polypeptides) are synthesized and secreted by the pineal that have a definite antigonadotrophic and/or antigonadal effect on mammalian and avian organisms (1, 90, 97).

The pineal is photoreceptive in nature and produces certain indoleamines whose synthesis is highly dependent upon the daily photoperiod. In this respect, there is a circadian rhythm in biogenic amine synthesis with the peak synthesis occurring during the dark phase of the cycle (90). There are, however, additional factors other than light that alter pineal activity and function. Among these are environmental temperature (114, 68), stress (69), radiation (24) and food deprivation (115). Each of these stressors have been demonstrated to activate the pineal; however, little work has been done to elucidate the mechanisms involved in these phenomena.

The dramatic changes in adrenal structure and function during stress reported by Selye (104) lead one to postulate that changes in the pineal organ

that occur during stress are mediated through the adrenal gland. In this investigation, the effects of adrenal glucocorticoids and catecholamines on two key enzymes involved in mammalian pineal indoleamine metabolism were ascertained.

Part II of this paper examines the influence of arginine vasotocin (AVT)[1] on testicular MAO of various species. AVT is a small polypeptide

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[1]The following abbreviations will be used in the text:

ACTH--adrenocorticotrophic hormone
AVT--arginine vasotocin
c-AMP--cyclic 3',5'-adenosine monophosphate
COH--compensatory ovarian hypertrophy
COMT--catechol-o-methyltransferase
CNS--central nervous system
CRF--corticotrophic releasing factor
DNA--deoxyribonucleic acid
DOPA--3,4-dihydroxyphenylalanine
E--epinephrine
FSH--follicle stimulating hormone
GAS--general adaptation syndrome
HIOMT--hydroxyindole-o-methyltransferase
5-HIAA--5-hydroxyindole acetic acid
5-HT--5-hydroxytryptamine (serotonin)
5-HTP--5-hydroxytryptophan
5-HTPD--5-hydroxytryptophan decarboxylase
LH--luteinizing hormone
M--molar
MAO--monoamine oxidase
MW--molecular weight
NAT--N-acetyltransferase
NE--norepinephrine
R--roentgen
RNA--ribonucleic acid
s. c.--subcutaneous

ml--milliliter
μl--microliter
g--gram
mg--milligram
μg--microgram
kg--kilogram
nU--nano-Units

consisting of eight amino acids that has been isolated from the mammalian pineal gland (12) and has been shown to have antigonadal properties (97). The mechanism of action of AVT has not been clearly elucidated, however, there is some evidence that AVT may act through central and/or peripheral mechanisms.

Since there is a paucity of information regarding the target enzyme for the secretory products of the pineal, the effects of AVT on testicular MAO of various species was ascertained. MAO was chosen as a target enzyme since recent investigations have shown that the pineal gland appears to act through serotonergic mechanisms (23, 103).

REVIEW OF LITERATURE

The Pineal Gland

General considerations

The pineal gland has been an organ of interest dating back to around 300 B. C. at which time the organ was described by Herophilus and Erasistratus (1). All of the early observations of the pineal were, however, based on the superstitious beliefs of the time. It was not until about the middle of the twentieth century that valid scientific research concerning the biological activity of this gland was reported.

Pineal development in vertebrates results from an evagination of the neuroepithelial cells of the diencephalic roof. As the fetus develops, these embryonic mesenchymal cells either stay closely adherent to the epithalmus underneath the corpus collosum or migrate to the surface of the brain to assume a supracollosal position (41). The pinealocyte or pineal parenchymal cell is the cell-type of greatest interest to pineal researchers since it is unique to the gland. Although the predominant belief among researchers is that the pinealocyte is ectodermally derived, there are still some who believe that this cell has mesodermal origins (28).

Quay (90) has outlined the embryonic development of the pinealocyte. He indicates that pineal cell morphogenesis occurs during embryonic life. The proliferation of the pinealocytes drops rapidly at birth at which time

significant postnatal growth is mainly by pinealocyte hypertrophy with some increase in glial and stromal tissues.

The pineal is also a well vascularized organ. Goldman and Wurtman (33) have estimated the blood flow through the rat pineal to be 2-4 ml per minute per gram. This is the rough equivalent of the amount of blood passing through the neurohypophysis.

Biochemical activities

Although active lipid and carbohydrate metabolism have been described in the pineal (90), much interest has been generated toward the biochemical and physiological actions of pineal indoleamines. Melatonin (5-methoxy-N-acetyl-tryptamine) is by far the most studied of these indoleamines. Melatonin was first isolated and identified by Lerner and co-workers (54, 55) following studies to isolate the pineal constituent responsible for blanching amphibian skin. This molecule is a derivative of the amino acid tryptophan, the only indole amino acid and one of the essential amino acids required by man and other mammals. Serotonin, another indoleamine derivative of tryptophan, is also a precursor of melatonin and has been demonstrated to be present in exceptionally high concentrations in the pineal gland (6).

Research concerning the activities of pineal enzymes involved in indoleamine metabolism has given students much insight into the biochemical activities of this gland. Most of what is known about pineal enzyme control and function comes from organ culture studies.

The initial step in pineal indoleamine metabolism follows the uptake of tryptophan by the pinealocyte. Tryptophan is oxidized in the 5-position to form 5-HTP. This step is catalyzed by the enzyme tryptophan hydroxylase. Lovenberg et al. (57) have demonstrated that this enzyme requires the presence of O_2, ferrous iron and a reduced pteridine cofactor. These workers found that this enzyme has a high activity in the pineal glands of all species examined (56). In reviewing the activity of tryptophan hydroxylase, Cardinali and Wurtman (11) suggested that the high substrate K_m for this enzyme relative to the levels of free tryptophan likely to be present in the pineal organ, indicates that the enzyme probably functions without being saturated with substrate. Moreover, changes in pineal concentration of tryptophan are likely to influence the rate at which this amino acid is hydroxylated.

Hydrozylation of tryptophan results in 5-HTP. This product then serves as the substrate for the nonspecific enzyme 5-HTPD (58), that exists in high concentrations in the pineal (34). The high enzyme concentrations combined with the rapidity of the metabolic step leave low levels of 5-HTP in the mammalian pineal (89). The enzyme 5-HTPD is ubiquitous in the mammalian system. It requires a pyridoxal phosphate cofactor and participates in the biosynthesis of catecholamines (58).

Quay (90) has reviewed the literature concerned with the enzymes of 5-HT formation. Because tryptophan hydroxylase is activated by c-AMP and is stimulated by NE, while neither c-AMP or NE stimulates the formation of

^{14}C-5-HT from ^{14}C-5-HTP, Quay has speculated that hydroxylation is the rate-limiting step in 5-HT formation.

Once formed, pineal 5-HT may be metabolized by one of several routes. A portion of 5-HT is oxidatively deaminated by MAO to 5-hydroxyindole acetaldehyde, an unstable intermediate, which is then either oxidized to 5-HIAA or reduced to 5-hydroxytryptophol (11).

Of greater significance to pineal physiologists is the conversion of 5-HT to melatonin. Melatonin is the O-methylated and N-acetylated derivative of 5-HT and satisfies at least many of the criteria for being a hormone. The formation of melatonin from 5-HT is a two-step process that begins with acetylation to form N-acetylserotonin. The enzyme involved in this reaction has been named by Klein (47) as acetyl coenzyme A N-acetyltransferase or serotonin N-acetyltransferase. This enzyme transfers an acetyl group from acetyl coenzyme A to the amino group of 5-HT (125). Klein, Weller and Moore (47) have suggested that NAT may be the rate limiting enzyme in melatonin production by limiting the amount of N-acetylserotonin available for O-methylation.

N-acetylserotonin is converted to melatonin by the transfer of a methyl group from S-adenosylmethionine to the 5-hydroxy group on the indole ring by the enzyme HIOMT. Both the enzymes NAT and HIOMT may influence the rate at which the pineal synthesizes melatonin (11, 24).

As opposed to pineal morphogenesis which begins to decline near parturition, pineal enzymatic activities appear shortly after the onset of the postnatal period, and are complete near the time of puberty in laboratory rodents and possibly sheep and cattle. These activities predate puberty by many years in man (90). In his study of 5-HT related enzymes, Zweig (133) examined the appearance of 5-HTPD, MAO and HIOMT in the newborn rat pineal. He found that MAO, which appeared on day-1 and reached adult levels on day-18, was one of the first pineal enzyme activities to appear. The activity of 5-HTPD appeared on day-6 but reached adult levels within the next two succeeding weeks. The enzyme activity of HIOMT was found to lag behind that of MAO and 5-HTPD. This enzyme was barely detectable at day-6 and achieved only 10 percent of the adult activity by day-12. Adult activity was achieved by 34 days of age. Klein and Lines (44) found no HIOMT activity at 10 days or earlier, but concurred with Zweig's findings on the rapid increase of HIOMT during the second and third postnatal week.

Ellison, Weller and Klein (25) studied the development of pineal NAT activity in the rat. Their findings indicate that NAT is detectable in the rat pineal as early as 4 days before birth and that a circadian rhythm begins on the fourth day after parturition. Moreover, NAT activity develops most rapidly during the second postnatal week and reaches adult magnitude by the end of the third week.

Physiological activities: Innervation, activation and biorhythms

During the past decade investigations of several pineal enzymes (HIOMT, 5-HTPD and NAT) in intact and surgically prepared animals housed under continuous light or darkness have established that in mammals the sympathetic innervation to the pineal organ mediates its metabolic responses to environmental lighting (45, 110, 129). Pineal innervation in mammals is exclusively autonomic. With the possible exception of some parasympathetic fibers in some kinds of lower primates, this autonomic innervation is believed to be entirely sympathetic (53).

Perhaps the first clues to link the biochemical activities of the pineal, and the roles played by the secreted indoleamines, came from the observations of pineal circadian rhythms. Circadian rhythms are those rhythms having a cycle length of about 1 solar day. The components of the circadian rhythms of pineal indoleamine metabolism have been studied intensively in recent years. The events of the metabolism of pineal indoleamines and the control factors involved can be outlined, but certain limitations must be imposed: (a) Most of the research conducted on pineal indoleamine rhythms has involved the use of the laboratory rat and is not necessarily applicable to all species, and (b) the relationships described below pertain only to the adult animal. The adult rhythm in contrast to the infant, has an exogenous drive mechanism imposed on it by means of its sympathetic innervation (90).

The first pineal circadian rhythm was detected and demonstrated in rat 5-HT content by Quay in 1963 (85, 87). Experiments in the modification of the times of either the onset of light or darkness succeeded in significantly modifying the timing of the rise and fall of 5-HT. Rat pineal contents of 5-HIAA (88) and melatonin (59) were also found to follow circadian rhythms. These findings are consistant with the idea that the metabolism of 5-HT to 5-HIAA is most active during the day, and the pathway to melatonin formation is predominant at night.

The events of the adult rat's pineal circadian rhythm can be described sequentially from the uptake of tryptophan to the secretion of melatonin. At the onset of light, NE has fallen to its daily low, presumably due to prior release. The synthesis of 5-HT increases at this time through the previously increased activity of tryptophan hydroxylase. This process, the rate-limiting step in 5-HT production, is accelerated by NE and requires mediation by the second messenger c-AMP (107). The concentration of c-AMP in the rat pineal increases during the daily light period to nearly 6 times of that found at the end of the dark period, possibly due to increased synthesis (20). Shein and Wurtman (108) have demonstrated that both NE and c-AMP stimulate ^{14}C-5-HT production from ^{14}C-tryptophan, but do not increase the amount of this indoleamine when incubated with ^{14}C-5-HTP. Thus, the decarboxylating enzyme is not effected by daily fluctuations of NE or c-AMP. It is interesting to note, however, that denervation of the pineal organ has been reported to result in either no change (34) or an elevation of the 5-HTPD activity (110) of

this gland. Moreover, the pineal glands of rats kept in constant light environments have about twice the 5-HTPD activity as the pineal glands of litter-mates kept in constant darkness. Blinding or sympathetic denervation prevents the effect of constant light exposure on this enzyme (110). Further studies have revealed that neither 5-HTPD or MAO have a demonstratable circadian rhythm in the rat pineal (110).

The enzymatic steps prominent during the nocturnal phase of pineal metabolism are dramatic. NAT has been demonstrated to have a circadian rhythm in the rat pineal gland with night time values enhanced 15 to 50 fold over the daytime activity (18, 45). The activity of this enzyme in pineal cultures is stimulated by NE acting through c-AMP as a second messenger (107, 113, 123).

Once N-acetylserotonin is formed, it is rapidly O-methylated through the action of HIOMT to form melatonin (42). Axelrod, Wurtman and Snyder (3) demonstrated a naturally occurring diurnal rhythm in HIOMT activity. Their results show a 1.6 to 3 fold increase in enzyme activity several hours after the end of the light photoperiod. Puromycin was shown to block the rise of HIOMT after the onset of darkness. This suggests the effect of illumination on the formation of this enzyme may be mediated by protein synthesis.

The circadian rhythms of the mammalian pineal gland that involve the production of 5-HT and melatonin, and the enzyme activities involved, are mediated by post-ganglionic sympathetic nerves arising from the superior cervical ganglion. As was discussed above, 5-HPTD activity may be increased

after sympathetic denervation. Wurtman and colleagues (129) have shown that changes in HIOMT activity in response to light or darkness are prevented by removal of the superior cervical ganglia. Klein, Weller and Moore (47) have demonstrated that superior cervical sympathectomy abolishes the NAT rhythm by the elimination of the central input into the gland. These investigators concluded that neural signals regulate the NAT rhythm in the normal rat through the release of NE from the sympathetic terminals within the gland. Volkman and Heller (122) reported that daytime levels of NAT rose after sympathetic stimulation but later returned to baseline levels whether or not stimulation was continued. From these findings Volkman and Heller made two conclusions: (a) The reduction of NAT activity after nervous stimulation may be due to a process intrinsic to the pineal and (b) since the sympathetic fibers contain NE, excitation of these fibers presumably results in the release of NE from the vesicles in the sympathetic endings and a subsequent increase in pineal NAT activity. Further studies have demonstrated that the NAT activity is modulated by the neural release of NE from the sympathetic nerves via beta adrenergic receptors and that the increase in enzyme activity is due to the synthesis of new enzyme molecules (10, 17).

The close relationship that exists between the phasing of the diurnal rhythms in NAT and the pineal melatonin concentration in vivo (45, 100) and the magnitude of stimulation of NAT and ^{3}H-melatonin production in vitro (43, 45) has lead to the conclusion that NAT acts as the rate-limiting enzyme

in melatonin production by determining the amount of N-acetylserotonin available for O-methylation.

Although environmental light does have a definite effect on pineal biochemical activities, the activities are not necessarily completely controlled by photic input to the gland. In observing the effects of continuous light or continuous darkness on pineal biochemical activities (45, 74, 92), Quay (90) concludes:

> (1) Continuous light and continuous darkness are not opposite in their effects. The particular results of these treatments depend upon which pineal constituent one measures. (2) Continuous light suppresses all of the pineal's circadian rhythms in indoleamines. It is likely that this represents a complex and widespread modification of pineal metabolism, rather than a metabolically sharply localized effect. Continuous light leads to an increased pineal content of c-AMP, along with suppression of rhythms and depression of peak values in pineal 5-HT and N-acetyltransferase. These circumstances raise doubts concerning the primacy of c-AMP and c-AMP-mediated activities for the daily stimulation of pineal synthesis of 5-HT during the light period and N-acetylserotonin and melatonin during the dark period. (3) Continuous darkness on the other hand has very little effect on pineal indoleamine rhythms or levels, apparently including those of HIOMT activity and melatonin. It can be concluded that light is not required for these rhythms, which are thus truly endogenous. The effect of light, superimposed by the pineal's sympathetic innervation, is to synchronize on a daily basis the pineal's indoleamine rhythms with that of the daily light-dark cycle. (90, pp. 162-163)

Pineal indoleamines: Effect on pituitary-gonadal axis

The mammalian pineal organ is thought to secrete substances that are antigonadotrophic (130). As discussed above, the probable sequence of events leading to the secretion of melatonin is as follows: The sensory input (darkness) increases the activity on the sympathetic neurons causing the release of

NE from the terminals of the pineal organ. NE activates the adenyl cyclase system and increases the activity of enzymes in the melatonin pathway, i.e., tryptophan hydroxylase, HIOMT and NAT. The process of the conversion of an electrical signal (from the sympathetic nerves) to a chemical signal (pineal indoleamines) has led Wurtman and Anton-Tay (127) to describe the pineal as a neuroendocrine transducer.

Research into the effects of pineal secretions on the reproductive system have been extensive, however, it is quite difficult to formulate generalizations concerning the responses of the reproductive system to this gland. The pineal is implicated in mammalian reproductive functions, but due to the wide variation of effects seen among species, the actual role the pineal plays has yet to be clearly delineated.

The research conducted to date leaves some debate on where the pineal substances act, i.e., centrally or peripherally. In support of the central theory of action, Reiter (97) believes that this theory seems reasonable because it would be somewhat inefficient to allow gonadotrophin secretion and then block its action at the target organ.

All studies conducted to date confirm that the hamster, an animal very sensitive to changes in photoperiod and the inhibitory influence of the pineal, is affected by the pineal at the neuroendocrine level. In support of the central theory of pineal action, Reiter (93) has demonstrated that the ovaries and uteri of pineal stimulated animals respond to treatment with gonadotrophins.

He has also shown that male hamsters, which have their sexual organs involuted due to pineal stimulation, also have depressed LH levels (94).

Restriction of the amount of light to which hamsters are exposed leads to involution of their sexual organs (98). Accompanying this atrophy is an observed reduction in anterior pituitary levels of LH (95) and FSH (99) and a diminished concentration of plasma LH (94). These changes are nullified by the surgical removal of the pineal (37) or interruption of the pineal's neural input (97).

Reiter (97) has outlined the probable sequence of events leading to pineal induced gonadal regression. In the absence of long daily photoperiod with illumination of more than 12.5 hours per day (32), the pineal is activated to synthesize and release an antigonadotrophic factor. The nature of this factor remains unknown in the hamster, but it probably intervenes with gonadotrophin releasing factor mechanisms in the hypothalmus which results in a cessation of pituitary gonadotrophin release.

In rats, the pineal plays a somewhat lesser role. This is probably due to the fact that rats are somewhat less photosensitive and therefore experimental results are less statistically reliable (97). However, several studies have been conducted in an attempt to show that rat pineal indoles may act on the CNS by inhibiting gonadotrophin release. Kamberi and co-workers (39) have demonstrated that melatonin injected into the third ventricle of rats decreased peripheral LH and FSH titres while prolactin blood levels were

elevated. These changes were not observed when the melatonin was administered through the hypothalmo-hypophyseal portal system.

Fraschini et al. (29) have demonstrated testicular hypertrophy and an increase in the weight of the ventral prostate and seminal vesicles of adult rats following pinealectomy. An increase in pituitary LH was also demonstrated. These data show that pinealectomy stimulates synthesis and release of pituitary LH and suggests that the pineal gland exerts an inhibitory influence on LH secretion. In the same study, Fraschini placed pineal fragments or melatonin in the median eminence or reticular substance of the midbrain. The result was a depletion of pituitary LH. These studies suggest that the pineal gland can modify pituitary function by acting on receptors in higher centers and that these receptors may participate in the regulation of synthesis and/or the release of the gonadotrophins, especially LH (30).

In peripheral studies, daily injections of microgram amounts of melatonin in rats decreased the incidence of estrus and reduced rat ovarian weights (128) while in the hamster, melatonin has repeatedly failed to exhibit gonad-inhibiting activity (101). Evidence for action of melatonin at the testicular level has been reported by Ellis (23) whose studies have demonstrated that melatonin interferes with androgen production by acting on 17α-hydroxypregene-17, 20-lyase and 17β-hydroxysteroid dehydrogenase in the androgen biosynthetic pathway.

Sorrentino, Vaughan and co-workers (72, 118) have completed some excellent studies on the actions of pineal constituents by using the method of

compensatory ovarian hypertrophy (COH). This method involves the observation of increased growth of one ovary following the ablation of the other in the same animal. The enlargement of the remaining ovary is thought to be due to increased circulating FSH (5) levels, but LH could also be involved. In the rat, daily injections of melatonin were found to curtail the rise in FSH titres associated with COH (72). In the mouse, where the remaining ovary enlargement is quite uniform and ranges between 30 and 60 percent, as little as 25 μ g of melatonin significantly blocks COH (118).

Adrenal Gland and Pineal Organ

Responses to Stress

Stress

A biological entity cannot live without experiencing some degree of stress. In its medical sense, stress is essentially the rate of wear and tear in the body (105). Whenever stress is present, certain changes in the structure and chemistry of the body occur. Some of these changes represent signs of damage; others are manifestations of the body's adaptive reactions which are activated in defense against stress. When an organism is stressed, the nervous system and endocrine organs are activated in such a way as to maintain homeostasis and thereby to resist the stressful agent.

Selye (104) has defined stress as the state manifested by a specific syndrome which consists of all the nonspecifically induced changes within a biological system. The sum of all these nonspecific systemic reactions of the

body which ensue upon long-continued exposure to stress has been termed the general adaptation syndrome (104). One of the most fundamental observations in connection with the GAS was the finding that many of the morphologic, functional and biochemical changes elicited by various systemic stressor agents are essentially the same, irrespective of the specific nature of the eliciting stimulus.

Selye found that the GAS was general in nature in that it was elicited only by those stresses that are generalized (i.e., they affect large portions of the body--temperature changes, physical exersion, immobilization, starvation, etc.). The reaction is adaptive in that it helps in the acquisition and maintenance of a state of inurement. The reaction can also be called a syndrome; its individual manifestations are coordinated and partly interdependent.

Selye has subdivided the GAS into three specific reactions to the stressor: The first is termed the alarm reaction and is defined as the sum of all nonspecific phenomena elicited by sudden exposure to stimuli which affect large portions of the body and to which the organism has not adapted. The alarm reaction includes such physiological actions as hypothermia, hypotension, hemoconcentration, tissue breakdown and deranged capillary and cell membrane permeability. In other words, the alarm reaction is associated with the development of the symptoms of shock. Moreover, each of the above reactions are indicative of general tissue damage.

The alarm reaction can also be characterized by phenomena that act as a defense against shock, i.e., adrenal enlargement and the physiological reactions associated with corticoid release (rise in blood pressure, hyperglycemia, hyperthermia and an increase in blood volume). This "counter-shock" mechanism represents a transition into the second stage of the GAS, namely, the stage of resistance. This stage represents the sum of all non-specific systemic reactions elicited by prolonged exposure to stimuli to which the organism has become adapted.

The stage of resistance represents an increased resistance to the stressor agent and a decreased resistance to other stimuli. In this stage, the physiological, biochemical and morphological deviations from the normal are reversed.

The final stage of the GAS is that of exhaustion. This stage develops as a result of prolonged over-exposure to the stressor to which adaptation had occurred but could no longer be maintained. Thus, if exposure to a stressful agent continues, adaptation fails and further resistance becomes impossible.

There are, of course, many alarming stimuli with which an organism can come into contact with. Among these are trauma, burns, a change in diet (i.e., fasting), nervous stimuli (i.e., emotional stress caused by the immobilization of an animal), asphyxia, exercise and infection. Also included in this group are drugs and other toxic compounds such as atropine, insulin, colchicine and histamine.

Stress and adrenal function

The mammalian adrenal gland occupies a position immediately superior to the kidney at each side of the body. The gland is liberally supplied with both efferent and afferent blood vessels that enter a central sinus. The mammalian adrenal consists of two distinct regions: An inner medulla (of neural origin and hence not capable of regeneration) which is the source of the catecholamines E (adrenaline) and NE (noradrenaline), and an outer cortex (of mesodermal origin and hence capable of regeneration) which is the source of the mineralacorticoids and glucocorticoids. The mineralocorticoids are mainly concerned with electrolyte balance while the glucocorticoids are involved in carbohydrate metabolism. Due to neural impulses stimulating secretion directly, the release of the medullary hormones is rapid, while the release of the glucocorticoids (the hormones involved in the GAS) is slower and mediated by ACTH which passes to the gland from the pituitary via the blood stream.

The reaction of the adrenals during the stress reaction has been described in detail by Selye (104). Of particular interest is the relative activities of the medulla and cortex during the reaction. At the onset of GAS, the adrenal medulla discharges its contents (NE and E) into the blood stream. There is occasional cytolysis of medullary and cortical cells and also a hypertrophy of cortical cells is observed. As the reaction continues into the stage of resistance, the medullary activity approaches normal, but mitotic activity and hypertrophy of the cortex occurs. This results in an increase in the total

width of the adrenal cortex. Some cytolysis of the cortical cells is also observed under severe stress.

A reasonable mechanism of action of adrenal activation due to stress has been outlined by Brain (9). Brain proposes that stressful stimuli modify neural activity in various regions of the brain. This activity produces changes in brain monoamines that modify the production of corticotrophin releasing factors by cells in the hypothalmus. The CRF passes to the pituitary initiating the release of ACTH which then travels to the adrenals and causes the production and release of glucocorticoids (cortisol and corticosterone).

Thus, an animal under systemic stress experiences an increase in adrenal function and weight. One of the early studies that demonstrated the effect of stress on adrenal weight was conducted by Herrington (36). The study involved exposing rats to various emotional stressors (intermittent bell ringing, air blasts and cage vibrations) each hour for several hours. Herrington observed an increase in adrenal weight but a decrease in testicular weight. In two recent studies, McCarty and Richardson (63, 64) found that in male and female mice, there was a significant increase in adrenal weight and decrease in final body weight associated with decreased living space. The results of these experiments support work completed by Christian (13, 14) who reported the stress response associated with crowding in caged populations of small rodents to be characterized by increased adrenocortical activity and decreased reproductive function.

Stress and pineal function

There have been many published observations of the responses of the pineal gland to stressful factors. One of the earliest stressful factors studied was the continuous exposure of an animal to light. Fiske and colleagues (26) reported a reduction in pineal weights of rats exposed to continuous light for 25 weeks when compared with controls or with rats kept in darkness.

Several biochemical changes in the pineal have also been observed during continuous light stress. A depressed lipid content (84) and diminished cellular glycogen (86) was reported by Quay, while Halaris and Matussik (35) reported swollen mitochondria. The detection of light by the lateral eyes has also been demonstrated to significantly inhibit pineal RNA and protein synthesis (76).

It has been suggested that the above observations may be due to the changes in the secretory activity of the pituitary, gonads, thyroid or adrenal cortex since each of these glands is affected by light. This hypothesis was tested by Fiske and colleagues (27) by placing hypophysectomized, gonadectomized, adrenalectomized or thiouracil treated rats in continuous light or under usual day-night conditions. In each instance, continuous light significantly reduced pineal weight. These data indicated that a change in the activity of these endocrine organs was not necessary for, and could not explain, the response of the pineal to light. This view is further supported by Wurtman (130) who demonstrated that HIOMT continues to respond to shifts in light and darkness after hypophysectomy and ovariectomy.

A great abundance of the literature concerning the effects of stress on the pineal has originated from the laboratory of Miline and colleagues. For example, the pineals of rats immobilized for a period of 24 hours, showed a hypertrophy of the nuclei and nucleoli of the pinealocytes, a hypertrophy of the mitochondria, an enlargement of the Golgi apparatus and a degranulation of the sympathetic nerve endings (69). Moreover, placing rats in cold environmental temperatures resulted in similar structural changes (68). In another study conducted by Miline and co-workers (67), hibernating bats were subjected to an auditory stimuli. The histophysiological reaction of the pineal was found to be involutive. These results were in contrast to many of the observations of pineal changes that occur during stress. The pineals of adult male rats also exhibited atrophic changes when they were exposed to the pungent odor of isonitrile (66). All of these studies have led Miline and colleagues to postulate a role for the pineal in mediating adaptation during stress (69).

Another factor which in some instances in stressful, is locomotor activity. Ralph et al. (91) reported that the amount of melatonin in the rat pineal is closely linked with the locomotor activity of the animal. Although pineal changes observed during periods of stress are many and varied, the observations made should eliminate any narrow interpretation of the pineal being affected only by radiant energy perceived through the lateral eyes.

Stressful agents have also been observed to influence pineal indoleamine metabolism. Previous mention has been made of the reduced activity of NAT and HIOMT after continuous exposure to light. Many other stressors

have been demonstrated to affect pineal indoleamine metabolism. Ulrich et al. (114) have shown an increase in pineal NAT in suckling rats placed in dark and exposed to lowered environmental temperatures. This in contrast to animals exposed to constant light at the same temperatures that showed increased pineal 5-HT levels. Chronic cold has also been demonstrated to increase catecholamine levels in the brain, while acute stress of various types (foot shock, exposure to cold) has been demonstrated to reduce endogenous content of NE in the brain (7). The changes in brain catecholamine levels could be a possible explanation of the changes in NAT following cold stress in that NE and isoproterenol, when injected into rats, increases the enzyme activity (18).

Ellis et al. (24) have demonstrated that x-irradiation as a stressor activates pineal enzymes. Radiation localized to the heads of adult male rats with 450 R increased pineal HIOMT activity and pineal biosynthesis of melatonin from tryptophan-3-^{14}C. Injections of histamine-PO_4 caused inhibition of rat pineal melatonin synthesis.

Recently, Lynch and co-workers (60) found that melatonin and NAT activity of the rat pineal increased rapidly in response to physical immobilization or insulin induced hypoglycemia. This response can be blocked by injecting propranolol, a beta-adrenergic blocking agent. It was found that prior destruction of the sympathetic nerve terminals with 6-hydrozydopamine does not block, but actually potentiates the increase in melatonin content and NAT activity after induced hypoglycemia or immobilization. These findings suggest the influence

of circulating catecholamines and glucocorticoids from the adrenal may act on the pineal. Moreover, the activity of NAT is rapidly elevated in vivo after administration of catecholamines, MAO inhibitors or theophylline. Inhibitors of protein synthesis or propranolol completely blocks this increase in activity of NAT. A superinduction of NAT activity was also observed upon administration of L-DOPA or MAO inhibitors in denervated rat pineals (18).

Starvation has also been observed to alter pineal activity. A recent study by Urry and Ellis (115) indicated an increase in pineal NAT activity, pineal weights and pineal HIOMT activity after restricting rats to one-third their normal food intake when compared with other animals.

Food deprivation or immobilization has also been shown to increase rat brain tryptophan and 5-HIAA (16, 83). There is now considerable evidence that brain 5-HT synthesis is influenced by cerebral tryptophan concentrations (49). These apparent changes of brain 5-HT synthesis are consistent with the belief that tryptophan hydroxylase (the rate limiting enzyme) is functional even though it is non-saturated by its substrate tryptophan (22, 31). The increase in brain tryptophan that occurs after food deprivation or immobilization appears to result from an increase in the small fraction of plasma tryptophan that is not bound to plasma proteins (48).

Regulation of Monoamine Oxidase

General considerations

Amine oxidases are widely distributed in nature and catalyze the following reaction:

$$R\text{-}CH_2\text{-}NH_2 + O_2 + H_2O \dashrightarrow RCHO + NH_3 + H_2O_2$$

MAO enzymes catalyze the irreversible oxidative deamination of NE, E, 5-HT, dopamine and many other monoamines. The physiological role of MAO is unknown; however, one function may be to inactivate toxic amines that are endogenously produced or injected. This function is illustrated by the occurrence of severe hypertensive reactions in patients receiving MAO inhibitors after ingesting food high in tyramine. Apparently, the ingested tryamine induced NE release from the nerve endings thus stimulating receptors involved in the hypertensive reaction.

MAO plays a major role in the metabolism of transmitter amines that diffuse from neuronal storage vesicles into the neuroplasm and a minor part in terminating the action of the transmitter amines that are released by the nerve impulses onto receptor sites (50, 51). Although NE is a substrate for MAO, there is considerable evidence that this enzyme does not play a major role in the inactivation of circulating catecholamines, except in the deamination of the O-methylated derivatives formed from administered catecholamines. It is believed that COMT is the enzyme mainly responsible for the inactivation of circulating E and NE (2).

The existence of multiple forms of MAO in various tissues of the same animal has been recognized for several years. Yang, Gordis and Neff (131) have recently postulated and described the existence of MAO in the sympathetic nerve and the pineal gland. Their research compared the MAO in the pinealocyte and in the pineal sympathetic fibers and found the enzyme to have different characteristics. The predominant enzyme in the ganglion was inhibited by low concentrations of clorgyline (0.1 mM), exhibited a lower apparent K_m for tyramine than the enzyme in the pinealocyte, was readily inactivated by trypsin and was relatively heat stable. In contrast, the pinealocyte MAO was inhibited by 0.1 mM clorgyline, was not readily inactivated by trypsin nor was it heat-labile. Moreover, these enzymes were found to have different substrate specificities.

Neff and Gordis (75) have further clarified the properties of the MAO enzymes involved in pineal biochemical activities in a recent review. Enzyme-A is the dominant enzyme in the sympathetic nerve endings. Apparently MAO enzyme-A deaminates 5-HT, NE and normetanephrine, whereas, enzyme-B (which is found in the pinealocytes) deaminates benzylamine and is relatively insensitive to clorgyline. Both enzymes deaminate tryamine, dopamine and tryptamine. The ratio of the activity of the ability of enzyme-A to metabolize tryamine and 5-HT varies among itssues; therefore, Neff and Gordis suggest that MAO enzymes A and B represent classes of enzymes rather than single entities.

The pineal is totally innervated by sympathetic nerves that originate from the superior cervical ganglion. Glands from normal animals contain both sympathetic nerve endings and pineal gland cells, whereas, glands from superior cervical ganglionectomized animals contain primarily pinealocytes. Changes that occur after ganglionectomy are assumed to be associated with the loss of sympathetic nerve endings. For example, the fall of MAO activity in the pineal after ganglionectomy is most likely due to the loss of sympathetic MAO (75).

Adrenal gland: Effects on MAO

The activity of MAO might be regulated by modifying its synthesis or catabolism or both. As was mentioned previously (133), rat pineal MAO appears on the day after birth and reaches adult levels by day-18. Shih and Eiduson (109) have demonstrated by gel electrophoresis that molecular forms of MAO in the chick brain change with age. Thus, the action of any regulating factor may be limited by changes in various forms of MAO present in the tissues.

In a recent study completed by Parvez and Parvez (80), the activity of MAO was measured in the brain, heart, liver and spleen of rats 10 weeks after adrenalectomy. Another group of adrenalectomized rats received 5 mg of hydrocortisone daily for a period of 10 days. The MAO activity of the two groups was then measured. There was a significant rise in MAO activity following adrenalectomy in the tissue homogenate, mitochondrial and supernatant fractions of all the organs studied. Hydrocortisone administration

decreased MAO activity to the level of normal rats. Parvez and Parvez suggest from these results that the adrenocorticoids serve as a rate-limiting factor in the control of MAO activity. They tested this theory by blocking the biosynthesis of glucocorticoids with Metopirone. A single injection of 75 mg of this drug increased cerebral, hepatic and cardiac MAO from control levels. These results led Parvez and Parvez to speculate that adrenocorticoids present in the circulation of normal rats regulate MAO activity in most organs.

Csaba, Went and co-workers (15, 126) examined the activity of MAO and 5-HTPD in rat and mouse tissues after adrenalectomy and cortisone treatment. They found no substantial change in 5-HTPD activity 24 hours or 12 days after adrenalectomy. Protracted cortisone treatment caused a small decrease in lung 5-HTPD activity while in the skin, pylorus, duodenum and liver the activity remained unchanged. With the exception of the duodenum, adrenalectomy had no effect on rat MAO activity in the lung, pylorus or liver. In the small intestine, MAO activity was found to be significantly higher 24 hours and 12 days after the operation. Both single and prolonged treatment with cortisone reduced MAO activity in the liver and raised it in the pylorus, while no change was observed in the lungs or duodenum.

In the mouse, 5-HTPD activity showed no change in the lungs, stomach, small intestine or liver on the first or twelfth day following adrenalectomy. Cortisone in either single or prolonged treatment failed to induce a change in 5-HTPD. MAO activity remained unchanged in liver, lungs, stomach and small intestine of adrenalectomized animals, whereas, both single and prolonged

cortisone treatment significantly decreased MAO activity in the liver, lung, stomach-pylorus and small intestine.

Parvez and Parvez (81) have proposed a possible molecular mechanism by which glucocorticoids inhibit MAO and other enzymes of metabolic significance.

> There is abundant evidence available that glucocorticoids regulate many enzymes of metabolic significance by induction of protein synthesis of new enzyme molecules. The exact connection between receptor sites for glucocorticoids and their effects on protein synthesis remains tenuous and subject to further extensive studies at the genetic level. Now it is generally accepted that these hormones probably exert their effects at the level of RNA transcription to DNA. Previous studies also provide evidence that changes in enzyme activity are thought to occur on the amount of enzyme protein present in the tissues and consequently the regulatory factors. The regulatory factors affect the rate of synthesis as well as break down of enzyme protein. The biosynthesis of active enzyme protein might eventually be dependent on a sufficient amount of hormonally stimulated effectors or other cofactors. (81, p. 1261)

Stress: Effect on MAO

Various stressors have also been shown to change the activity of MAO in tissues. Many of these investigations have employed "physical" stresses based on intense stimuli of a single type, i.e., swimming, cold exposure and electric shocks. It is, therefore, difficult to discriminate the psychological effects of fear and frustration from the physical effects of extensive muscular effort, debilitation and the exhaustion of tissue resources (124). However, a recent study conducted by Maura and colleagues (62) described changes in MAO following stress involved with emotional responses. A combination of environmental stresses (auditory stimulation, flashing lights and cage oscillations)

were applied acutely and chronically to newborn rats from 10 days after birth until the age of 4 months. MAO activity was measured in the liver, brain, heart, stomach, fundus, adrenals and blood platelets. Acute environmental stress was ineffective in altering MAO levels in all organs; however, psycho-environmental stress, chronically applied from birth until full maturity decreased MAO activity in heart, liver, brain and fundic homogenates. MAO in liver, brain and heart tissues were found to be more sensitive to stress than adrenal, platelet and fundic homogenates.

The above results could represent either an alteration of MAO synthesis and/or catabolism, and inhibition caused by increased adrenal cortical activity or a combination of both.

Other factors influencing MAO

There is little doubt that MAO can be influenced by a variety of factors other than adrenal glucocorticoids. Urry, Frehn and Ellis (116) have demonstrated a dependence of testicular MAO activity on hypophyseal hormones in both immature and mature animals. The increase in total testicular MAO following FSH injection into hypophysectomized rats indicates that FSH may be the prime hypophyseal factor responsible for maintaining testicular MAO activity. Prolactin and HCG had no effect on testicular MAO. It was also demonstrated that adrenalectomy does not alter testicular MAO, indicating that the adrenal corticosteroids may have no effect on the enzyme in the testis. Urry and co-workers suggest that testicular MAO may respond differently to inhibitors than the same enzyme from other tissues. This

hypothesis supports previous findings of the existence of multiple forms of MAO in various tissues (132).

MAO Involvement with pineal enzymes

MAO enzyme-A deaminates sympathetic nerve 5-HT and NE in the pineal organ (75). Studies on the inhibition of pineal MAO have demonstrated dramatic changes in pineal indole metabolism. Deguchi and Axelrod (18) described the action of various compounds on NAT activity. They found that this enzyme activity was rapidly and markedly elevated in vivo after administration of L-DOPA, NE, E, isoproterenol, theophylline and the MAO inhibitors Catron and pargyline. Deguchi and Axelrod (18) reasoned that since there is almost no NE in the denervated pineal organ, it is possible that inhibitors of MAO might elevate the concentration of circulating NE or might act directly on the receptor site itself.

Klein and Weller (46) have also demonstrated that harmine, another inhibitor of MAO, can increase the concentration of NAT in cultured rat pineal organs.

Dgeuchi and Axelrod's (19) belief that MAO inhibitors (pargyline, Catron and harmine) may stimulate the adrenergic beta receptor in the pineal has been refuted by Illnervoa (38). Illnerova argues that pargyline could not act on the pineal receptors because she has demonstrated that administration of this drug in the evening does not prevent a decrease in NAT activity after a short light stimulus at night, whereas, the administration of isoproterenol

before the stimulus does prevent the decrease. Illnerova further argues that a sudden light stimulus at night--obviously inhibiting NE release from sympathetic nerve endings--causes a decrease in enzyme activity. Pargyline does not prevent this decrease, so it obviously does not act on the receptors in the same manner as isoproterenol. Illnerova continues by clarifying that not even NE breakdown by MAO is decisive for raised NE levels. Even with completely inhibited MAO, the NAT activity rapidly decreases. Thus, it would appear that effective NE is broken down after light by re-uptake into the nerve endings or by the action of COMT.

Non-Indolic Pineal Substances

General considerations

The abundance of evidence suggesting that pineal antigonadotrophic activities are indolic in nature is by no means unequivocal. Researchers investigating the chemical nature of pineal hormones have aligned themselves into two schools of thought. One group of workers maintains that the pineal hormones are indoleamines while the other group contends the pineal substance is a polypeptide. With few exceptions, the polypeptides have been promoted predominantly by scientists in Europe and Asia while American workers have conducted most of the research on the indoleamines. The two schools of thought are not mutually exclusive and investigators do not deny the possible antigonadal effects of several different categories of pineal substances.

One of the first studies to ascertain whether or not the pineal gland contains polypeptides was conducted by Milcu and co-workers (65). These men isolated a polypeptide in bovine pineal and concluded that the substance was arginine vasotocin or a hormone with similar structure. Cheesman (12) later confirmed by mass spectroscopic analysis that the pineal contained AVT.

Gonadal effects of pineal substances

A few years before Cheeseman had confirmed the presence of AVT in the pineal, Pavel and Petrescu (82) and Moszkowska and Ebels (71) had demonstrated this substance to be antigonadotrophic. The results of these studies indicated that the peptide interfered with the action of the gonadotrophin or gonadal hormones at the peripheral level, but a central effect was not precluded. The indoleamines are believed to act at a central level.

Several other, yet unidentified, hormonally active pineal fractions have been separated from pineal tissue. After gel filtration on Sephadex G-25, Moszkowska, Ebels and colleagues (21, 70, 72, 73) successfully isolated several constituents from bovine pineal powder which exhibited activity when incubated with rat anterior pituitary glands. At least two of these fractions modulated FSH, one acting in a stimulatory manner, and the other being inhibitory. Another fraction was found to inhibit LH secretion. Purification and identification of these fractions has proven difficult and their exact chemical nature remains unknown. It is known, however, that the substances are not melatonin and probably have a molecular weight of less than 500.

Using the COH bioassay, Benson, Matthews, Vaughan and colleagues (4, 61, 119) have demonstrated gonad inhibiting properties of pineal extracts thought to be small polypeptides (MW 500-1000). Whether or not this material is related to AVT is not known. In another study, Vaughan and co-workers (121) demonstrated that AVT inhibited the development of the ovaries, testis, ventral prostates, seminal vesicles and coagulating glands when injected into immature rodents. Similar effects were also found in immature hamsters (120). These studies suggest that AVT might mediate the effects of the pineal gland on normal sexual development.

Several studies have been conducted to ascertain if melatonin-free pineal extracts act centrally. Orts, Benson and Cook (77, 78) have demonstrated that crude, melatonin-free extracts of rat pineal glands inhibits COH, delays vaginal opening time, reduces the incidence of constant estrus and significantly inhibits the concentration of serum LH. These experiments were supported in a recent review by Reiter (96) who reported on previous findings of Pavel and co-workers. These men demonstrated that synthetic AVT inhibits COH when injected into the third ventricle, yet, has no influence when injected intravenously.

In summarizing the antigonadal effects of pineal constituents, Reiter (96) states:

> It seems that neither the pineal indoles nor the pineal polypeptides consistently yield unequivocal results in reference to their gonadotrophin-inhibiting ability. The question of the specific pineal substance(s) responsible for the gonad-regulating capability of the pineal obviously remains unanswered. ... Perhaps both the indoles

and the polypeptides are secreted by the pineal gland and act through different mechanisms on the neuroendocrine-gonadal system. The indoles may act on the brain where they modify monoamine or catecholamine metabolism, while the polypeptides may act on the anterior pituitary in a manner similar to the action of other polypeptides, the hypothalamic releasing factors. (96, p. 285)

METHODS OF PROCEDURE

Assays

MAO assay

MAO activity was measured according to the methods of Urry et al. (117). This procedure involved measuring the amount of ^{14}C-5-HT that was converted to 5-HIAA and 5-hydroxyindole acetaldehyde. These products were then extracted into ethyl acetate, dried down and counted in a liquid scintillation counter.

NAT assay

NAT activity was measured using individual pineal glands with thin-layer chromatographic isolation techniques as described by Klein, Weller and Moore (47).

Pineal Organ Experiments

Animal care

Unless otherwise specified, male rats (Holtzman strain) were maintained under natural lighting in a professional animal care facility. The room temperature in this unit was maintained at 71 F with a relative humidity of 35 percent. Laboratory chow and water were given to all animals ad libitum except in the case of the starvation experiment. All animals were sacrificed by decapitation.

Bovine pineal MAO: Effects of cortisol

A bovine pineal organ was obtained from a female animal minutes after the animal's death. The organ was maintained on ice during transport back to the laboratory. The gland was homogenized (5 ml water per gram tissue) and seven 10 μl samples were taken from the tissue homogenate for incubation in each of five treatments: (a) control, (b) 10^{-4}, (c) 10^{-5}, (d) 10^{-6}, and (e) 10^{-7} M cortisol. Each group was then assayed for MAO. Aliquots of cortisol (hydrocortisone alcohol--Sigma Chemical Co.) dissolved in methanol, were transferred to assay tubes and the alcohol evaporated with dry nitrogen gas at 45 C. The assay mixture was added to the tubes and the assays conducted as described above. The mean and standard error of mean of each group was calculated and compared to the control group by use of the student's t-test and then plotted to give a dose-response curve.

Rat pineal NAT: Effects of cortisol and norepinephrine

Male rats weighing 250 to 300 grams were used in each of four experiments described below. In experiment one, rats were divided into three groups: (a) control intact (eight animals), (b) adrenalectomized (nine animals) and (c) adrenalectomized cortisol injected (10 animals). All animals were adrenalectomized in the laboratory under ether anesthesia.

Each animal in the adrenalectomized injected group received 3 mg of cortisol suspended in 0.1 ml corn oil, s.c., 24 and 3 hours before sacrifice. The animals in the other two groups each received 0.1 ml injections of corn oil, s.c., during the same periods of time. Due to the light sensitive nature

of the pineal, animals were quickly sacrificed by decapitation between 11 p.m. and midnight after only brief (not more than 30 seconds) exposure to dim light. The pineals were quickly removed, weighed, homogenized and assayed for NAT activity as described above.

Experiment two was conducted in a similar manner to the first experiment except that the animals were injected as described above 6 and 3 hours before the assay began. All animals in experiment two were also put on a 14 hour light schedule (6 a.m. to 8 p.m.) for the week preceeding the assay. NAT activity was measured as described above.

Experiment three was conducted in the same manner as experiment two with the following modifications: The adrenalectomized injected group received 6 mg cortisol, s.c., at 10 a.m. 2 days before the assay, 3 mg cortisol at 10 a.m. and 4 p.m. the day before the experiment and 3 mg cortisol at 10 a.m. and 8 p.m. the day of the experiment. All control and adrenalectomized animals received injections of corn oil during the same time periods described above. Pineal organs were assayed for NAT activity as described above.

Experiment four was conducted using three groups of adrenalectomized animals (six animals in each group). Group one (control) was injected, s.c., with 0.1 ml corn oil 24 and 3 hours before the assay. Group two received 3 mg of cortisol suspended in 0.1 ml corn oil during the same time periods. The animals in group three received the cortisol injections described for group two and in addition, an injection of NE (Sigma Chemical Co.) at a dosage

of 1.5 mg/kg in 0.1 ml of 0.9 percent NaCl 3 hours before the start of the experiment. All animals were kept on a 15 hour light schedule as described above. Pineal organs were assayed for NAT activity as described above.

Pineal NAT activity: Adrenal effects in starved rats

Nine adrenal demedullated and eight intact control animals were obtained from Hormone Assay Laboratories, Inc. All animals were fed 8 grams (approximately one-third of their normal daily food intake) of rat chow daily and water ad libitum for 23 days. On day-23, all animals were sacrificed by decapitation and the pineals were quickly removed, weighed and assayed for NAT activity. The testis, pituitaries and adrenals were also removed and weighed. Adrenal glands from each group were fixed and examined for the presence and/or absence of adrenal chromaffin tissue and for evidence of lipid droplets.

Arginine Vasotocin Experiments

Animal care

Male laboratory rats, golden hamsters and rabbits were maintained under natural lighting conditions in a professional animal care facility with a room temperature of 71 F and a relative humidity of 35 percent. All animals were fed Purina laboratory chow and water ad libitum. Animals were sacrificed by decapitation or paracervical dislocation before removal of testicular tissue.

Testicular MAO activity: Effects of AVT

Synthetic AVT (8-arginine oxytocin) was obtained from Spectrum Medical Industries, Inc. The stock solution in 0.1 M acetic acid was diluted in instant buffer to 10, 1, 0.1 and 0.01 nU of activity per tube. Seven samples of decapsulated homogenized testicular tissue was incubated with each of the above concentrations of AVT and a control containing buffer only. The incubation mixtures were then assayed for MAO as described above. This procedure was followed on experiments using rabbit, rat and hamster testicular tissue.

PART I

EFFECTS OF THE ADRENAL GLAND ON THE RAT PINEAL ORGAN

RESULTS

Pineal Organ Experiments

Bovine pineal MAO: Effects of cortisol

Bovine pineal MAO was significantly inhibited when incubated with cortisol at 10^{-7} M concentration. No significant inhibition was seen at concentrations greater than 10^{-7} M (Figure 1).

Rat pineal NAT activity: Effects of cortisol and norepinephrine

In experiments one, two and three, adrenalectomized rats showed a decrease in NAT activity as measured in counts per minute per incubation when compared to control animals (Table 1). These observed decreases were not statistically significant, but clearly represented a consistent trend towards a decreased activity. Various regimes of cortisol administration in experiments one, two and three raised enzyme activity above the adrenalectomized values, however, this increase in activity in each case was not statistically significant (Table 1).

NAT activity was raised in experiment four from the control adrenalectomized group after injection of 3 mg cortisol 24 and 3 hours before the assay. This increase, like that demonstrated in the previous three experiments, was not significant. When cortisol treated animals were injected with

Figure 1. Inhibition of bovine pineal MAO activity in vitro with various concentrations of cortisol (N = 7 for each group). Vertical bars represent the mean ± standard error of mean of the MAO activities expressed as counts per minute on a per incubation basis. Statistical comparisons were made between the treated and control groups.

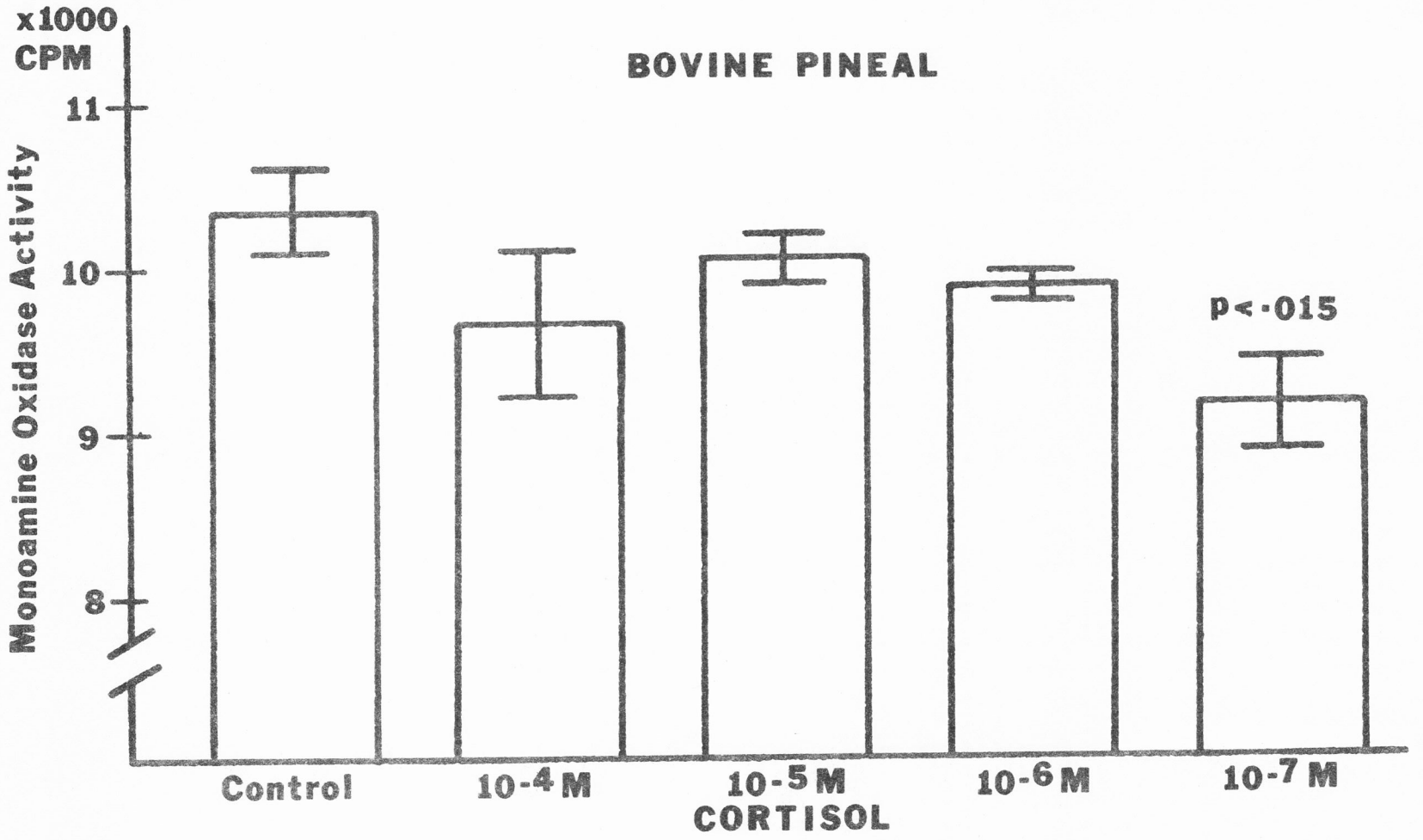

BOVINE PINEAL
x1000
CPM
Monoamine Oxidase Activity
11
10
9
8
p<·015
Control
10-4M
10-5M
10-6M
10-7M
CORTISOL

Table 1. Effect of cortisol administration on rat pineal NAT activity in vivo. Activity is represented in counts per minute per incubation and is the mean ± the standard error of mean.

Treatment	Number of animals	Exp. 1* (CPM)	p value	Exp. 2** (CPM)	p value	Exp. 3*** (CPM)	p value
Control	8	1161 ± 111		1369 ± 141		1004 ± 70	
Adrenalectomy	9	996 ± 87	<0.3	1170 ± 90	<0.3	918 ± 86	>0.5
Adrenalectomy + Cortisol	10	1032 ± 76	<0.5	1208 ± 106	<0.5	920 ± 100	>0.5

*3 mg cortisol injected 24 and 3 hours before assay.
**3 mg cortisol injected 6 and 3 hours before assay.
***6 mg cortisol injected 10 a.m. 2 days before assay.
3 mg cortisol injected 10 a.m. and 4 p.m. 1 day before assay.
3 mg cortisol injected 10 a.m. and 4 p.m. on day of assay.
p values represent comparisons between the treatment the value is associated with and the treatment located directly above that value.

1.5 mg/kg NE, NAT activity was significantly increased over control values (Figure 2).

Pineal NAT activity: Adrenal effects in starved rats

All food-deprived rats showed definite signs of weakness and slugishness at the end of the starvation period. Rat body, pineal, pituitary and testis weights are outlined in Table 2. Body weights after starvation were found to be approximately 50 percent of those before the starvation period. There was no significant differences found between control and demedullated animals after starvation in the pineal, pituitary and testis weights on a per 100 gram body weight basis. A significant increase in body weight of the starved demedullated animals was noted when compared to the starved controls.

A histological study of the adrenal glands of the starved demedullated and starved control animals (Figures 3 and 4) revealed lipid droplets representative of steroid biosynthesis in the cortical region of the glands. Confirmation of actual demedullation was also made in the demedullated group while intact chromaffin tissue was observed in the control animals. The adrenals of the demedullated group were found to be significantly larger than those of the control group (Figure 5).

Pineal NAT activity was also found to be significantly higher in the demedullated group than among the controls when calculated on a per incubation basis (Figure 5).

Figure 2. Pineal NAT activity in adrenalectomized (A), adrenalectomized plus cortisol treatment (A C), and adrenalectomized plus cortisol and norepinephrine (A C N) treated rats (N = 6 for all groups). Vertical bars represent activity in counts per minute per incubation and are the mean $\pm$ the standard error of mean. The p value represents a comparison of the ACN group with the A group.

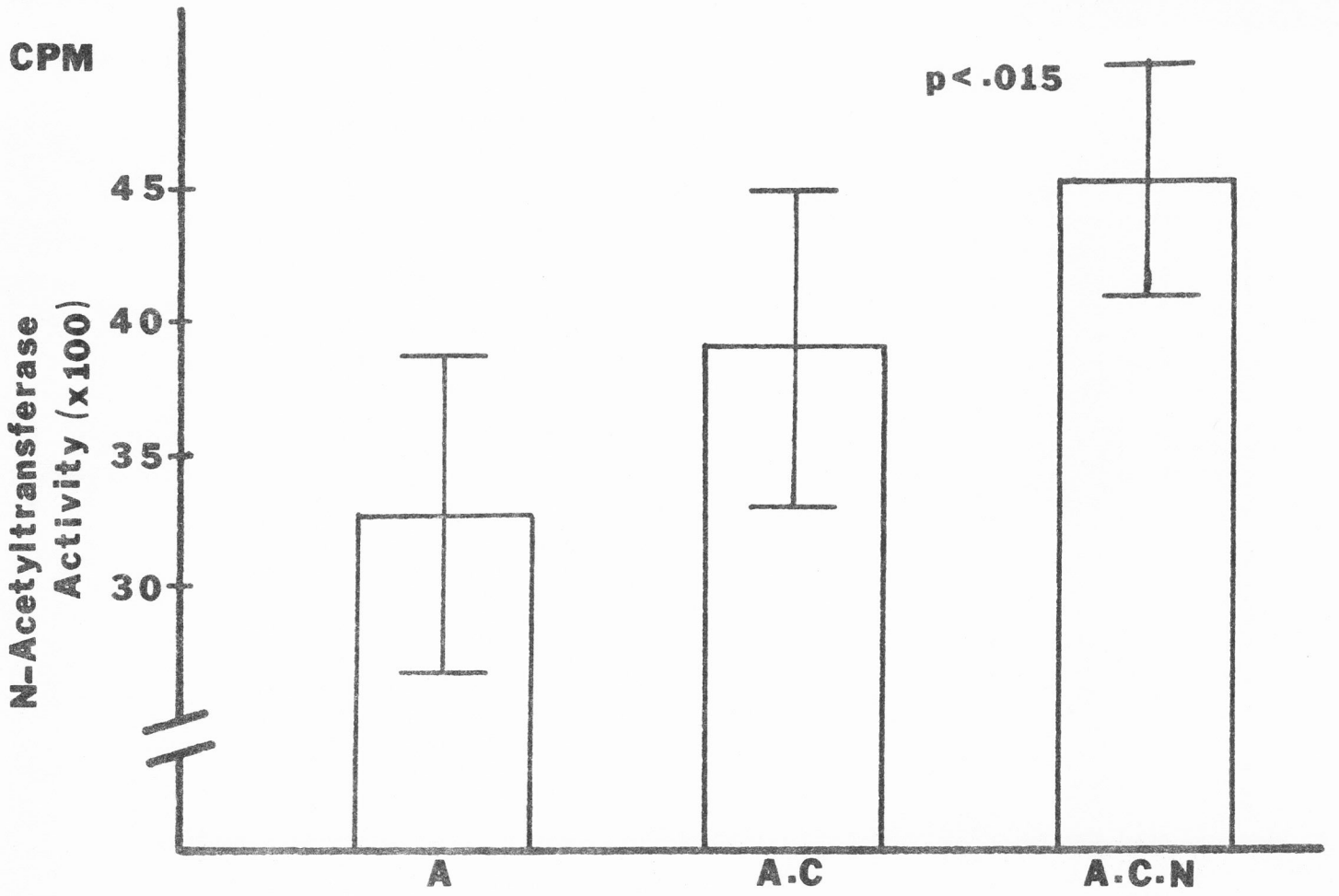

CPM
p < .015
45
40
35
30
N-Acetyltransferase Activity (x100)
A
A.C
A.C.N

Table 2. Body, pineal, pituitary and testis weights of control and adrenal demedullated animals before and and after food deprivation. Weights are represented as the mean ± the standard error of mean. Statistical comparisons are made between the starved demedullated and starved control animals.

Treatment	Number of Animals	Initial Body Weight (g)	p value	Terminal Body Weight (g)	p value	Pineal Weight mg/100 g body weight	p value	Pituitary Weight mg/100 g body wt.	p value	Testis Weight mg/100 g body weight	p value
Control	8	293.2 ± 5.5		146.4 ± 2.8		0.673 ± 0.114		3.04 ± 0.26		1573 ± 59	
Demedullated	9	283.3 ± 4.3	<0.3	157.9 ± 2.8	<0.6	0.582 ± 0.063	<0.5	3.39 ± 0.08	<0.2	1611 ± 44	>0.5

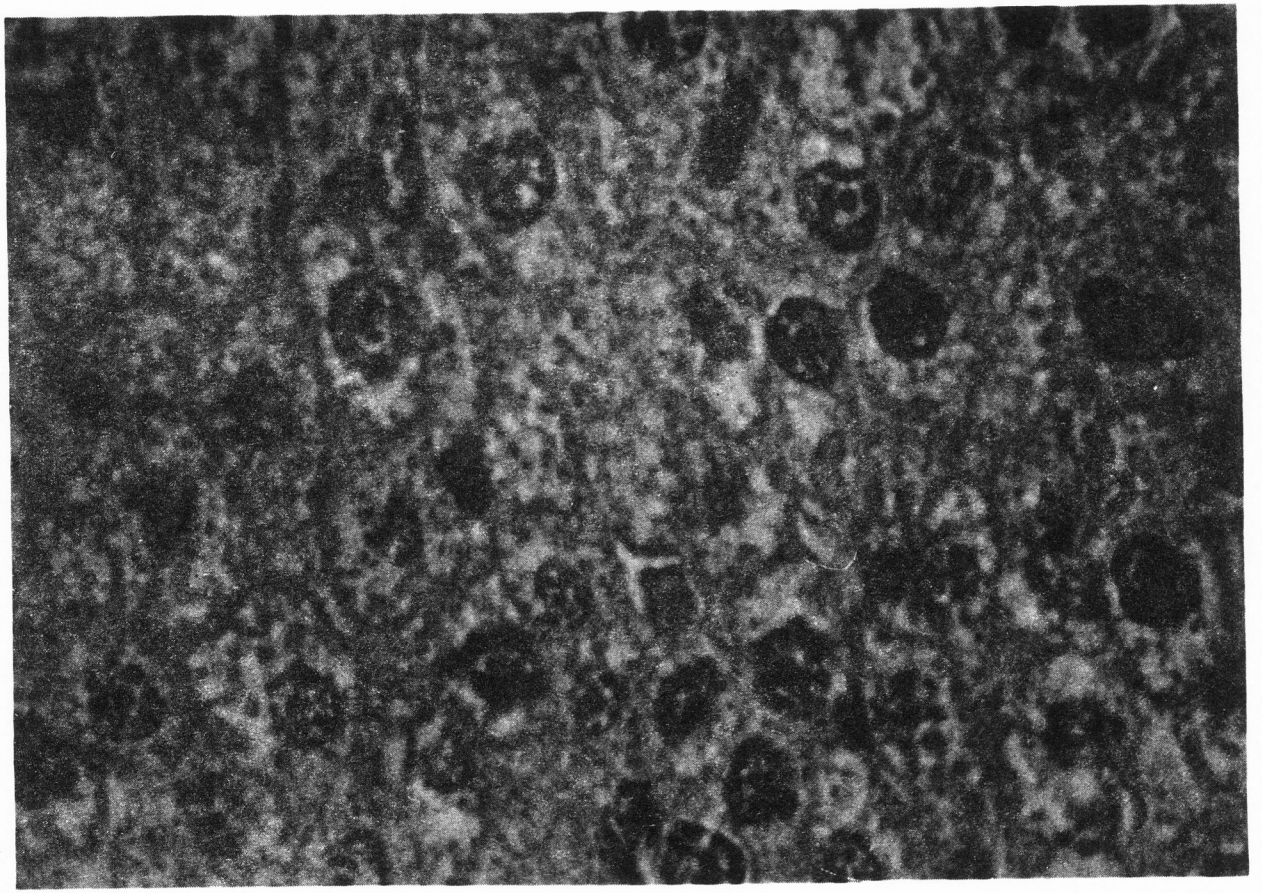


Figure 3. Adrenal cortex of starved control rats.

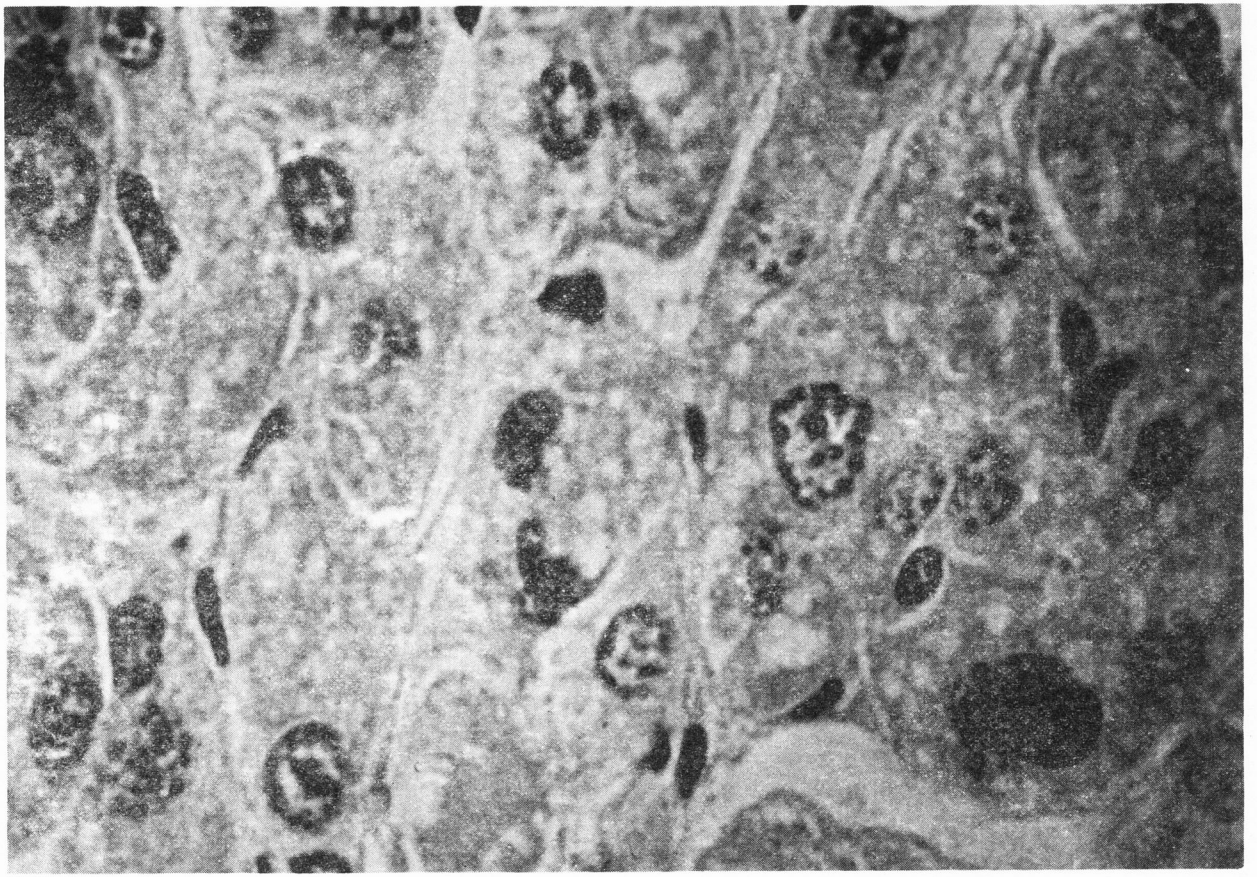
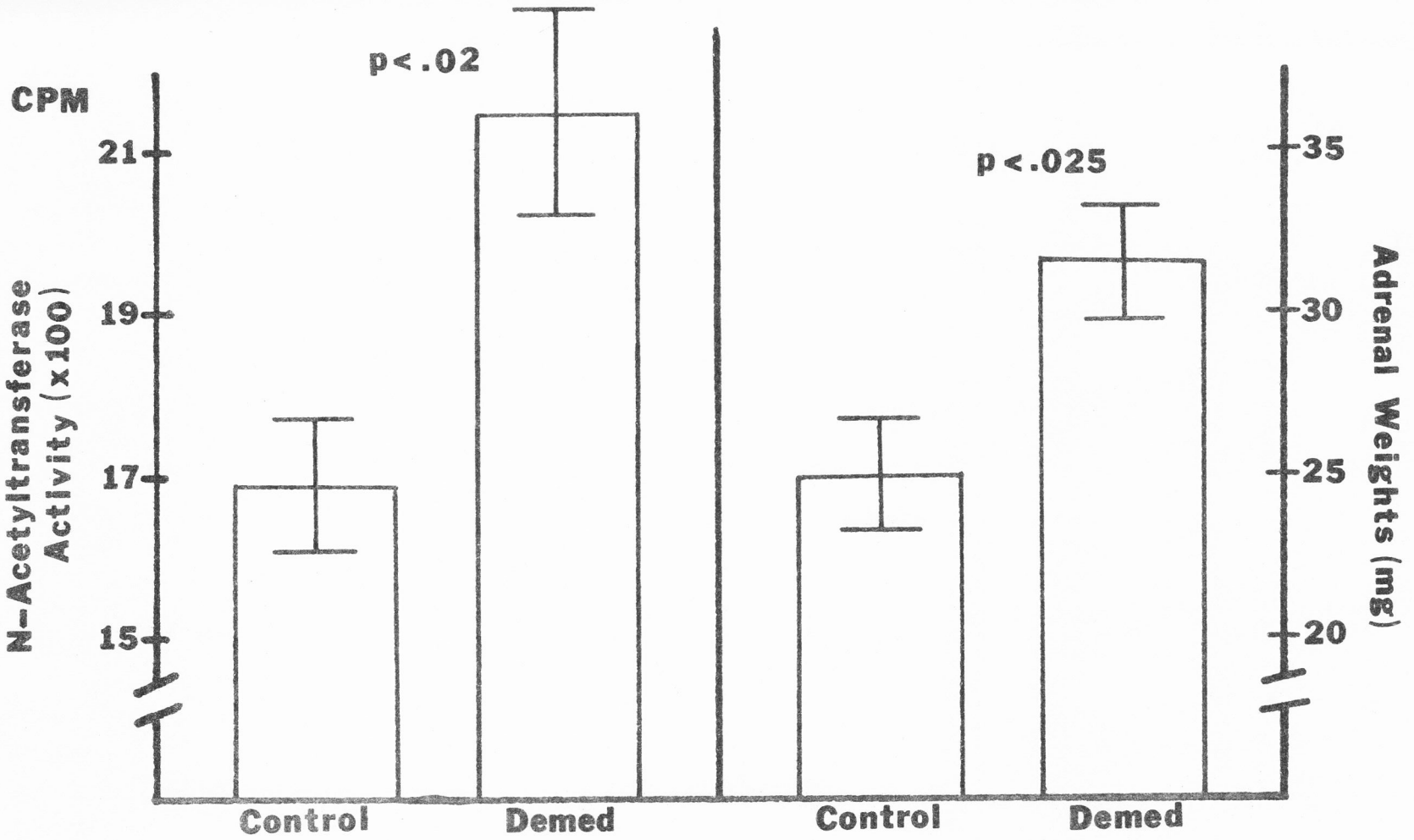


Figure 4. Adrenal cortex of starved demedullated rats showing cortical cell hypertrophy and lipid droplets representative of steroid biosynthesis.

Figure 5. Pineal NAT activity and adrenal weights for control (N = 8) and adrenal demedullated (N = 9 rats. Activities are represented as counts per minute per incubation. Adrenal weights are represented in mg per 100 g body weight. Bars represent the mean $\pm$ the standard error of mean.


p < .02
p < .025
CPM
21
19
17
15
N-Acetyltransferase Activity (x100)
35
30
25
20
Adrenal Weights (mg)
Control
Demed
Control
Demed

DISCUSSION

The observed inhibition of pineal MAO activity when tissue homogenate was incubated with cortisol lends credence to the hypothesis of Parvez and Parvez (80) that cortical steroids present in the circulation of normal animals regulate MAO activity in most organs. If Parvez and Parvez are correct, the observed decrease in MAO activity following adrenal activation would result in an increase in the monoamine concentration of the tissues involved. An increase in the NE content of the sympathetic nerves and surrounding medium of the pineal would result in an activation of the adenyl cyclase system and a stimulation of NAT activity (18). The results of experiments one, two and three, which involved the measurement of NAT activity after cortisol administration, while not statistically significant alone, do corroborate this conclusion.

Several investigators have demonstrated an increase in NAT activity following pineal MAO inhibition. Deguchi and Axelrod (18) demonstrated that the MAO inhibitors pargyline and Catron increase NAT activity 20- or 40-fold, respectively. The pargyline induced increase in NAT activity has also been observed by Illnerova (38). Illnerova warns, however, that the increase in enzyme activity is the result of a complex function of both darkness and the rhythmic ability of the organism to respond to darkness. Thus, although MAO inhibition during the normal daylight hours results in an increase in NAT

activity, even with completely inhibited MAO, the NAT activity decreases at night upon exposure to a sudden light stimulus.

Adrenergic drugs have also been shown to increase the activity of pineal NAT. Deguchi and Axelrod (18) demonstrated that E, NE, L-DOPA and isoproterenol stimulated this enzyme. These workers suggested that the adrenergic drugs and MAO inhibitors may act directly on the pineal receptor. Illnerova (38) negates this possibility by pointing out that the evening administration of pargyline does not prevent a decrease in NAT activity after a short light stimulus, whereas, the administration of isoproterenol before the stimulus does prevent the decrease. Thus, two mechanisms may be working to increase the enzyme activity. The results of experiment four support this conclusion. The administration of cortisol caused a small but detectable increase in NAT activity while the administration of both cortisol and NE raised the enzyme activity to a statistically significant level. This result was not surprising due to the fact that NE has been demonstrated to significantly increase NAT activity when administered alone (18).

Morphological changes in the pineal gland during stressful situations have been studied and elucidated by Miline and co-workers (66, 67, 68, 69). Many laboratories have also produced considerable evidence of biochemical changes in the pineal during stress (24, 60, 114). Starvation, hypoglycemia and physical immobilization have been shown to increase pineal NAT activity. Lynch et al. (60) attribute this increase in activity following stress to be due to the action of circulating catecholamines arising from the adrenal medulla.

This hypothesis is supported by the work of Deguchi and Axelrod (18) who observed a rapid increase in NAT activity after injecting various natural and synthetic catecholamines in rats. The above data strongly implicate both the adrenal medulla and cortex in pineal activation during stress.

In the starvation experiment, food deprived demedullated animals showed an increased NAT activity over starved intact controls. The adrenal glands of the demedullated group were significantly larger than those of the control group. A histological examination of the cortical region of the demedullated and control groups revealed increased lipid droplets in the cytoplasm of the demedullated adrenals which are representative of steroid biosynthesis. The adrenal cortex enlargement represents a standard reaction of the body to a stressor as described by Selye (104). The increase in the size of the adrenals of the demedullated group over those of the control group is not easily explained, but could represent a compensation reaction to the paucity of chromaffin tissue. The adrenal cortex, therefore, may play a significant role in pineal activation during the stress reaction. Care must be taken, however, to avoid simple explanations of the complex interactions occurring in the mammalian system. Lynch and co-workers (60) demonstrated a marked increase in NAT activity in response to insulin induced hypoglycemia. The starved rats in my experiment (Table 2) lost approximately 50 percent of their body weight after the 23 days of food deprivation. The observed weight loss suggests a severe loss of carbohydrate, lipid and protein stores which

would result in a hypoglycemic condition. Therefore, the blood sugar levels could be indirectly responsible for the increased enzyme activity.

The activation of the adrenal during the stress reaction results in an increase in pineal NAT activity. An increase in this enzyme's activity results in an increase in melatonin production. Since melatonin is believed to be antigonadal in most instances, one could expect any stressor that activates the adrenal and thereby the pineal, to be deleterious to gonadal growth and function. This conclusion is supported by the work of Herrington (36) who demonstrated an increase in adrenal weight and a decrease in testicular weight upon exposing rats to emotional stressors. In this respect, Christian (13) reported that the stress response associated with crowding in caged populations of small rodents was characterized by increased adrenocortical activity and depressed reproductive function.

PART II

EFFECT OF ARGININE VASOTOCIN ON RAT, HAMSTER AND RABBIT TESTICULAR MONOAMINE OXIDASE

RESULTS

Arginine Vasotocin Experiments

AVT: Effect on rat testicular MAO

Synthetic AVT was found to inhibit rat testicular MAO *in vitro* at 1, 0.1, and 0.01 nU[1] of activity (Figure 6). The inhibition was dose-dependent with the lowest concentration of AVT inducing the greatest inhibition of the enzyme.

AVT: Effect on hamster testicular MAO

Hamster testicular MAO was found to respond in an oscillatory manner when incubated *in vitro* with various amounts of AVT (Figure 7). At a concentration of 1 nU of activity per incubation, AVT was found to slightly stimulate the enzyme activity while lower concentrations of AVT inhibited the enzyme. Significant inhibition was observed with 0.01 nU AVT. The lower threshold of AVT inhibition was not established.

AVT: Effect on rabbit testicular MAO

Synthetic AVT did not alter the activity of rabbit testicular MAO (Figure 8) with the concentrations of polypeptide used.

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[1] 1 nU = 1.87×10^{-15} M AVT.

Figure 6. Effect of AVT on rat testicular MAO activity (N = 7) in each group). Activities are represented as counts per minute per incubation and are the mean ± the standard error of mean. Statistical comparisons are between treatment and control groups.

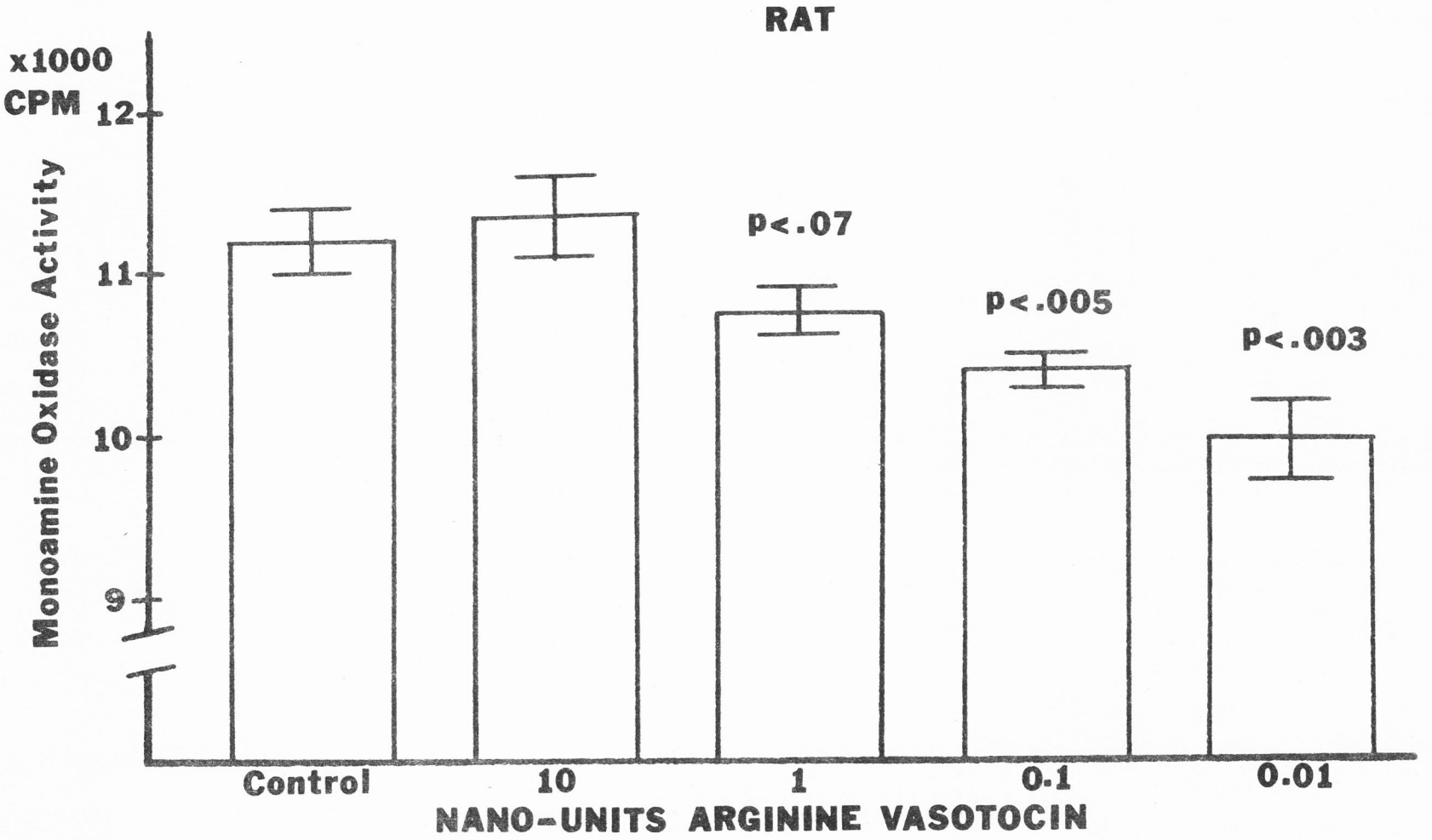

RAT
x1000
CPM
12
11
10
9
Monoamine Oxidase Activity
p<.07
p<.005
p<.003
Control
10
1
0.1
0.01
NANO-UNITS ARGININE VASOTOCIN

Figure 7. Effect of AVT on hamster testicular MAO activity (N = 7 in each group). Activities are represented as counts per minute per incubation and are the mean ± the standard error of mean. Statistical comparisons are between treatment and control groups.

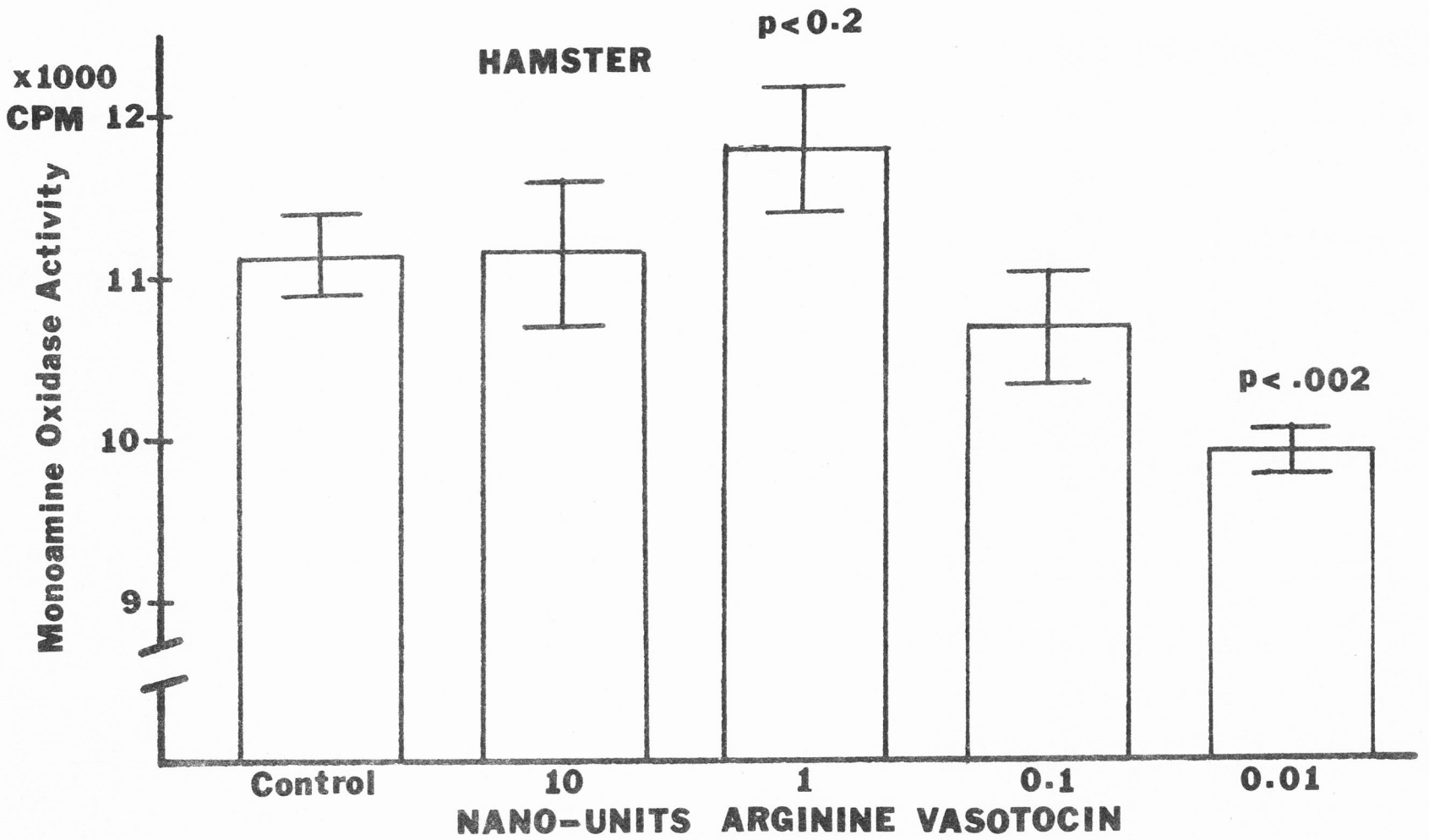
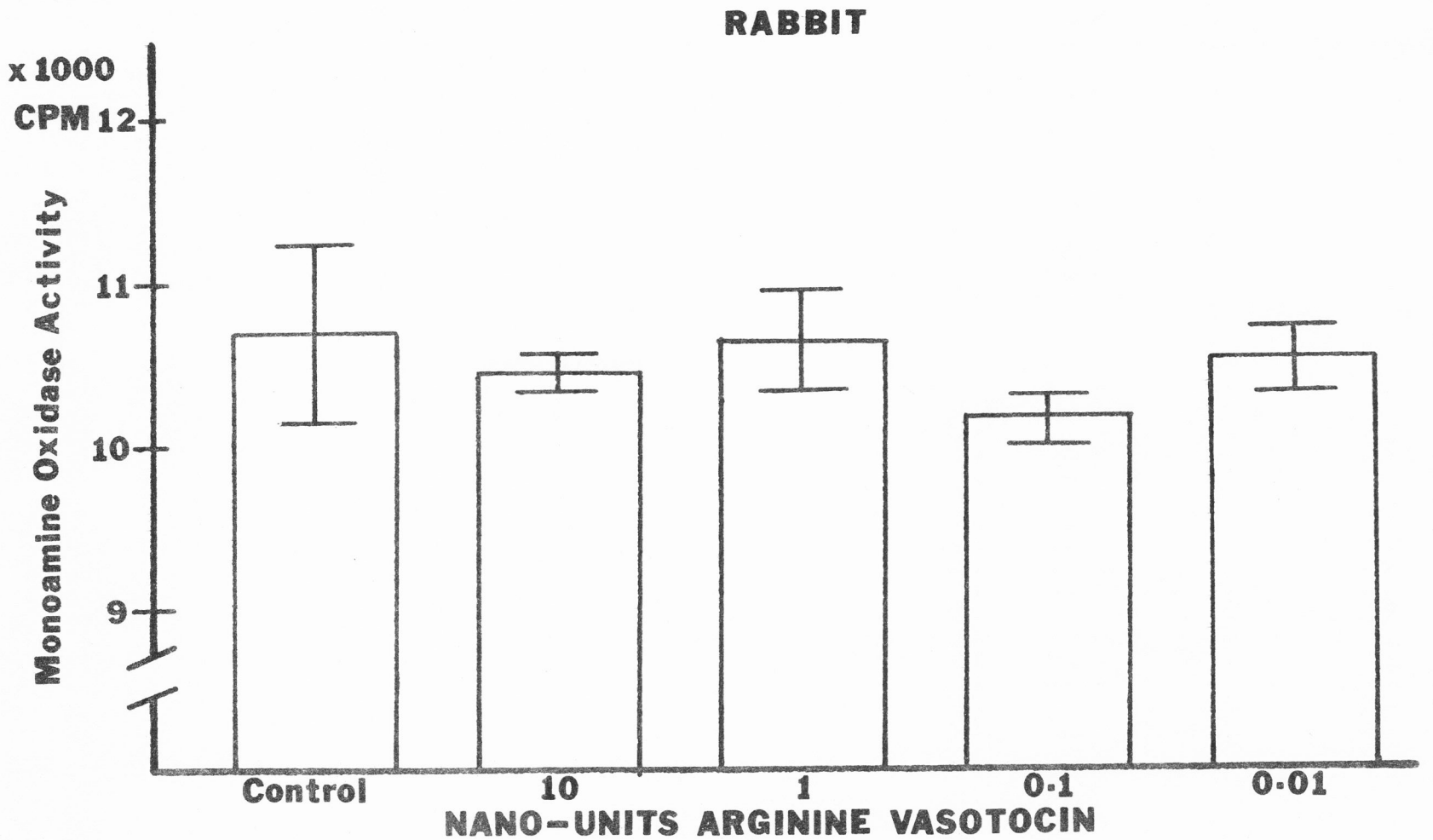

HAMSTER
p<0.2
p<.002
x1000
CPM
12
11
10
9
Monoamine Oxidase Activity
Control
10
1
0.1
0.01
NANO-UNITS ARGININE VASOTOCIN

Figure 8. Effect of AVT on rabbit testicular MAO activity (N = 7 in each group). Activities are represented as counts per minute per incubation and are the mean ± the standard error of mean. No significant differences between treated and control groups were observed.


RABBIT
x 1000
CPM 12
11
10
9
Monoamine Oxidase Activity
Control
10
1
0·1
0·01
NANO-UNITS ARGININE VASOTOCIN

DISCUSSION

The inhibition of testicular MAO by AVT has not been reported to date. These findings are significant because the primary route of 5-HT degradation in the testis is through the enzyme MAO. Serotonin has been demonstrated to be deleterious to testicular tissue by Salgado and colleagues (102) and Boccabella and co-workers (8) in rats and O'Steen and co-workers (79) in mice. Kormano and colleagues (52) found that 5-HT caused severe detrimental changes to the gametogenic elements of the rat testis. Seibel and Bush (106) on the other hand, reported that injections of 5-HT failed to alter reproductive organ weights in male hamsters. Ellis (23) has shown that 5-HT inhibits rat testicular androgen synthesis through a non-competitive mechanism. More recently, Segal and co-workers (103) have demonstrated the mean 5-HT excretion in oligospermic and azoospermic men to be significantly higher when compared to a fertile control group.

The results of this investigation demonstrate that there are different forms of MAO in the testicular tissue of various species on the basis of their response to AVT. The existence of multiple forms of MAO has been recognized for several years (75, 131) and therefore, the differential actions of AVT in rat, hamster, and rabbit testicular MAO were not surprising.

The inhibition of MAO in the rat and hamster necessarily result in an increase in testicular 5-HT. Thus, inhibition of MAO by AVT may

represent a possible peripheral pathway of pineal control of gonadal function. This conclusion is supported by the recent isolation of AVT in the pineal gland (12), and the finding that this polypeptide decreases testis weights in mice (12) and reduces growth of ventral prostate and accessory sex organs in hamsters and mice (120).

SUMMARY AND CONCLUSIONS

The purpose of this paper was twofold: (1) To examine the effects of adrenal glucocorticoids and catecholamines on pineal MAO and N-acetyltransferase activity, and (2) to observe the effect of AVT (a pineal polypeptide) on testicular MAO of various species. Pineal NAT activity was shown to increase upon injection of the adrenal glucocorticoid cortisol, and upon injection of a mixture of the glucocorticoid and the adrenal catecholamine, NE. Pineal MAO was inhibited when incubated with cortisol. The activity of the enzyme NAT was also increased in starved demedullated rats when compared to starved intact controls.

These experiments strongly implicate both the adrenal catecholamines and glucocorticoids in pineal NAT activation during stress. The glucocorticoid cortisol was found to inhibit MAO in the pineal while increasing NAT activity. This observation suggests that adrenal glucocorticoids can affect pineal melatonin synthesis possibly by altering the NE content of the sympathetic nerves and surrounding medium of the pinealocyte. The increase in NAT activity in starved adrenal demedullated rats corroborates this conclusion.

Arginine vasotocin was found to inhibit rat and hamster testicular MAO activity while no effect was seen on rabbit MAO. The observed inhibition suggests that this pineal polypeptide can act peripherally to increase the 5-HT content of the testicular tissue in certain species. Since 5-HT has been shown

to be deleterious to testicular structure and function, these experiments implicate the pineal to act in a peripheral antigonadal role.

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