CENTRAL MECHANISMS INVOLVED IN RELEASE OF PROLACTIN AND CORTICOSTERONE IN RESPONSE TO RESTRAINT STRESS.

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ABSTRACT

CENTRAL MECHANISMS INVOLVED IN
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IN RESPONSE TO RESTRAINT STRESS

By

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The purpose of these experiments was to investigate the mechanisms controlling the stress-induced rise in serum prolactin and plasma corticosterone.

1. The first study examined the relationship between time and the release of prolactin and corticosterone after 3-minute supine immobilization stress in male rats. Serum prolactin and plasma corticosterone concentrations were observed before and 0, 5, 10, 15, 30, and 60 minutes after this stress. Prolactin rose rapidly after immobilization stress and reached a maximum level by 5 minutes of the onset of stress. By contrast, the concentration of plasma corticosterone rose gradually in response to restraint stress and did not achieve a maximum until 15 minutes after the initiation of stress. The elevation in the levels of these hormones after acute stress was only transient. The levels of both prolactin and corticosterone declined shortly after their initial rise and returned to pre-stress values within 60 minutes after stress. The hypothalamus is believed to be the site where prolactin and corticosterone release in response to stress is regulated.

2. The second study indicated that central monoamines were intimately

involved in regulating prolactin and corticosterone release in response to restraint stress. Pre-treatment of male rats with drugs, which altered the levels of dopamine, norepinephrine, serotonin, and acetylcholine, produced marked changes in the pattern of prolactin and corticosterone release observed before and 15, 30, and 60 minutes after immobilization stress. Alteration in amine activity changed peak levels, the time of the peak, and the rate of decline of these hormones after stress. In addition, the reaction of each hormone to these drugs was different. Enhancement of catecholamine activity by l-dopa lowered the maximum stress-induced increases of both prolactin and corticosterone. Depletion of catecholamine levels by α-methyl dopa, however, elevated the resting level of prolactin but inhibited the release of prolactin after the application of restraint stress. By contrast, α-methyl dopa did not increase the non-stress level of corticosterone but delayed the peak rise of corticosterone consequent to restraint stress.

When both catecholamine and serotonin activities were increased by the MAO inhibitor, iproniazid, this potentiated the release of corticosterone in response to stress but had the opposite effect on prolactin release. Specific depletion of serotonin stores by parachloroamphetamine partially inhibited the stress-induced rise of prolactin 15 minutes after stress. However, this drug did not adversely affect the rise of corticosterone in response to stress but did accelerate the rate at which this hormone returned to pre-stress levels.

The cholinomimetic agent, pilocarpine, not only elevated the non-stress level of corticosterone, but also potentiated its release in response to immobilization stress. Both pilocarpine and its antagonist, atropine

sulfate, inhibited the stress-induced rise of prolactin. Atropine sulfate, on the other hand, did not adversely affect the response of corticosterone to stress.

These findings suggest that the neurotransmitters, norepinephrine and serotonin may play a role in mediating the response of prolactin and corticosterone to acute stress. What appears to be important in the stress-induced secretion of these hormones is not the action of one monoamine but an interaction between norepinephrine, dopamine, and serotonin, all of which are increased during acute stress.

3. The possible interaction of corticosterone and prolactin in response to restraint stress was examined in the third study. A single injection of either corticosterone or hydrocortisone acetate reduced the basal secretion of prolactin in male rats, but did not adversely affect the pattern of prolactin release 15, 30, and 60 minutes after immobilization stress. In contrast, adrenalectomy did not affect the resting level of prolactin secretion, but did result in an increased release in response to restraint stress over that observed in sham-operated controls and in adrenalectomized animals given corticosteroid replacement treatment. It was demonstrated that the effect of either removal or administration of glucocorticoids on prolactin release is mediated by the hypothalamus, since there was no difference in pituitary prolactin content under either condition. Both the non-stress and stress-induced changes in prolactin secretion were releated to alterations in turnover of catecholamines and serotonin after corticosteroid administration and adrenalectomy.

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INTRODUCTION

One of the prominent themes of scientific interest for many years has been how the body participates in what Seyle has termed the "general stress reaction". It has been shown that virtually every organ and chemical constituent of the body are involved in this reaction. The nervous and endocrine systems play an important part in maintaining resistance during stress. Both systems help to keep the structure and function of the body at homeostasis despite exposure to stress producing agents or stimuli. During stress the release of 4 of the 6 hormones secreted by the anterior pituitary gland may be increased. The concentration of ACTH and prolactin in the blood rise rapidly after application of such stressful stimuli as surgical trauma, cold, ether anesthesia, or restraint. Both Dunn et al. (1972) and Krulich et al. (1973) demonstrated that the levels of plasma LH are increased after application of 2 minute ether stress. A similar but smaller rise was also observed for FSH. In addition, the secretion of TSH was shown to be enhanced in response to ether stress. However, other workers have found that the secretion of TSH may decline after such stresses as surgical trauma, introduction to novel stimuli, and injection of saline or nembutal (Duncommun et al., 1966 and Kraicer et al., 1963). The secretion of GH appears to be inhibited by stress. Stresses such as etherization and cardiac puncture, cold, hypoglycemia, and intense exercise all have been shown to depress the levels of GH in rats (Schalch et al., 1968 and Collu et al., 1973). What is

significant is that all hormones secreted by the anterior pituitary are changed simultaneously during the stress raction. Other stimuli may also induce alteration in the release of all anterior pituitary hormones. There is evidence that estrogen, thyroid hormones, suckling, and underfeeding also can modify the secretion of all anterior pituitary hormones. Since stress initiates a change in all anterior pituitary hormones simultaneously, perhaps there is a common mechanism involved in controlling their secretion in response to stress. The studies reported here were designed to determine if there were common factors involved in initiating the stress-induced rise of ACTH and prolactin, two hormones known to be elevated in response to acute stress. The first experiment examined the relationship of time to levels of serum prolactin and plasma corticosterone after 3 minutes of restraint stress. The second experiment determined whether the pattern of release of these hormones after this stress could be changed by altering the turnover of biogenic amines. The third study investigated the interaction between adrenal steroid secretion and release of prolactin in response to stress.

MATERIALS AND METHODS

A pilot study examining the response of serum prolactin and plasma corticosterone to a variety of non-specific stresses demonstrated the great sensitivity of both hormones to the influence of environmental stimuli. It was found that noise produced by opening and closing cage doors and introduction to novel stimuli, such as transferring animals from their animal quarters to a strange room greatly elevated the level of serum prolactin and plasma corticosterone over control animals ($F=13.06$, $P<.002$). Further, non-handled rats displayed an increased concentration of prolactin and corticosterone over handled rats (Duncan's $P<.05$). The results of this study demonstrated the need to avoid non-specific stresses and to standardize the conditions for each experiment. Therefore, 24 hours before each experiment, all experimental animals were placed in individual cages and isolated in a separate animal room, which was not entered for at least 18 hours before the start of the experiment. Before placing the animals in separate animal quarters, the animals were kept in groups of 3-5 rats per cage for a 5-9 day period of acclimatization under constant temperature ($25\pm2^{\circ}C$) and controlled lighting (fluorescent illumination 5 A.M.-7 P.M.). Wayne Lab Blox (Allied Mills, Chicago, Illinois) and tap water were made available ad libitum. Experimental studies were carried out between 9 A.M. and 3 P.M. to avoid the naturnal diurnal rise in both serum prolactin and plasma corticosterone that occurs in the late afternoon. All stress and collection procedures were preformed outside

the animal quarters in a separate laboratory adjacent to the animal quarters. A second pilot study investigating the variation between different stress and blood collecting technics revealed that 1 minute etherization and cardiac puncture produced the greatest variation among the different methods tested. Etherization and orbital sinus puncture plus etherization and decapitation exhibited less but still a high degree of variation. Decapitation alone produced the least variation of all methods compared. For this reason, decapitation was chosen as the blood collecting method and 3-minute supine immobilization was selected as the stress. The experimental procedure employed for each study is outlined in each experimental section.

RELATION OF TIME TO THE SECRETION OF PROLACTIN AND CORTICOSTERONE IN RESPONSE TO RESTRAINT STRESS

INTRODUCTION

Stimulation of the pituitary-adrenal axis by stressful stimuli has long been recognized. The interaction of this axis with a variety of stresses has been reviewed by several authors (Ganong, 1963, Mangili et al., 1966, and Sayers and Sayers, 1947). One criterion for a stressful stimulus has been a rise in the level of adrenal corticoids.

Although the rapid reaction of ACTH to stress has been well established, the quick response of prolactin to stress has only recently been recognized. Nicoll and Meites (1960) were the first to demonstrate that stress might play a role in the release of prolactin. They found that five days of continuous exposure to a variety of stresses such as cold, restraint, starvation, or an injection of formaldehyde, induced lactation in estrogen-primed female rats. Since then, several acute stress conditions have been found to stimulate prolactin secretion in a variety of species. Grosvenor et al. (1965) demonstrated that laparotomy, bleeding, and cervical stunning depleted pituitary prolactin stores in lactating rats. Subsequently, Neil (1970) found that these acute stresses were accompanied by an elevation in plasma prolactin in the rat. Stress induced by prolonged ether inhalation or soon after pentobarbital administration elevated serum prolactin in both male and female rats (Wuttke and Meites, 1970, Wakabayshi et al., 1971). In addition, Dunn et al. (1972) observed that ether stress markedly increased serum prolactin in male rats throughout a 24 hour test period. He noted that both ether

stressed and non-stressed rats exhibited a circadian periodicity in prolactin levels. In contrast, stress abolished the rhythmic secretion of LH and dampened the daily peaks found in non-stress corticosterone secretion. Stress has also been found to be a stimulus for prolactin release in cows, goats, and humans (Meites and Clemens, 1972). Increased levels of serum prolactin were observed in cows after 10 minutes of noise and restraint stress (Raud et al., 1971). Both emotional anxiety and surgical trauma have been shown to promote prolactin release in human patients. Less traumatic situations, such as intense exercise, also were found to increase plasma prolactin in normal men and women (Noel et al., 1972).

Since the previous studies did not investigate the relation of time to the release of both prolactin and corticosterone after acute stress, the objective of the present study was to examine this relationship. The experiment was designed to test the rapidity and duration of the release of these hormones in response to 3 minutes of supine immobilization.

PROCEDURE

Animals used in this experiment were male Sprague-Dawley rats (250-300g) purchased from Spartan Research Animals, Haslett, Michigan. One hour before the start of the experiment, the rats were given an intraperitoneal injection of .5 ml. of phosphate buffer saline in .1% gelatin suspension. Non-stress blood samples were obtained by rapid decapitation after animals were removed from their animal quarters to an adjacent laboratory (time<20 sec.). Animals were stressed by subjecting them to supine immobilization in a plastic rat restrainer. Following supine immobilization, blood samples were removed at 0, 5, 10, 15, 30, and 60 minutes by decapitation. Half of the trunk blood was collected in glass tubes for serum samples to be used for prolactin assays and half in heparinized tubes for plasma to be used for corticosterone assays. At the termination of the experiment, blood samples were centrifuged and serum and plasma samples were stored at -20°C. until assayed. In addition, each anterior pituitary was removed and weighed following decapitation. At the end of the experiment the pituitaries were homogenized, diluted, and stored frozen until assayed. Both serum and pituitary prolactin were measured by a double antibody radioimmunoassay (Niswender et al., 1969) and an average of four dilutions was expressed in terms of a purified rat prolatin reference standard (NIAMD-RAT-PROLACTIN-RP1). Plasma corticosterone concentrations were measured by the fluormetric procedure of DeMoor and Steeno (1963).

RESULTS

The release of prolactin in response to restraint stress occurs with great rapidity. Figure 1 shows the changes in the levels of serum prolactin in male rats subjected to 3-minute supine immobilization. Prolactin levels started to rise immediately after stress, although no significant difference was found between levels at time 0 and non-stress levels ($P>.05$). When the concentration of serum prolactin was compared to both non-stress and stress levels at the other time intervals, a maximum level of serum prolactin was reached between 5-10 minutes after the beginning of stress (Duncan's $P<.05$). However, this may not represent the actual peak. Terkel et al. (1972) noted that 6 minute ether stress enhanced prolactin secretion in non-suckling lactating female rats. Prolactin levels started to rise between 1-2 minutes and reached a maximum 3-4 minutes after exposure to ether anesthesia. The increased levels of prolactin release produced by restraint stress in the present study were not sustained. Prolactin levels started to decline 15 minutes after stress (Duncan's $P<.05$), even though at this time the levels were still statistically greater than non-stress levels (Duncan's $P<.05$). Prolactin levels continued to fall at 30 and 60 minutes after stress. At 60 minutes, the concentration of serum prolactin was not statistically different from the non-stress state ($P>.05$). These results extend and confirm those of Krulich et al. (1973) who noted a consistent "biphasic change" in the concentration of plasma prolactin in rats decapitated 0, 10, 30, 60, and

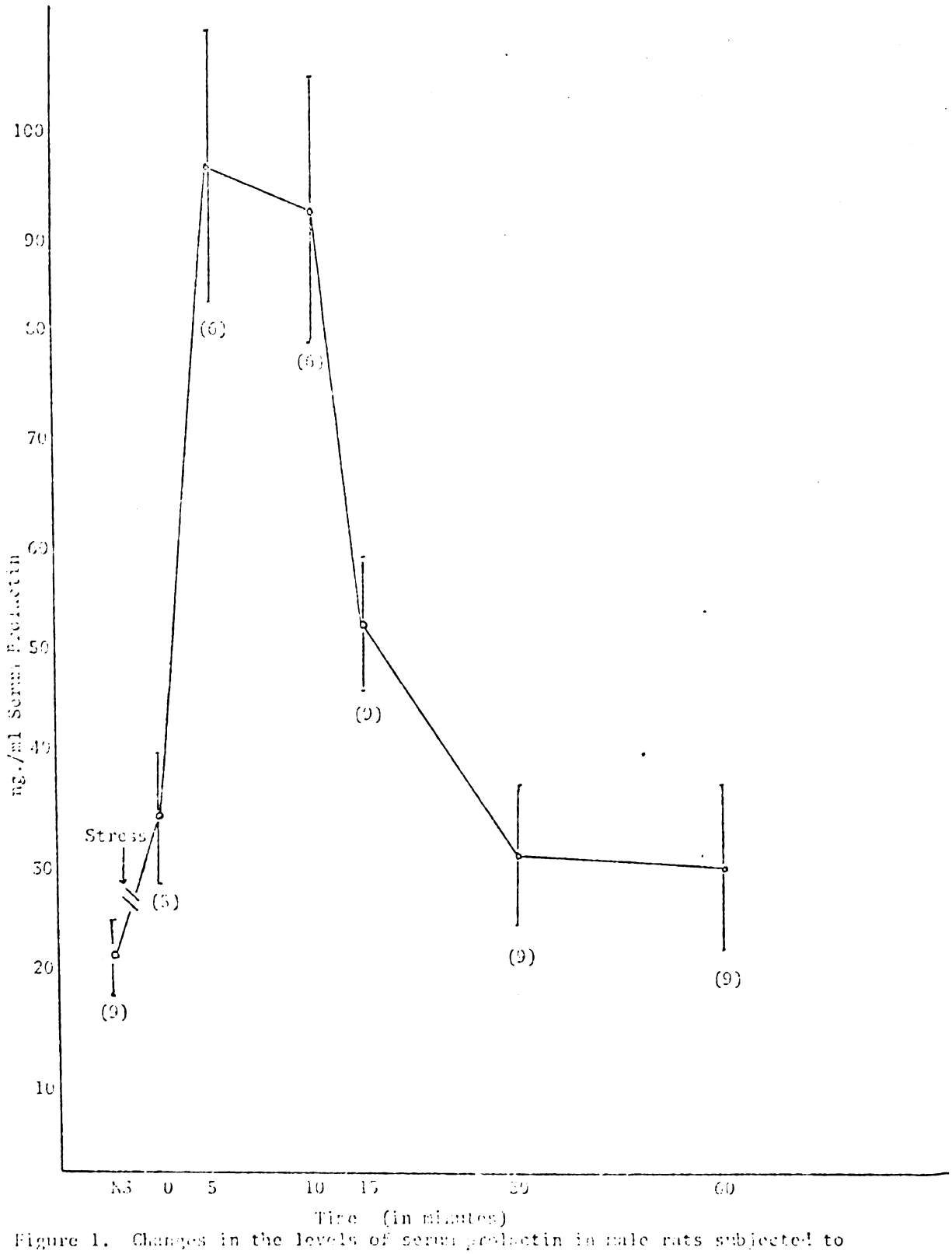


Figure 1. Changes in the levels of serum prolactin in male rats subjected to 3-minute supine immobilization. The circles represent the mean serum prolactin concentrations, the vertical lines the SEM, and the numbers in parentheses the number of blood samples taken.

120 minutes after acute stress. The stresses used in their experiment included 2 minutes of ether inhalation, repeated ether inhalation, or exposure to a novel situation. In their study prolactin levels rose within 10 minutes of application of stress, followed by a decline to slightly below initial levels 2 hours after stress.

The fall in pituitary prolactin content seen in Table 1 appeared to reflect the rise in serum prolactin following three minute immobilization stress. However, analysis of variance indicated no significant difference ($P>.025$) in the concentration of prolactin when all time periods were examined. This may be because the amount of prolactin released into the general circulation in response to acute stress is relatively small compared to the pituitary stores of prolactin.

Figure 2 shows the changes in the levels of plasma corticosterone in male rats subjected to 3 minutes of supine immobilization. Stimulation of the adrenals after acute stress is slow relative to prolactin. Duncan's New Multiple Range test showed that there was no significant elevation in the levels of plasma corticosterone at either 0, 5, or 10 minutes after stress. However, the concentration of corticosterone appeared to gradually increase following stress until it reached a maximum level 15 minutes after stress. At this time there was a 3-fold increase in plasma corticosterone levels above non-stress levels ($P<.05$). Further analysis revealed there was no significant difference between the levels of corticosterone at 15 and 30 minutes following restraint stress ($P>.05$), even though at 30 minutes the levels appeared to decline. The levels of plasma corticosterone 60 minutes after stress were not statistically different from non-stress levels ($P>.05$).

Table 1.

Effect of 3-Minute Supine Immobilization Stress on Pituitary Prolactin Content.

Group	n	Pituitary Prolactin Content ng./Anterior Pituitary
Non-Stress	5	21,509.5 ± 3079.6
Minutes After Stress		
0	5	20,499.5* ± 4082
5	6	17,399.0 ± 1320.8
10	6	18,777.0 ± 2440
15	6	15,182 ± 115.5
30	6	15,214.8 ± 540.1
60	5	15,145.5 ± 725.9

* Standard Error of Mean

n represents the number of samples taken

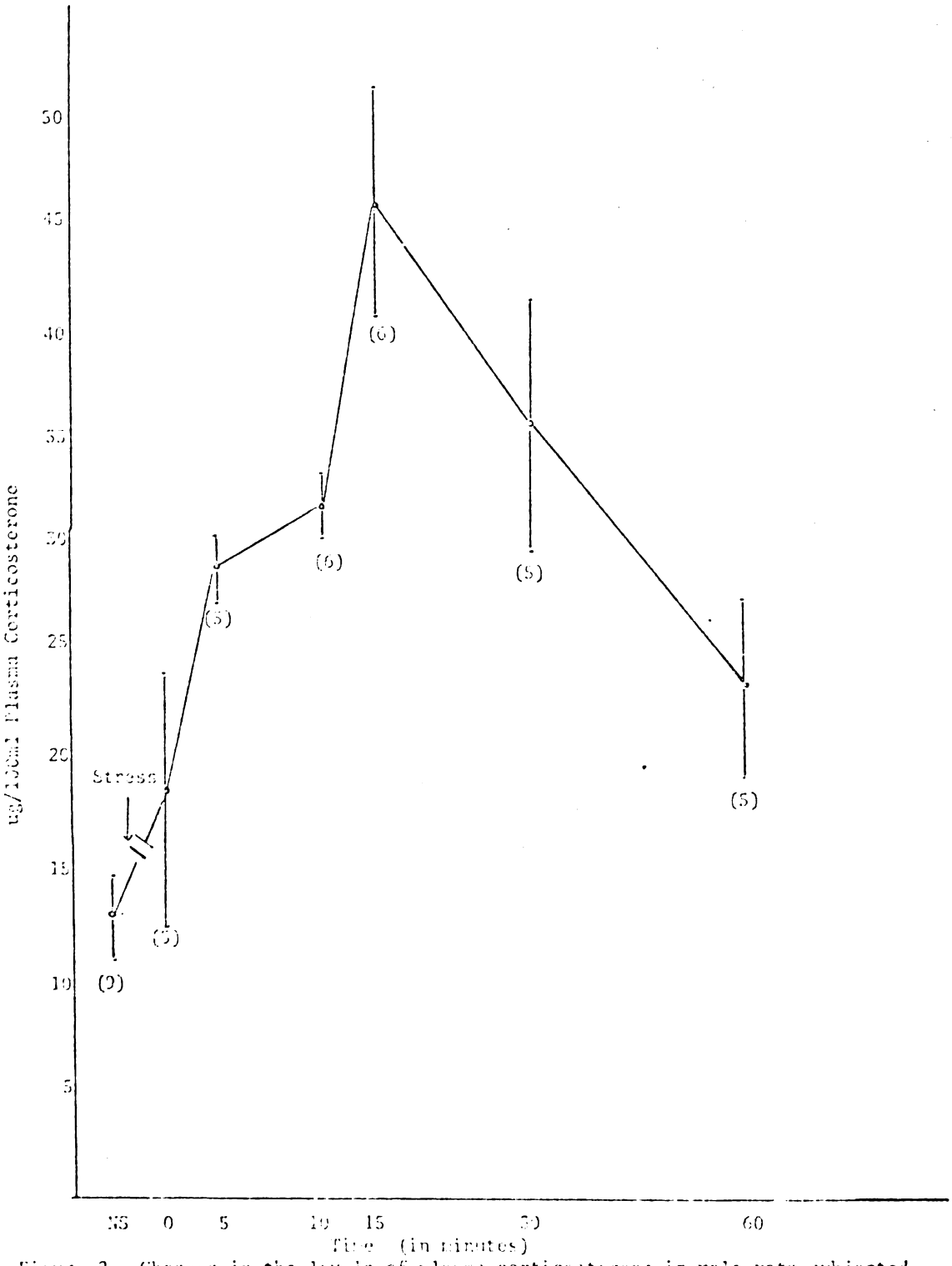


Figure 2. Changes in the levels of plasma corticosterone in male rats subjected to 3-minute supine immobilization. The circles represent the mean corticosterone concentration, the vertical lines the S.E., and the numbers in parentheses the number of blood samples taken.

DISSCUSION

The secretion of prolactin and corticosterone in response to acute stress appears to display a definite pattern. Prolactin levels rose rapidly after 3-minute immobilization stress and achieved a maximum level within 5 minutes of the onset of stress. By contrast, the concentration of plasma corticosterone rose gradually in response to this stress and did not reach a maximum level until 15 minutes after the initiation of stress. Following this rise, the stress-induced release of each hormone declined. Both prolactin and corticosterone at 60 minutes after stress had returned to levels which were not statistically different from pre-stress values. Activation of the adrenal in response to stress is slow when compared to the rapid release of ACTH after stress. Hodges (1971) followed the concentration of ACTH in the blood of rats at various time intervals after laparotomy under ether anesthesia. He observed a significant rise in ACTH by one minute after stress. A maximum level was reached 2.5 minutes after the beginning of anesthesia. The concentration of ACTH started to decline 5 minutes after stress and continued to fall at 10, 20, and 40 minutes after stress. At 40 minutes after stress the concentration of ACTH was barely detectable. One criticism of the experiment is that the concentration of pituitary ACTH after the application of stress was not compared to the concentration prior to stress. A low initial concentration could have resulted in a smaller release of ACTH in response to stress. It is thus difficult to assess the true

significance of the rapid elevation of serum ACTH after ether anesthesia and laparotomy. However, the findings of Hodges (1971) demonstrate the rapidity with which stress promotes the release of ACTH. His study also shows that the release of ACTH in response to stress exhibits a pattern similar to that outlined for prolactin release after restraint stress.

When this pattern is examined, there are two main events which must be explained in terms of existing mechanisms regulating the function of the anterior pituitary. One event involves the rapid rise of both blood prolactin and ACTH after the initiation of stress. The rapidity with which the release of ACTH and prolactin occurs after acute stress is in agreement with the existence in the hypothalamus of neural or neurohumoral mechanisms controlling the function of the pituitary gland. The hypothalamus appears to be the principal mediator of the stress response. Disruption of the functional integrity of the hypothalamus by lesions placed in the medial basal hypothalamus or median eminence, by pituitary stalk section or by pituitary transplantation has been shown to prevent stress-induced release of ACTH (Mangili et al., 1966). It seems likely that the hypothalamic influence on pituitary ACTH secretion in response to stress is mediated by CRF. Venikos-Danellis (1964) demonstrated in female rats that ether or ether and surgical stress caused a rapid and marked increase in CRF activity in the median eminence approximately one minute after stress. The enhanced CRF activity one minute after stress correlates with the elevated levels of ACTH found by Hodges (1971) 2.5 minutes after laparotomy under ether anesthesia.

The influence of the hypothalamus on prolactin secretion is different

from ACTH The predominant action of the hypothalamus on prolactin secretion is inhibitory. Suppression or removal of this tonic inhibition by either disruption of the hypothalamo-pituitary connection or by appropriate drug administration has been shown to increase the release of prolactin from the anterior pituitary (Meites et al., 1972). These findings have led some workers to interpret the rapid rise of prolactin following stress as a result of acute inhibition of PIF release (Wakabayshi et al., 1971, Meites et al., 1972). In addition to a PIF, recent evidence suggests that prolactin secretion may also be influenced by a releasing factor (PRF) (Meites and Clemens, 1972). Valverde et al. (1973) found that ether stress further elevated the levels of prolactin in rats pre-treated with reserpine (500ug/100g). Since reserpine has been shown to lower PIF activity and enhance prolactin release (Meites, 1970), they proposed that the stress augmented rise in plasma prolactin seen in reserpine-treated animals was due to stimulation of PRF rather than to acute inhibition of PIF. Whether acute suppression of PIF or stimulation of PRF is the mechanism governing the prolactin response to stress is not known. Only by directly measuring the respective PIF and PRF activities after stress can this question be answered.

Since catecholamines (dopamine and norepinephrine) and serotonin have been shown to have a profound influence on the release of the hypothalamic hypophysiotropic hormones and the anterior pituitary hormones, it is relevant to determine how stress affects these neurotransmitters. A rapid elevation in the levels of these 3 amines has generally been observed within only a few minutes of the inception of stress (Kato et al., 1967,

Breitner et al., 1963). Welch and Welch (1968a) also found that restraint stress produced a marked increase in the levels of norepinephrine, dopamine, and seretonin in various parts of the mouse brain within one to five minutes. The rise in monoamines during stress appears to proceed and parallel the maximal stress-induced release of ACTH at 2.5 minutes and prolactin at 5 minutes. No direct correlation has been made between the changes in brain monoamines and the release of the anterior pituitary hormones after a stressful stimulus. However, from this indirect evidence it would appear that changes in brain monoamines in response to stress might play a role in mediating the stress-induced release of ACTH and prolactin.

The second event is the decline of ACTH and prolactin following their stress-induced rise. One possible explanation for this event involves the changes in brain monoamines following acute stress. The effects of physical stress on brain catecholamines and serotonin are "biphasic". The initial tendency at the onset of stress is for the levels of these amines to be elevated. Bliss et al. (1971) found that the levels of homovanillic acid, the principal dopamine metabolite, increased in the brains of mice during electric foot shock. At the termination of one hour of electric foot shock the levels of homovanillic acid remained elevated for 30 minutes and then began to return to normal levels. Depending upon the type, intensity, and duration of the stress, the levels of brain amines may be decreased. The concentration of brain norepinephrine and dopamine was shown to decline in mice as a result of intense fighting (Welch and Welch, 1969) and in rats by electric foot shock (Bliss et al., 1968,

Thierry et al., 1968). Whereas low frequency stimulation of the midbrain raphe with implanted electrodes tended to elevate the levels of serotonin, stimulation at higher frequencies lowered it (Sheard and Aghajanian, 1968). Brain norepinephrine also has been reported to decrease as a result of intense exercise for one hour (Gordon et al., 1966 and four and eight hours of restraint stress (Corrodi et al., 1968). Thus, it would appear that the utilization of catecholamines and serotonin in response to stress follows a pattern similar to that found for the release of ACTH and prolactin. The evidence indicates that both brain monoamines and the anterior pituitary hormones ACTH and prolactin rise after the inception of acute stress. This stress-induced rise is then followed by a decline in brain amines and hormones to normal levels or lower than normal levels depending upon the type, intensity, or duration of the stress. The rise and subsequent fall of brain monoamines after stress therefore, might explain the increase and decline or prolactin and ACTH levels after acute stress. However, it has been reported that hypothalamic catecholamines inhibit both ACTH (Ganong, 1971) and prolactin (Meites et al., 1972). Therefore, the relation of hypothalamic biogenic amines to stress-induced increases in blood prolactin and ACTH is not entirely clear.

Another possible explanation for the decline of ACTH and prolactin may involve the "short loop" feedback mechanism. It has been found that the hypophysiotrophic area of the hypothalamus is sensitive to feedback from anterior pituitary hormones. Thus, these hormones may influence their own secretion through what has been termed the "short loop"

feedback mechanism. Prolactin implants in the median eminence region resulted in decreased levels of serum and pituitary prolactin in intact and ovariectomized female rats. The reduction in prolactin concentration was linked to an increase in hypothalamic PIF activity (Clemens and Meites, 1968). A similar autoregulatory mechanism exists for ACTH (Mangili et al., 1966). ACTH or anterior pituitary tissue implanted into the hypophysiotropic area inhibited ACTH release (Halasz and Szentagothai, 1960). Therefore, the decline in ACTH and prolactin seen following a stress promoted increase of these hormones might be the result of feedback inhibition. Both ACTH and prolactin released after stress might possibly feed back on neurons in the hypophysiotropic area of the hypothalamus to suppress the release of CRF or enhance the release of PIF. Consequent to the action of these hormones on their respective hypophysiotropic factors, the secretion of ACTH and prolactin would be reduced. This mechanism, however, does not appear to be important since prolactin release is maintained as long as a stressful stimulus is continued.

A third possible explanation involves the role of adrenal corticoids in suppressing the secretion of ACTH by feedback inhibition. Corticoids implanted into the medial basal hypothalamus resulted in a decrease in hypothalamic CRF and pituitary ACTH content (Chowers et al., 1967). Inhibition of ACTH secretion has also been reported following injection or implantation of corticoids into other brain areas, such as the midbrain, septal region, and amygdaloid nuclei. In addition, the pituitary has also been established as another probable site of feedback inhibition of ACTH by adrenal corticoids (Ganong, 1970). Thus, corticosterone, which

has also been shown to rise in response to restraint stress, may be involved in suppressing ACTH secretion. This feedback inhibition of ACTH by adrenal corticoids does not appear to operate under acute stress conditions, since the concentration of ACTH starts to fall before the levels of plasma corticosterone has increased significantly.

RELATION OF BIOGENIC AMINES TO THE STRESS RESPONSE OF PROLACTIN AND CORTICOSTERONE

INTRODUCTION

Since several pharmacological agents, which either mimic central monoamine neurotransmission or interfere with synaptic transmission, have been shown to enhance both ACTH and prolactin secretion, a question arises as to whether both hormones are controlled by the same monoamine regulatory mechanism. The major emphasis of existing evidence has suggested a stimulatory role for the neurotransmitter, serotonin, in the release of both prolactin and ACTH. A single injection of serotonin into the third ventricle of male rats produced a marked increase in serum prolactin levels (Kamberi et al., 1971a). Although systemic administration of serotonin has no effect on serum prolactin concentrations, an injection of 5-hydroxytryptophan (5-HTP), the immediate precursor of serotonin, did prove stimulatory to the release of prolactin in both proestrous female rats and hypophysectomized rats with an anterior pituitary graft (Lu et al., 1970). 5-HTP also has been found to be capable of stimulating the pituitary-adrenal axis (Fiore-Donati et al., 1959). Oral administration of 5-HTP (150 mg) resulted in increased levels of both ACTH and cortisol in human male subjects (Imura et al., 1973). Direct application of serotonin to the median eminence or different areas of the hypothalamus, midbrain, and forebrain has produced significant elevations in the tonic secretion of corticosteroids (Krieger et al., 1970, Naumenko et al., 1968). It has also been shown that serotonin may play a role in mediating the circadian periodicity of the pituitary-adrenal axis (Krieger et al., 1969). Scapagnini

et al. (1971) observed that there was a direct correlation between the variations in serotonin content of the amygdala and hippocampus and plasma corticosterone levels. Both values were low at 8 A.M. and high at 8 P.M. Koch et al. (1971) and Dunn et al. (1972) independently showed that the concentration of prolactin is higher during the late afternoon than in the morning. Thus, serotoneric neurons may play a part in mediating the diurnal changes in both prolactin and ACTH levels. The important role played by the serotonergic system in ACTH regulation has been indicated by the findings of Naumenko et al. (1968) and Popova et al (1972). Interruption of neural afferents to the hypothalamus either by midbrain transection in guinea pigs or complete deafferentation of the medial basal hypothalamus in rats did not prevent the stimulation of the pituitary-adrenal axis by 5-HTP or serotonin administration. Direct action of 5-HTP on the adrenal cortex was ruled out by finding that hypophysectomy completely abolished the stimulatory effect of 5-HTP on corticosterone secretion. In contrast, while regional injections of carbacol or norepinephrine into the hypothalamus or midbrain significantly increased the levels of plasma corticosteroids in intact guinea pigs, both of these compounds were ineffective in midbrain transected animals. These results strongly suggest that the terminal neurons in the hypophysiotrophic area of the hypothalamus stimulating the release of CRF are serotonergic in nature.

The main difference between the regulation of ACTH and prolactin release is that prolactin secretion is controlled principally by an inhibitory factor and perhaps a releasing factor (Meites et al., 1972). ACTH secretion is governed mainly by a releasing factor (CRF) (Mangili et al.,

1966). From the evidence presented it appears that serotonin overcomes the dominant inhibitory influence of the hypothalamus on prolactin secretion by either suppression of PIF or release of PRF, although neither hypothesis has been proven conclusively. CRF, on the other hand appears to be unique among the hypothalamic hypophysiotrophic hormones in that serotonin has been shown to be stimulatory to its release. In contrast, this neurotransmitter has been suggested to inhibitory to the releasing factors controlling gonadotrophin secretion (Kamberi et al., 1979 and 1971a).

The dominant inhibitory influence of the hypothalamus on prolactin secretion appears to be maintained by dopaminergic neurons present in the hypothalamus and median eminence. The correlation made between the dopaminergic system and inhibition of prolactin secretion is based upon several lines of evidence. A single injection of dopamine into the third ventricle was reported by Kamberi et al. (1971b)to depress the concentration of serum prolactin in male rats. This decrease in serum prolactin levels was accompanied by an increase in PIF levels in pituitary stalk portal blood. Increasing the concentrations of catecholamines, particularly dopamine, either by enhanced synthesis or reduced metabolism has resulted in the inhibition of prolactin release. L-dopa, the immediate precursor of dopamine, was shown to depress serum prolactin and increase hypothalamic PIF content in hypophysectomized female rats with an anterior pituitary graft (Lu and Meites, 1972). The monoamine oxidase inhibitors, pargyline, iproniazid, and also the catechol-o-methyl transferase inhibitor, pyrogallol, all caused a reduction in serum prolactin (Lu and Meites, 1971, Clemens and Meites, 1972). In contrast, pharmacological agents which reduce hypothalamic catecholamine activity by interfering with synthesis,

storage, or receptor interaction of these amines, have been shown to enhance prolactin secretion. Chlorpromazine, pimozide, and halperidol, drugs which block central dopamine and norepinephrine receptors all have been shown to stimulate prolactin secretion (Meites and Clemens, 1972). In addition, reserpine, which decreases monoamine transmission by inhibiting the uptake and storage mechanism of amine granules, was reported to enhance prolactin secretion by suppressing PIF (Ratner et al., 1965). Inhibition of catecholamine biosynthesis by α-methyl-p-tyrosine (AMPT), α-methyl-m-tyrosine (AMMT), or α-methyl dopa has proved stimulatory to prolactin release. A single injection of these drugs caused a marked increase in serum prolactin levels within 30 minutes of administration and all resulted in reduced pituitary prolactin content except AMMT (Lu and Meites, 1970). From this evidence it would thus appear that dopaminergic neurons tonically stimulate PIF secretion and thereby mediate the inhibitory influence of the hypothalamus on prolactin secretion.

The exact neuroendocrine role of the neurotransmitter, norepinephrine, on prolactin and ACTH is difficult to define. Meites and Clemens (1972) found that disulfram, an agent which inhibits norepinephrine biosynthesis, caused a significant reduction in the level of serum prolactin in ovariectomized rats. Likewise, Donoso et al. (1972) showed that a single injection of DL-DOPS (DL-threo-3,4,hydroxyphenylserine 200mg/kg) which selectively increases norepinephrine biosynthesis, caused a significant elevation in plasma prolactin in ovariectomized rats. These results suggest that while norepinephrine probably has no role in controlling tonic prolactin secretion, it may be stimulatory to prolactin release under special conditions.

With regard to ACTH secretion, it has been found that both reserpine and chlorpromazine are stimulatory to ACTH secretion. Bhattacharya and Marks (1969) found that a single injection of either agent into female rats produced a marked rise in plasma and adrenal corticosterone concentrations and was accompanied by a dramatic fall in CRF content in the median eminence. If these effects are related to blockade of central catecholamine neurotransmission, it would mean that norepinephrine or dopamine released from their various terminal systems of the brain would act to inhibit the secretion of ACTH. This view is also supported by the observation that an intaventricular injection of l-dopa (20mg), dopamine (4mg), or l-norepinephrine (5mg) suppressed the 17-hydroxyglucocorticoid response to laporatomy stress in dogs. In addition, an intraventricular injection of agents which have been shown to release catecholamines, such as tyramine (20mg) and α-ethyltyramine (8mg), reduced the concentration of 17-hydroxyglucocorticoids after surgical stress (Van Loon et al., 1971). However, since the doses needed for ACTH inhibition were large relative to the levels of amines normally present in the hypothalamus, it was suggested that suppression of ACTH release by catecholamines might be secondary to vasoconstriction of the portal blood vessels. This possiblity was ruled out by the finding that an intraventricular injection of angiotensin II, a proven vasoconstrictive agent, failed to prevent the adrenocortical response to laparotomy stress (Ganong, 1971). In addition, stimulation of the hypothalamus in animals pretreated with α-ethyltyramine overcame this agent's inhibition of ACTH secretion (Ganong et al., 1965). Thus, a catecholamine precursor, catecholamines, and two drugs that are capable of releasing catecholamines were shown to inhibit the release of ACTH when

injected into the third ventricle of dogs. Further evidence for a possible adrenergic inhibition of ACTH secretion is based on experiments using pharmacological agents which inhibit catecholamine synthesis and release. Van Leon et al. (1971) found that either a systemic injection (100mg/kg) or an intraventricular injection (20mg) of AMPT, which depletes brain norepinephrine and dopamine, increased plasma corticosterone levels in male rats. When rats were given l-dopa along with AMPT, the depletion of catecholamines was partially prevented and the mean increase in plasma corticosterone was less than in those rats receiving AMPT alone. These authors also found a negative correlation between plasma corticosterone and the hypothalamic content of norepinephrine and dopamine after the administration of AMPT and l-dopa. When brain catecholamines were depleted, there was a marked increase in plasma corticosterone, but when catecholamine levels were high, a significant decrease in plasma corticosterone was found. Intraventricular administration of guanethidine (1mg/kg), which prevents catecholamine release from adrenergic neurons, caused a marked elevation in plasma corticosterone in male rats and was accompanied by a depletion in hypothalamic catecholamines (Scapagnini et al., 1972). Use of an inhibitor of dopamine-β-hydroxylase, which would prevent the conversion of dopamine to norepinephrine and thereby reduce the levels of brain norepinephrine, suggested that norepinephrine rather than dopamine might be the neurotransmitter responsible for inhibition of ACTH secretion. When the dopamine-β-hydroxylase inhibitor, FLA-63 was administered intraperitoneally to male rats, the hypothalamic concentration of norepinephrine declined, while that of dopamine remained unchanged. A marked rise in plasma corticosterone following FLA-63 administration was attributed to this

reduction in brain norepinephrine (Scapagnini et al., 1972). An inhibitory role for the norepinephrine terminal system on ACTH secretion is also indicated by results from lesion studies. Fuxe and Hökfelt (1971) reported that lesioning the ascending norepinephrine pathways produced an increase in the tonic secretion of corticosterone. All these pharmacological experiments would favor the view that increased release of norepinephrine from its various terminals in the brain acts to inhibit ACTH secretion.

The results obtained with psychoactive drugs have to be interpreted with caution, particularly since many workers report different results. Reserpine implants in the median eminence failed to alter ACTH secretion in response to various stimuli, including reserpine and chlorpromazine (Smelik, 1967). It has also been found that a stress-induced elevation in ACTH secretion was not changed by combine treatment with reserpine and α-methyltyrosine (Carr and Moore, 1968), which would block central catecholamine transmission. Furthermore, data recently have been obtained suggesting that the increase in plasma corticosterone observed after α-methyltyrosine administration was due to a non-specific stress (Kaplanski et al., 1972). Nembutal administration 30 minutes prior to α-methyltyrosine administration prevented the subsequent rise in plasma corticosterone found by Scapagnini et al. (1970) and Van Loon et al. (1971), even though catecholamine depletion was still present. In addition, repeated administration of 50 mg/kg α-methyltyrosine to male rats resulted in a decrease in brain catecholamine levels without any effect on ACTH secretion. These findings question the inhibitory role of norepinephrine neurons on ACTH secretion and demonstrate that intact function of hypothalamic

norepinephrine nerve terminals is not crucial in the regulation of ACTH

In addition to serotonin, dopamine, and norepinephrine, cholinergic neurons have also been shown to be capable of influencing ACTH and prolactin secretion. Krieger and Krieger (1970) and Endrocrzi et al. (1963) independently found that cholinergic chemical stimulation of the median eminence and different areas of the hypothalamus, midbrain and forebrain resulted in increased ACTH secretion in cats. On the other hand, injection or implantation of cholinomimetic drugs into other areas of the hypothalamus, midbrain, or forebrain was found to inhibit pituitary-adrenal activity. Naumenko et al. (1968) further demonstrated that carbacol injected into the hypothalamus of midbrain-transected guinea pigs failed to enhance or inhibit ACTH secretion. Thus, while cholinergic neurons are capable of relating information to the neurons controlling the production of CRF, this system is not essential to the regulation of ACTH secretion. The effects produced by cholinergic stimulation are probably the consequence of reflex mechanisms. This means that cholinergic stimulation serves as a source of efferent nervous impluses and activates the hypothalamic-pituitary-adrenal system through corresponding secondary mechanisms. With regard to prolactin secretion, Meites and Clemens, (1972) found that both acetylcholine and its antagonist, atropine sulfate, induced lactation in estrogen-primed rats and rabbits. However, the mechanism by which cholinergic neurons affect PIF and prolactin release has not yet been investigated.

The purpose of the present study was two fold: (1) to correlate the presumed rise and decline in brain amines in various stresses with the rise and fall of prolactin and corticosterone levels observed after stress.

(2) to investigate how dopamine, serotonin, norepinephrine, and acetylcholine, which can influence the secretion of these hormones, might affect their release in response to a stressful stimulus. Experiments were designed using psychoactive drugs to determine which monoamines are important in the release of ACTH and prolactin during the stress reaction. Three minute supine immobilization was chosen as the stress stimulus. Serum prolactin and plasma corticosterone concentrations were examined in relation to changes in catecholamines, serotonin, and acetylcholine, produced by specific drugs at 0, 15, 30, and 60 minutes after stress.

PROCEDURE

Animals used in the present study were adult male Sprague-Dawley rats obtained from Spartan Research Farms, Haslett, Michigan. The same procedures were used as outlined in the previous experiment for stressing, collecting blood samples, and assaying serum prolactin and plasma corticosterone. Drugs were dissolved or suspended in phosphate buffered saline .1% gelatin and given to groups of 5-6 rats. All drugs were administered intraperitoneally in .5 cc of the vehicle. The drugs, their doses, and schedule of administration employed in the present study are listed below in Table 2.

Table 2.

Drugs	Doses	Schedule of Administration
L-dopa (L-3,4,dihydroxyphenylalamine) (Hoffmann-LaRoche Inc.)	20 mg/rat	1 hour before stress
Iproniazid phosphate (Hoffmann-LaRoche Inc.)	40 mg/rat	"
α-methyldopa (L-3,4,hydroxyphenyl-2-metylalanine (Merk Sharp and Dohme)	80 mg/rat	"
P-Chloroamphetamine-HCl (Compound 511600) (Regis Chemical Co.)	0.83 mg/rat total dose 2.5 mg/rat	daily for 3 days before stress (1 hr.)
Atropine Sulfate (Sigma Chemical Co.)	0.8 mg/rat total dose 1.6 mg/rat	daily for 2 days before stress (1 hr.)
Pilocarpine nitrate (Nutritional Biochemical Corp.)	0.25 mg/rat	15 minutes before stress

RESULTS

1) EFFECTS ON CATECHOLAMINE ACTIVITY

The drug α-methyldopa, was used as an example of a drug causing depletion of brain catcholamine levels through synthesis inhibition. L-dopa was chosen for the opposite effect, since this drug enhances catecholamine neurotransmission through increased synthesis. Only the effects produced by 20 mg. of l-dopa are presented since further studies using higher doses of l-dopa did not change the response of prolactin or corticosterone. Iproniazid was selected as an example of a monoamine oxidase inhibitor. Use of such an agent would, therefore, enhance catecholamine and serotonin neurotransmission as a result of inhibition of interneuronal catabolism.

The stress-induced changes in serum prolactin and plasma corticosterone of male rats subsequent to treatment with these drugs are shown in Figures 3 and 4 and in Tables 3 and 4. Analysis of variance indicated a significant difference in the non-stress levels of prolactin and corticosterone among treatment groups ($F=11.83$, $P<.002$; $F=4.74$, $P<.002$ for prolactin and corticosterone respectively). Treatment of rats with α-methyldopa one hour prior to immobilization stress increased the resting levels of prolactin approximately 50% above the control level. In contrast, the same drug produced no significant change in the non-stress concentration of plasma corticosterone. Further, iproniazid administration, which failed to alter the levels of prolactin before stress, tripled the concentration of plasma corticosterone. Animals treated with l-dopa showed no

Table 3.

Effects of drugs which alter central monoamine neurotransmission on the stress-induced changes in serum prolactin of male rats.

Treatment	ng/ml Serum Prolactin Minutes After 3-Minute Supine Immobilization Stress Non-Stress		15		30		60	
Controls (PBS) .5cc/rat I.P.	20.4±** 3.7	(9)	52.4± 7.0	(9)	30.2± 7.1	(9)	28.0± 8.4	(9)
Catecholamine System								
L-dopa (200mg/rat)	10.3± 0.6	(5)	22.2±* 6.1	(6)	16.8± 2.1	(6)	9.8±* 0.4	(6)
α-methyl dopa (80mg/rat)	49.5±* 8.2	(6)	55.8±* 4.5	(6)	31.5± 5.5	(6)	40.6± 5.8	(6)
Catecholamine and Serotonergic System								
Ipromiarid (40mg/rat)	12.9± 0.8	(5)	16.9±* 1.2	(6)	13.0±* 1.1	(5)	10.6±* 0.7	(6)
Serotonergic System								
Para-Chloroamphetamine-HCl (2.5mg/rat)	13.7± 1.3	(5)	36.4±* 5.3	(6)	19.3± 3.5	(5)	24.3± 5.1	(6)
Cholinergic System								
Atropine Sulfate (1.6mg/rat)	10.3± 0.8	(6)	23.6±* 1.8	(6)	18.2± 0.2	(5)	11.6±* 0.3	(6)
Pilocarpine Nitrate (0.25mg/rat)	11.8± 1.6	(5)	22.4±* 0.6	(6)	9.6±* 0.4	(5)		

( ) number of animals in each group
ng/ml serum prolactin ± standard error of the mean**
Significantly different at P≤.05* (versus control)

Table 4.

Effects of drugs which alter central monoamine neurotransmission on the stress-induced changes in plasma corticosterone of male rats.

ug/100ml Plasma Corticosterone
Minutes After 3-Minute Supine Immobilization Stress

Treatment	Non-Stress	15	30	60
Controls (PBS) .5cc/rat I.P.	12.8 ± ** 2.0 (5)	46.6 ± 5.5 (6)	35.9 ± 5.9 (5)	23.9 ± 4.5 (5)
Catecholamine System				
L-dopa (20mg/rat)	11.8 ± 3.0 (5)	30.2 ± * 5.2 (6)	38.0 ± 8.1 (6)	26.6 ± 2.6 (6)
a-methyl dopa (80mg/rat)	14.8 ± 5.2 (5)	42.0 ± 1.4 (6)	57.2 ± * 3.0 (6)	26.0 ± 5.4 (6)
Catecholamine and Serotonergic System				
Iproniazid (40mg/rat)	36.4 ± * 6.8 (5)	52.0 ± 1.8 (6)	40.8 ± 4.9 (5)	29.7 ± 5.6 (5)
Serotonergic System				
Para-Chloroamphetamine-HCl (2.5mg/rat)	20.9 ± 2.8 (5)	48.2 ± 4.6 (6)	30.4 ± 4.7 (6)	14.3 ± 1.3 (5)
Cholinergic System				
Atropine Sulfate (1.6mg/rat)	16.4 ± 3.8 (6)	34.8 ± 2.4 (6)	39.9 ± 2.3 (6)	26.1 ± 5.6 (6)
Pilocarpine Nitrate (0.25mg/rat)	32.6 ± * 3.0 (5)	53.5 ± * 1.4 (6)	55.8 ± * 2.0 (5)	

( ) number of animals in each group
ug/100ml plasma corticosterone ± standard error of the mean**
Significantly different at P< .05* (versus control)

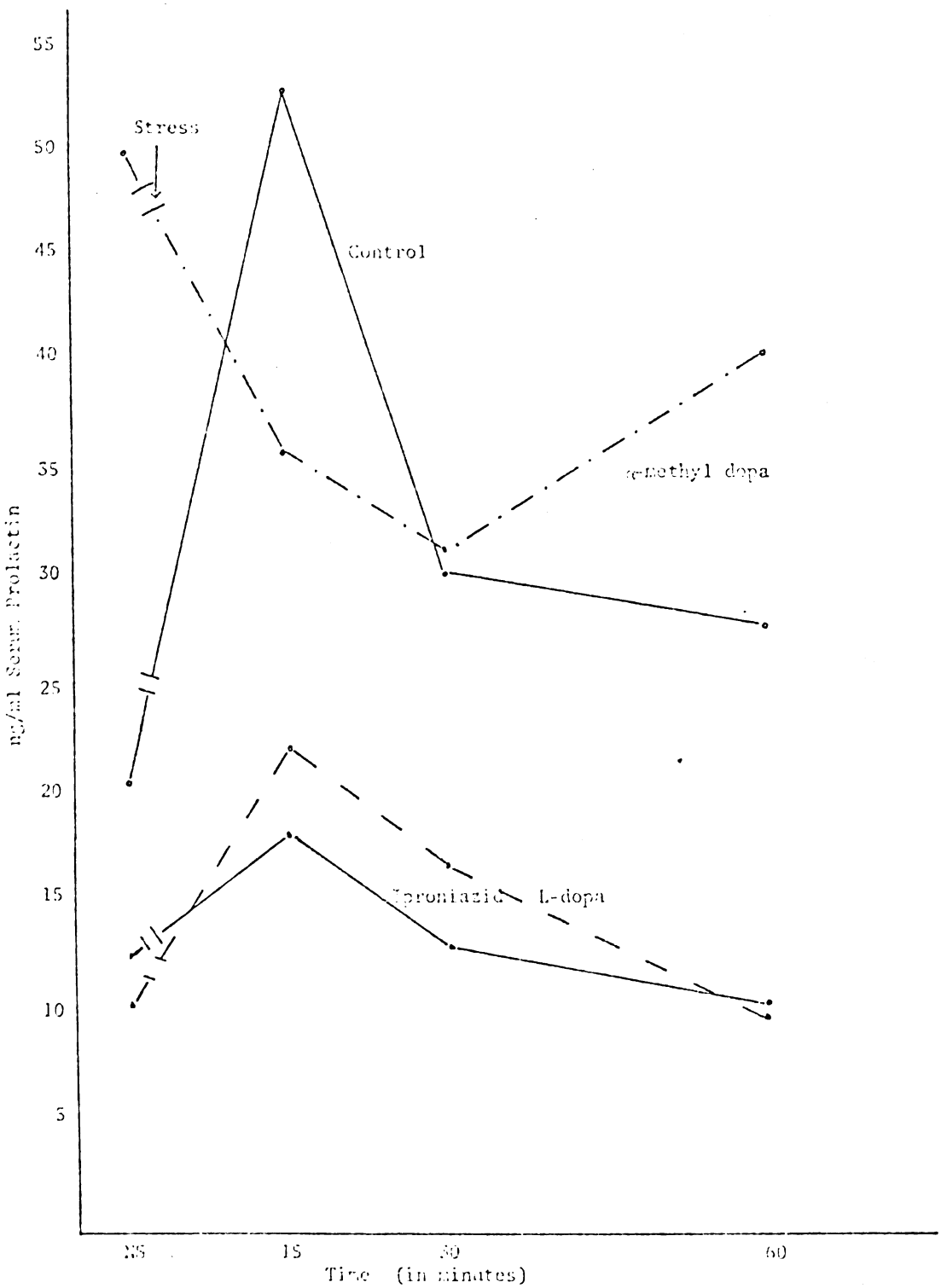


Figure 3. Effects of drugs which alter catecholamine neurotransmission on the stress-induced changes in serum prolactin of male rats.

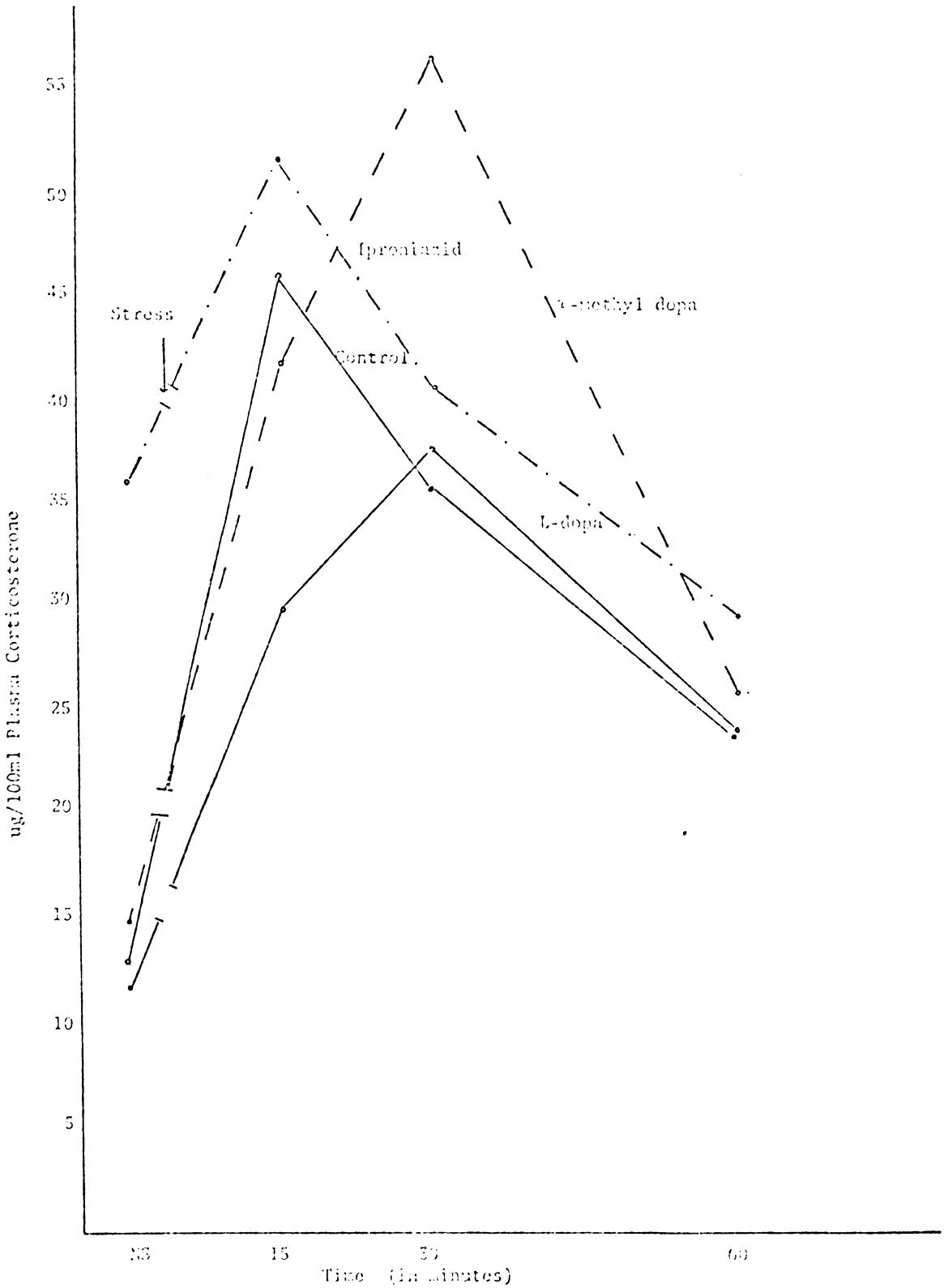


Figure 4. Effects of drugs which alter catecholamine neurotransmission on the stress-induced changes in plasma corticosterone of male rats.

significant change in the non-stress levels of either prolactin or corticosterone from control animals.

The previous study showed that 15 minutes post-stress represented the maximum response of plasma corticosterone to immobilization stress and also a time when prolactin levels were still increased over non-stress levels. Figures 3 and 4 demonstrate how either suppressing or enhancing catecholamine neurotransmission influences the peak response of plasma corticosterone and serum prolactin to acute stress. All three drugs, l-dopa, α-methyldopa, and iproniazid, blocked the elevation in prolactin levels seen 15 minutes after immobilization stress (Duncan's $P<.05$). In contrast, neither α-methyldopa nor iproniazid prevented the peak plasma corticosterone response to restraint stress. There was no significant difference ($P>.05$) in the concentration of plasma corticosterone between animals treated with these drugs and control animals. However, l-dopa did decrease the maximum levels of plasma corticosterone induced by acute stress (Duncan's $P<.05$).

The previous experiment demonstrated that 30 and 60 minutes post-stress represented the time interval when the concentration of corticosterone and prolactin returned to normal. Figures 3 and 4 show the effect of these psychoactive drugs upon the rate of decline of these hormones to pre-stress levels. The concentration of serum prolactin in animals treated with iproniazid and l-dopa was still lower than in control animals at both 30 and 60 minutes after immobilization stress (Duncan's $P<.05$). In contrast, these drugs did not cause a significant change ($P>.05$) in the levels of corticosterone 30 and 60 minutes after stress. However, α-methyldopa enhanced the concentration of plasma corticosterone 30 minutes after stress (Duncan's

$P<.05$), even though the levels of serum prolactin in animals treated with this agent were not statistically different from controls ($P>.05$). At 60 minutes after stress, there was no significant difference ($P>.05$) in the concentration of either prolactin or corticosterone between animals treated with α-methyldopa and control animals.

2) EFFECTS ON SEROTONERGIC ACTIVITY

Figures 5 and 6 reveal the stress promoted changes in serum prolactin and plasma corticosterone of male rats after treatment with pharmacological agents which affect serotonergic neurotransmission. Parachloroamphetamine (PCA) was chosen as an example of a drug which selectively reduces serotonergic activity. This pharmacological agent lowers the concentration of serotonin in the brain subsequent to inhibition of the rate limiting enzyme of serotonin biosynthesis, tryptophan hydroxylase. The action of PCA on the secretion of prolactin and corticosterone before and after stress was compared to that of iproniazid, since this monoamine oxidase inhibitor increases serotonergic activity in addition to catecholamine neurotransmission. Duncan's New Multiple Range test indicated that there was no significant difference ($P>.05$) in the resting concentration of either prolactin or corticosterone between thrice daily PCA (2.5 mg/rat total dose) treated animals and control animals. However, reduction in serotonergic activity in animals treated with PCA significantly reduced the non-stress levels of plasma corticosterone when compared to animals treated with iproniazid (Duncan's $P<.05$). In addition, rats which received PCA exhibited a lower maximal prolactin response to immobilization stress at 15 minutes than did control rats (Duncan's $P<.05$). However, this

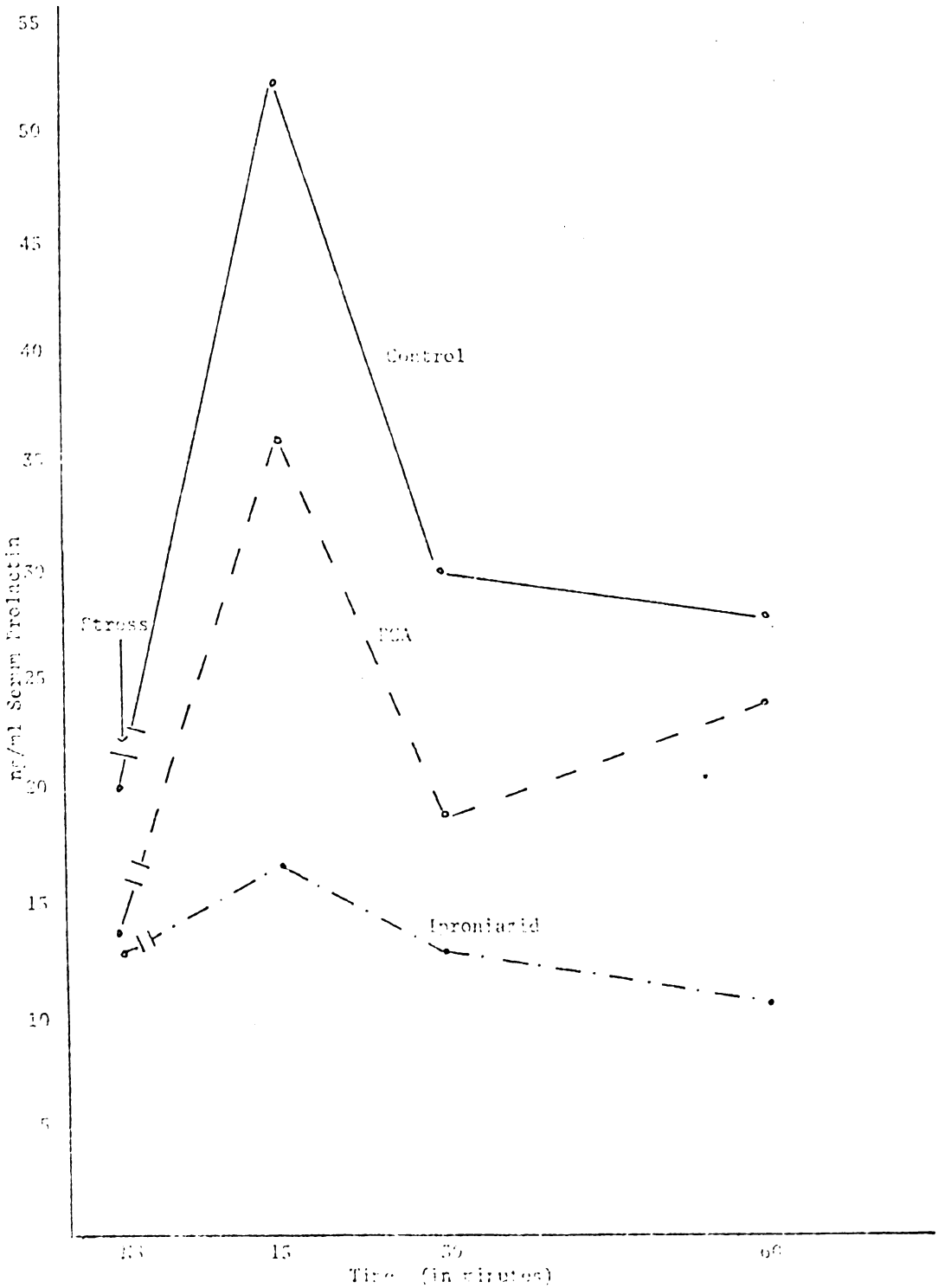


Figure 5. Effects of agents which affect serotonergic transmission on the stress-induced changes in serum prolactin of male rats.

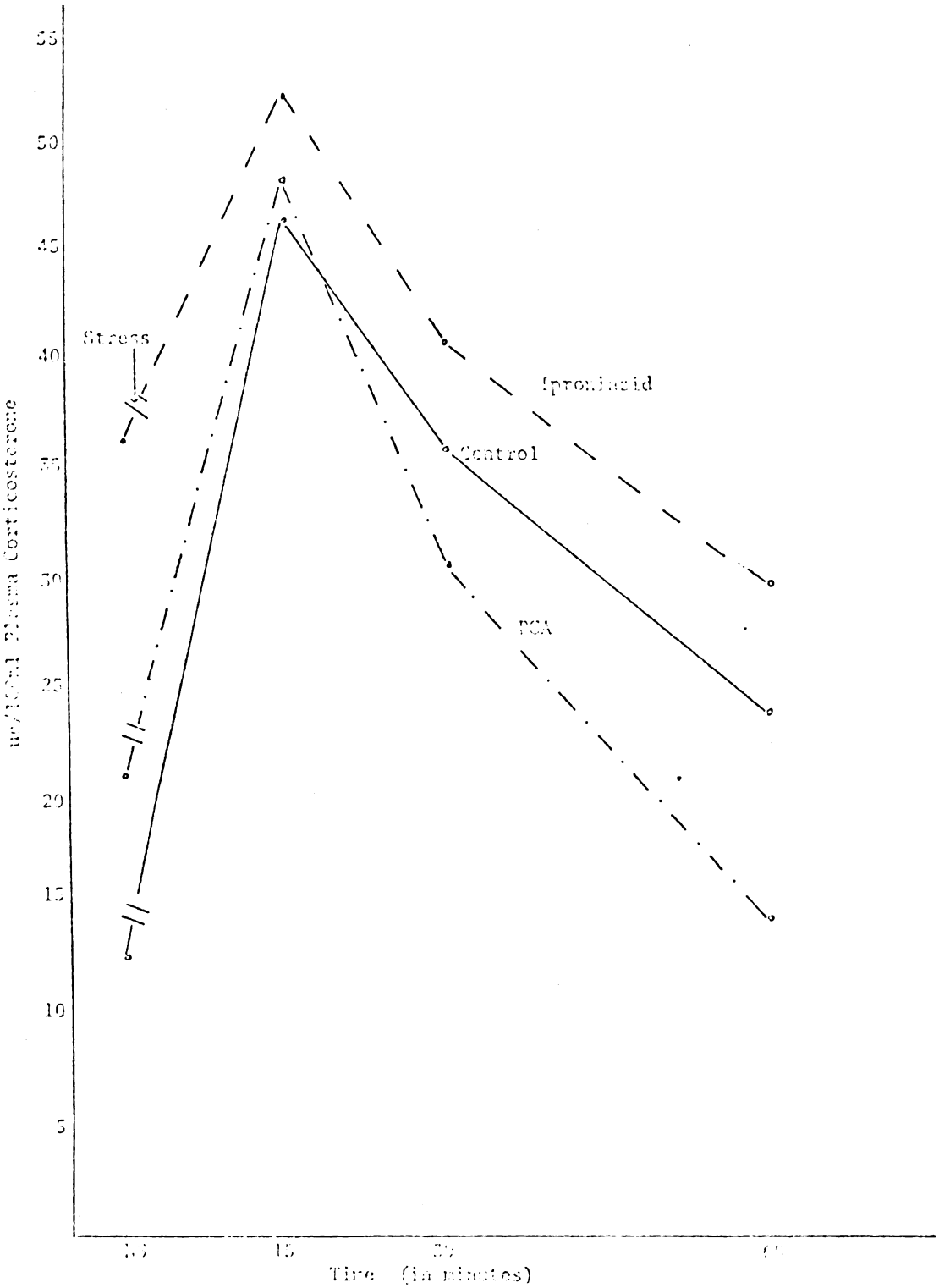


Figure 6. Effects of agents which affect serotonergic transmission on the stress-induced changes in plasma corticosterone of male rats.

inhibition of peak prolactin levels was not as great as seen with iproniazid (Duncan's $P<.05$). In contrast, specific depletion of serotonin stores by PCA had no effect on the maximum levels of plasma corticosterone observed 15 minutes after restraint stress. In addition, there was no significant difference ($P>.05$) in the concentration of corticosterone 15 minutes after stress when iproniazid and PCA treatments were compared. The rate of decline of both serum prolactin and plasma corticosterone to pre-stress levels was not significantly affected by PCA. At 30 and 60 minutes after immobilization stress, the concentration of corticosterone and prolactin in animals given PCA was not statistically different from control animals.

3) EFFECTS ON CHOLINERGIC ACTIVITY

Figures 7 and 8 show the stress-induced changes in serum prolactin and plasma corticosterone of male rats after treatment with pilocarpine and atropine sulfate. Pilocarpine was chosen as an example of a cholinomimetic agent. Atropine sulfate was selected as its antagonist. This agent blocks the action of acetylcholine at its receptor by competitive inhibition. The administration of either pilocarpine or atropine sulfate to male rats prior to stress produced no significant change ($P>.05$) in the resting concentration of serum prolactin from that found in controls. By contrast, while the administration of atropine sulfate produced no significant change ($P>.05$) in the non-stress concentration of plasma corticosterone, treatment of animals with pilocarpine did elevate the resting levels of corticosterone over control animals (Duncan's $P<.05$). Further, there was a significant difference in the levels of plasma corticosterone when

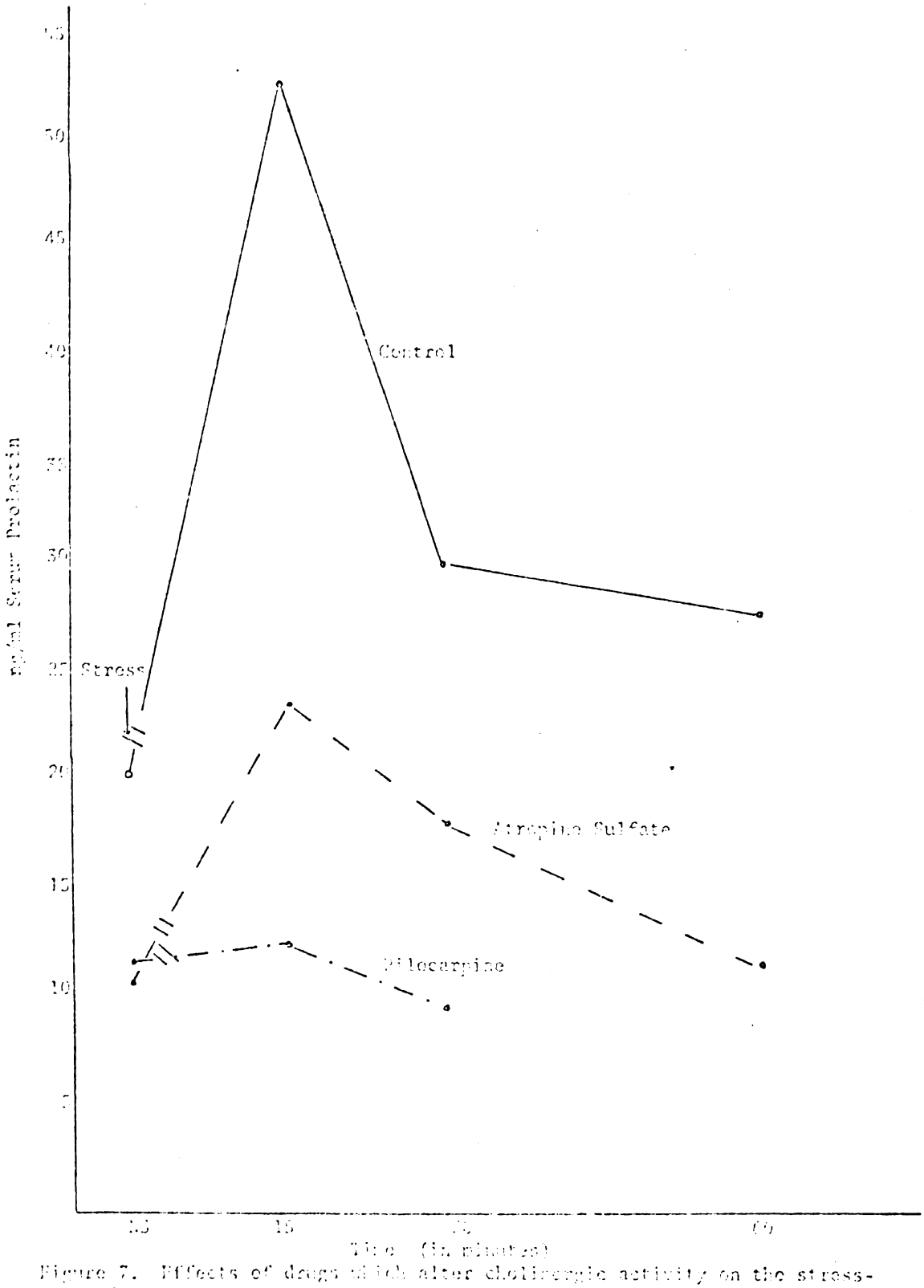


Figure 7. Effects of drugs which alter cholinergic activity on the stress-induced changes in serum prolactin of male rats.

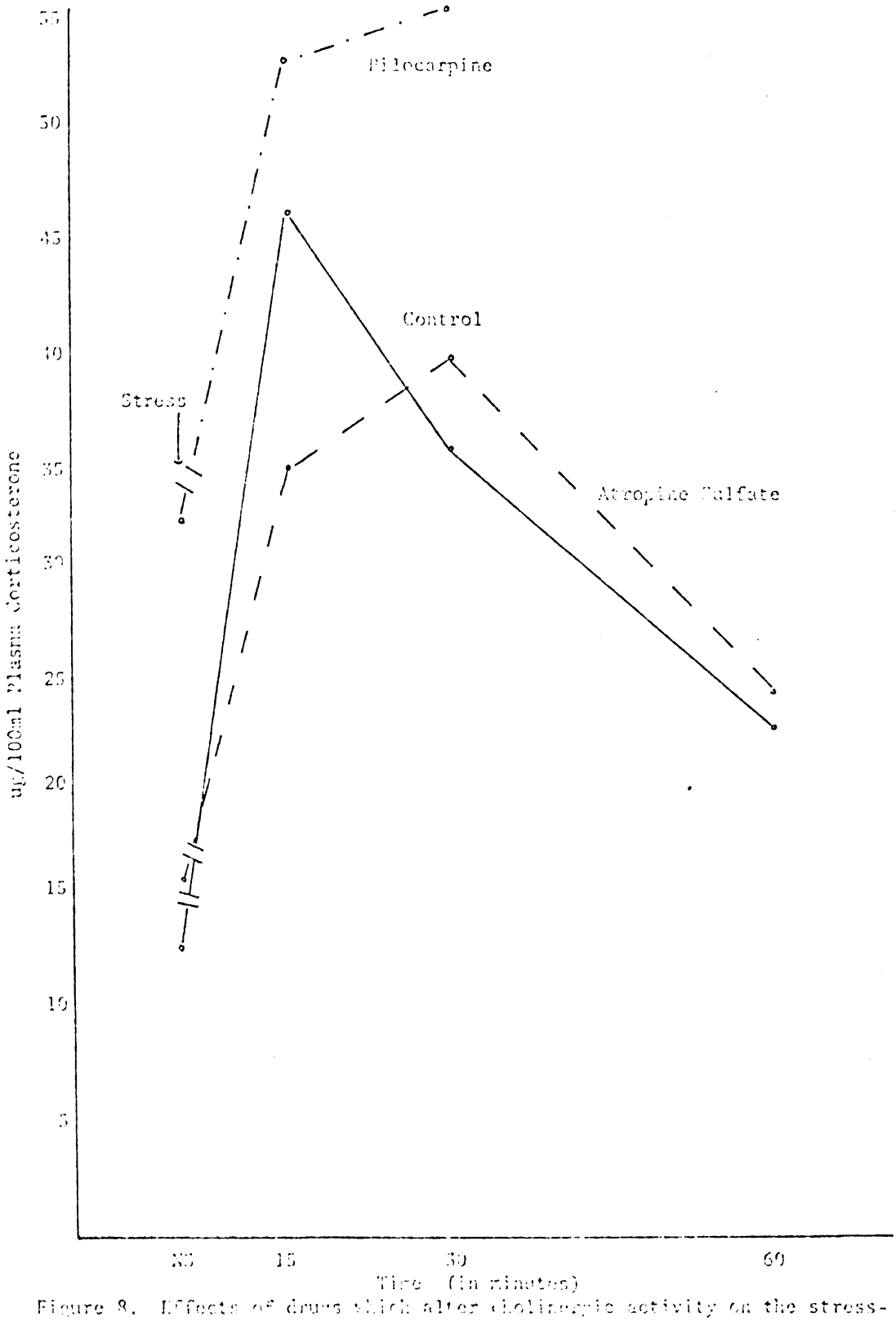


Figure 8. Effects of drugs which alter cholinergic activity on the stress-induced changes in plasma corticosterone of male rats.

pilocarpine treatment was compared to atropine sulfate treatment (Duncan's $P<.05$). Both pilocarpine and its antagonist, atropine sulfate, prevented the stress-induced rise of prolactin seen 15 minutes after stress (Duncan's $P<.05$). Atropine sulfate was equally as effective as l-dopa in suppressing the elevated levels of prolactin observed in response to stress. In addition, there was no significant difference ($P>.05$) between the response of atropine sulfate and pilocarpine treated animals 15 minutes after stress. In contrast, pilocarpine increased the maximum corticosterone stress-induced response (Duncan's $P<.05$). Atropine sulfate, however, produced no significant change ($P>.05$) in the concentration of plasma corticosterone 15 minutes after stress. At this time the levels of corticosterone of pilocarpine treated animals were elevated over those of atropine sulfate treated animals (Duncan's $P<.05$). The concentration of serum prolactin in animals given atropine sulfate and pilocarpine was still lower than controls at 30 minutes after stress (Duncan's $P<.05$). The levels of prolactin in atropine sulfate treated rats were also significantly decreased 60 minutes after stress (Duncan's $P<.05$). In contrast, there was no significant difference in the concentration of corticosterone when atropine sulfate treated animals were compared to control animals 30 and 60 minutes after stress ($P>.05$). However, pilocarpine did cause a marked rise in the levels of corticosterone 30 minutes after stress. The levels of corticosterone in pilocarpine treated animals were significantly greater than those observed in control animals (Duncan's $P<.05$) and in atropine sulfate treated animals (Duncan's $P<.05$).

Table 5.

Summary of the effect of drugs on monoamine neurotransmission and the secretion of prolactin and corticosterone in response to restraint stress.

Drug	Expected Effect on Monoamine Activity				Effect on Serum Prolactin and Plasma Corticosterone					
					Non-Stress		Peak		Duration of Peak	
	DA	NE	5-HT	ACH	P	C	P	C	P	C
L-dopa	+	+	o	o	o	o	-	-	-	o
a -methyl dopa	o	-	o	o	+	o	-	o	o	+
Iproniazid	+	+	+	o	o	+	-	+	-	+
Para-Chloro-amphetamine	o	o	-	o	o	o	-	o	o	-
Atropine Sulfate	o	o	o	-	o	o	-	o	-	o
Pilocarpine Nitrate	o	o	o	+	o	+	-	+	-	+

-=Decrease, +=Increase, o=No Change
DA=Dopamine
NE=Norepinephrine
5-HT=Serotonin
ACH=Acetylcholine

P=Prolactin
C=Corticosterone

DISCUSSION

It is difficult to define which particular central monoamine neurotransmitter is responsible for the rise and fall of prolactin and ACTH subsequent to a stressful stimulus. Part of the problem lies in our lack of understanding of the relationship between the liberation of amines at specific synapses by nerve impluses and the occurrence of specific postsynaptic events in the brain. The difficulty is further compounded by the many uncertainties that remain concerning the action of psychotrophic drugs on synthesis, release, uptake, and metabolism of monoamines or upon the interaction of amines with postynaptic receptors. Despite these problems, the results of this study do demonstrate that a change in the balance of central monoamine neurotransmission has a profound effect on the secretion of ACTH and prolactin in response to stress.

An example of the way in which alteration of central amine neurotransmission affects the stress-induced release of prolactin and ACTH is illustrated by l-dopa administration. While the administration of l-dopa one hour prior to stress had no effect on the pre-stress concentration of either prolactin or corticosterone, this drug did suppress the stress-promoted rise of these hormones. Furthermore, l-dopa appeared to be more inhibitory to prolactin secretion after stress than to corticosterone. There are two possible explanations for the inhibitory effect of l-dopa on the release of these hormones after stress. Glowinski and Baldessarini (1966) reported that the administration of l-dopa increased dopamine levels

throughout the brain, but had only a small effect on norepinephrine concentrations. The correlation between elevation of brain dopamine content subsequent to l-dopa administration and inhibition of ACTH and prolactin secretion is in aggreement with the findings of other workers (Ganong, 1970 and Meites et al., 1972). Another possible explanation is that the administration of l-dopa has recently been found to interfere with serotonergic transmission, which is stimulatory to both ACTH and prolactin (Ng et al., 1970 and 1971). These workers found that l-dopa rapidly reduces central serotonin stores from brain slices incubated in vitro. They also found that l-dopa may enter serotonergic neurons, undergo decarboxylation to dopamine, and subsequently be liberated in response to electrical stimulation. Therefore, dopamine formed in serotonergic neurons, as a consequence of l-dopa administration, may act as a false serotonergic transmitter. The suppression of the stress-induced rise of prolactin and corticosterone 15 minutes after stress by l-dopa may thus be explained in terms of enhanced brain dopamine levels or to interference of serotonergic neurotransmission subsequent to administration of this drug.

Monoamine oxidase (MAO) inhibitors have effects upon brain amines that are similar to the effects induced by stress. Bliss et al. (1968) found that the MAO inhibitor, iproniazid, increased the concentration of catecholamines and serotonin throughout the brain and prevented the depletion of these amines following electric foot shock stress. Various stressors, such as restraint, fighting, and d-amphetamine, also may elevate mouse brain norepinephrine, dopamine, and serotonin, within 5-10 minutes (Welch and Welch, 1968b). Likewise, MAO inhibitors may also enhance brain amine

levels with great rapidity. Welch and Welch (1968b) found that brain catecholamines and serotonin were significantly increased within ten minutes after the administration of pargyline, another monoamine oxidase inhibitor. Still other workers have found elevated brain monoamine levels when measured 30 and 60 minutes after pargyline (Spector et al., 1963, Everett and Wiegand, 1962). These worker also found that the increase of serotonin concentration was 20% greater than that of norepinephrine. MAO inhibitors mimic the effects of stress on brain monoamines and prevent their depletion after stress. Therefore, the administration of the MAO inhibitor, iproniazid, should enhance the response of prolactin and ACTH to stress and delay the return of these hormones to resting levels. Treatment of rats with iproniazid one hour before immobilization did appear to potentiate the release of corticosterone and partially delayed the decline of this hormone to pre-stress levels. However, the exact opposite was found for prolactin. The administration of iproniazid prevented the stress-induced rise of serum prolactin and accelerated its return to normal levels 30 and 60 minutes after stress. Although MAO inhibitors do elevate the concentration of brain amines and prevent their depletion after stress, they also have other effects. MAO inhibitors also have been shown to depress the spontaneous release of H^3-norepinephrine from sympathetic nerve endings and to prevent or diminish the release of this amine by catecholamine releasing agents such as reserpine, histamine, and nicotine (Bliss et al., 1968). Therefore, the reduction in prolactin secretion in response to stress observed after the administration of iproniazid might be the result of decreased norepinephrine release. If this assumption is correct,

it would implicate norepinephrine as playing a part in mediating the release of prolactin in response to stress.

The results obtained with α-methyldopa administration illustrates the distinction that must be made between the effects induced by this drug before stress and after a stressful stimulus. α-methyldopa administered to male rats one hour prior to stress did elevate the non-stress concentration of prolactin 50% above that found in controls. However, after immobilization stress, prolactin secretion was not further enhanced by α-methyldopa treatment rather it declined. Administration of this catecholamine depleting agent prevented the stress-induced rise in prolactin levels 15 minutes after stress. Prolactin levels remained low 30 minutes after stress, then started to rise above those found in control animals 60 minutes after stress. These results are in partial agreement with those of other workers (Lu and Meites, 1971), who found that a single injection of α-methyldopa into female rats increased serum prolactin over pre-treatment levels within 30, 60, and 120 minutes of injection. However, these workers did not observe that α-methyldopa decreased prolactin secretion after stress probably as a result of non-specific stresses inherent in their experimental design. They used serial bleeding by cardiac puncture under repeated etherization as their method for determining whether α-methyldopa enhanced prolactin secretion. Etherization alone has been shown to cause a 2-4 fold rise in prolactin secretion in 1-4 minutes of application (Terkel et al., 1972). Elevated prolactin levels induced by ether inhalation do not return to normal until 1-2 hours after stress (Krulich et al., 1973). Thus, the stress of injection, repeated etherization, and bleeding might

have masked the inhibition of prolactin release by α-methyldopa found after immobilization stress and decapitation in male rats. α-methyldopa caused transient reduction in the concentrations of dopamine and serotonin and also induced a prolonged decrease in brain norepinephrine (Glowinski and Baldessarini, 1966). If suppression of norepinephrine neurotransmission proves inhibitory to prolactin release under other specific stressful conditions, such as exercise, cold, electric foot shock, or d-amphetamine, it would further implicate norepinephrine as having a stimulatory role in the release of prolactin during stress.

Although α-methyldopa administration did not alter the pre-stress levels of plasma corticosterone, this drug did appear to delay the peak response of this hormone to immobilization stress. These results would suggest that while catecholamines, particularly norepinephrine, may not be an important factor in the regulation of tonic ACTH secretion, they may be involved in mediating the rise in ACTH during stress. This view is supported by the results of Lippa et al. (1973). These workers found that an intraventricular injection of 6-hydroxydopamine, which destroys adrenergic neurons, resulted in a significant though transient decrease in the resting levels of plasma corticosterone. However, chronic depletion of catecholamines by 6-hydroxydopamine impaired the ability of the pituitary-adrenal system to respond to a ketamine stressor 28 days after initial treatment.

The idea that norepinephrine may participate in the release of ACTH and prolactin in stress is based on the additional evidence that the synthesis and utilization of norepinephrine is increased after various types

of stresses: electric foot shock (Thierry et al., 1968a), cold and exercise (Gordon et al., 1966), and restraint (Corrodi et al., 1967). In addition, Corrodi et al. (1971) found that minor tranquilizers such as chlordiazepoxide (Librium) blocked the stress-induced activation of central norepinephrine neurons in immobilization stress. These drugs have also been shown to inhibit ACTH secretion (Gold and Ganong, 1967). It is not definietly known if the increase in norepinephrine turnover during various types of stress is related to the increased ACTH and prolactin secretion after stress. However, the evidence presented would suggest such a relationship. An experiment to determine if drugs such as chlordiazepoxide, which blocks the activation of central norepinephrine neurons, in immobilization stress would influence the increase in prolactin and corticosterone secretion found during this type of stress, might answer this question.

Since serotonin has been shown to be stimulatory to both the release of ACTH and prolactin, the increase in serotonin turnover found during various types of stress (Thierry et al., 1968b and Welch and Welch, 1968c) might be an important factor in the stress-induced rise of ACTH and prolactin. If this neurotransmitter participates in mediating the release of ACTH and prolactin in response to stress, then depletion of this amine by parachloroamphetamine (PCA) should prevent the rise in prolactin and corticosterone observed after immobilization stress. The administration of PCA for three days prior to stress did not affect the resting levels of either prolactin or plasma corticosterone. Likewise, Donso et al. (1972) found that blockade of serotonin biosynthesis by para-chlorophenylalanine did

not modify plasma prolactin levels. However, treatment of rats with PCA did partially inhibit the rise in serum prolactin induced by restraint stress. In contrast, PCA did not affect the stress-induced rise of plasma corticosterone, but did appear to accelerate the decline of this hormone to pre-stress levels 30 and 60 minutes after stress. Prezoisi et al. (1968) and De Schaepduver et al. (1969) also found that depletion of brain serotonin content subsequent to para-chlorophenylalanine administration failed to affect the ability of ACTH to be secreted in response to various stresses and in response to reserpine administration. However, these findings do not exclude a role for serotonin in mediating the increased secretion of ACTH and prolactin in response to stress. Fuller et al. (1973) found that either a single injection or multiple injections of parachloroamphetamine (20.6 mg/kg/rat) resulted in only a 50% reduction in brain serotonin content. His results and those of others (Deguchi et al., 1972) suggest that some serotonin neurons in the brain are not susceptible to depletion by parachloroamphetamine. Since serotonin levels are not totally depleted after parachloroamphetamine treatment, it is possible that a small amount of residual or newly formed serotonin could maintain effective activity in the serotonergic system. This could explain why PCA administration produced only partial inhibition of the stress-induced rise of prolactin and failed to alter plasma corticosterone levels in response to immobilization stress.

The exact role of acetylcholine during stress is not known. Maynert and Levi (1963) reported that electric foot shock stress failed to alter the levels of acetylcholine in rats. However, administration of

pilocarpine and its antagonist, atropine sulfate, did modify the release of ACTH and prolactin in response to immobilization stress. While pilocarpine and atropine sulfate administration had no effect on the non-stress levels of prolactin in the present study, both agents prevented the rise in prolactin after immobilization stress. The opposite effect was observed for corticosterone secretion. Pilocarpine not only increased the resting level of corticosterone secretion, but also potentiated the release of this hormone in response to stress. Atropine sulfate, on the other hand, did not impair the ability of the pituitary-adrenal system to respond to stress. In contrast, Hedge and Smelik (1968) found that implants of atropine sulfate in the hypothalamus did prove inhibitory to the release of CRF. Since both atropine sulfate and pilocarpine have been shown to principally affect peripheral cholinergic systems (Goodman and Gillman, 1970), the effect of these agents on the secretion of anterior pituitary hormones is probably secondary to stimulation of perpherial reflex mechanisms. Animals subsequent to pilocarpine administration exhibited sweating, salivation, watering eyes, and diarrhea. Naumenko et al. (1968) showed that central cholinergic stimulation of ACTH secretion was secondary to activation of peripheral reflex mechanisms. Whether inhibition of prolactin secretion is also the result of such a mechanism is not known. Perhaps by implanting more specific centrally acting cholinomimetic agents like physostigmine or its antagonist, scopolamine, into the hypothalamus and other areas of the brain, a clearer picture of the influence of the cholinergic system on the secretion of prolactin and ACTH might be obtained.

In summary, the results of the present study reveal several points. First, while pharmacological agents which alter central monoamine neurotransmission do not necessarily affect the tonic secretion of ACTH and prolactin, they do produce marked changes in the secretion pattern of these hormones in response to a stressful stimulus. Differences were observed between the acute stress response of ACTH and prolactin after treatment with drugs which altered the activity of adrenergic and serotonergic systems. Second, from the evidence presented, it would also appear that the secretion of ACTH and prolactin is closely regulated in response to acute stress. All drugs used in this study altered the individual patterns of prolactin and corticosterone secretion after initiation of immobilization stress. Whereas these agents did not always impair the ability of ACTH and prolactin to be secreted in response to restraint stress, they did alter the peak levels, the time of the peak, and the rate of decline of these hormones after stress. Third, the results of this study also implicate the neurotransmitters, norepinephrine and serotonin, as being important in mediating the release of prolactin and ACTH after immobilization stress. Furthermore, it would appear that what is important in the stress-induced secretion of ACTH and prolactin is not the action of one monoamine, but an interaction between norepinephrine, dopamine, and serotonin, all of which are increased during acute stress. This raises the interesting question as to how these neurotransmitters interact with hypothalamic neurons that contain CRF and PIF, and thus control the release of ACTH and prolactin.

POSSIBLE ROLE OF CORTICOSTERONE IN THE RESPONSE OF PROLACTIN TO STRESS

INTRODUCTION

Many physiological conditions and drugs which elicit ACTH release have also been shown to influence prolactin secretion. Elevated levels of ACTH have been reported after adrenalectomy, suckling and various stresses (Sydnor et al., 1954, Turner and Bagnara, 1971, and Ganong, 1963). In addition, the injection of different drugs which alter the balance of biogenic amines in the brain, such as chlorpromazine and reserpine, have been shown to produce marked changes in ACTH secretion.

All these factors also affect the release of prolactin from the anterior pituitary. It is known that different types of stresses cause elevated serum prolactin in both man and animals. Pseudopregnancy, a physiological consequence of increased prolactin levels has been induced in rats by adrenalectomy, surgical trauma, and also by the administration of reserpine and chlorpromazine (Swingle et al., 1951a and 1951b, and Baraclough and Sawyer, 1959). Lactation, another manifestation of elevated prolactin and ACTH levels, may be initiated by acute and chronic stress, suckling, and injections of morphine and serotonin (Nicoll et al., 1960 and Meites et al., 1959). Although it appears that many stimuli which elicit ACTH also cause the release of prolactin, the interrelation between the pituitary-adrenal axis and prolactin secretion remains unclear.

The objective of the present set of experiments was to study the influence of adrenal steroids on prolactin secretion in response to restraint stress, a condition shown to promote both ACTH and prolactin

release. The study was divided into two parts. The first part was designed to assess the effect of glucocorticoid administration on pituitary synthesis of prolactin and prolactin secretion. Experimental animals were normal male Sprague-Dawley rats purchased from Spartan Research Animals, Haslett, Michigan. The second part involves the influence of adrenalectomy and subsequent replacement with adrenal steroids on the secretion of prolactin.

PROCEDURE

The effect of glucocorticoid administration and removal of glucocorticoids on prolactin secretion was studied before and after restraint stress. Non-stress blood samples were obtained by rapid decapitation (time < 20 sec.) after animals were removed individually from their animal quarters to an adjacent laboratory. Stress blood samples were taken at 15, 30, and 60 minutes after 3 minutes of supine immobilization in a rat restrainer. Trunk blood was collected, centrifuged, and serum samples were frozen at -20°C. until assayed. Pituitaries were removed immediately following decapitation and at the end of the experiment were weighed, homogenized, diluted, and stored frozen until time for assay. Both serum and pituitary prolactin were measured by a double antibody radioimmunoassay (Niswender et al., 1969) and an average of four dilutions of each sample assayed were expressed in terms of purified rat prolactin reference standard (NIAMD-RAT-PROLACTIN-RP1).

All experimental substances used in the study were prepared on the day of the experiment in a phosphate buffer saline .1% gelatin suspension. Each animal received .5cc of their respective treatment substance intraperitoneally 4 hours before the start of the experiment. Intact male rats used in the first part of the study received a single injection of either hydrocortisone acetate (1mg/rat) or corticosterone (3mg/rat or 1mg/rat). Control animals received only the vehicle. Animals in the second part of the study were bilaterally or sham adrenalectomized under ether

anesthesia 9 days before the experiment. Adrenalectomized animals were maintained on 0.9% saline in the interim. Animals in this part of the study were divided into the following three groups: sham-operated controls, adrenalectomized controls, and adrenalectomized animals given a single injection of either hydrocortisone acetate (1mg/rat) or corticosterone (3mg/rat or 1mg/rat). These constituted the replacement steroids for three separate groups of adrenalectomized rats. Sham-operated and adrenalectomized controls received phosphate buffered saline .1% gelatin.

RESULTS

1) EFFECT OF GLUCOCORTICOID ADMINISTRATION ON PROLACTIN SECRETION IN THE INTACT MALE RAT BEFORE AND AFTER STRESS.

The effect of glucocorticoid administration on serum prolactin before and after 3-minute restraint stress in male rats is summarized in Table 6 and Figure 9. Analysis of variance indicated that there was a significant difference in the non-stress levels of prolactin between animals treated with glucocorticoids and control animals ($F=8.95$, $P<.003$). Both hydrocortisone acetate and corticosterone suppressed the resting levels of prolactin by 50%. Further analysis revealed that the prolactin levels in animals treated with glucocorticoids did not statistically differ from control animals at 15, 30, or 60 minutes after immobilization stress. Neither adrenal steroid inhibited the rise in serum prolactin after stress, nor was the pattern of prolactin release after acute stress changed by the administration of corticosteroids.

The pituitary prolactin content of control animals and animals treated with either hydrocortisone acetate (1 mg/rat) or corticosterone (3 mg/rat), measured before and after stress is presented in Table 7. Analysis of variance revealed that the pituitary prolactin content in control animals did not differ statistically from that of treated animals before and 15 minutes after restraint stress ($P>.025$). Additional analysis showed that there was no difference between the levels of pituitary prolactin in the control and hydrocortisone acetate group at either 30 or 60 minutes after

Table 6. Effect of Corticosteroid Administration on Serum Prolactin Before and After 3-Minute Restraint Stress in Male Rats.

Treatment	ng./ml Serum Prolactin							
	Non-Stress		Minutes After Stress					
			15		30		60	
Controls (PBS.1%Gelatin)	20.5 ± 1.4 **	(5)	62.5 ± 8.5	(6)	38.8 ± 10.2	(6)	38.4 ± 10.2	(6)
Hydrocortisone Acetate (1mg/rat)	10.9 ± 3.2 *	(5)	36.8 ± 6.7	(6)	28.2 ± 4.0	(6)	31.3 ± 4.6	(6)
Corticosterone (3mg/rat)	6.4 ± .03 *	(5)	42.6 ± 10.9	(6)	25.5 ± 5.6	(6)	23.1 ± 8.6	(5)
(1mg/rat)	10.0 ± .09 *	(5)	32.0 ± 6.2	(6)	28.6 ± 4.5	(6)	32.6 ± 4.1	(6)

( ) number of animals in each group
ng./ml serum prolactin ± standard error of the mean**
F=5.95, P <.003 *

Figure 9.

Effect of corticosteroid administration on serum prolactin before and after 3-minute restraint stress in male rats.

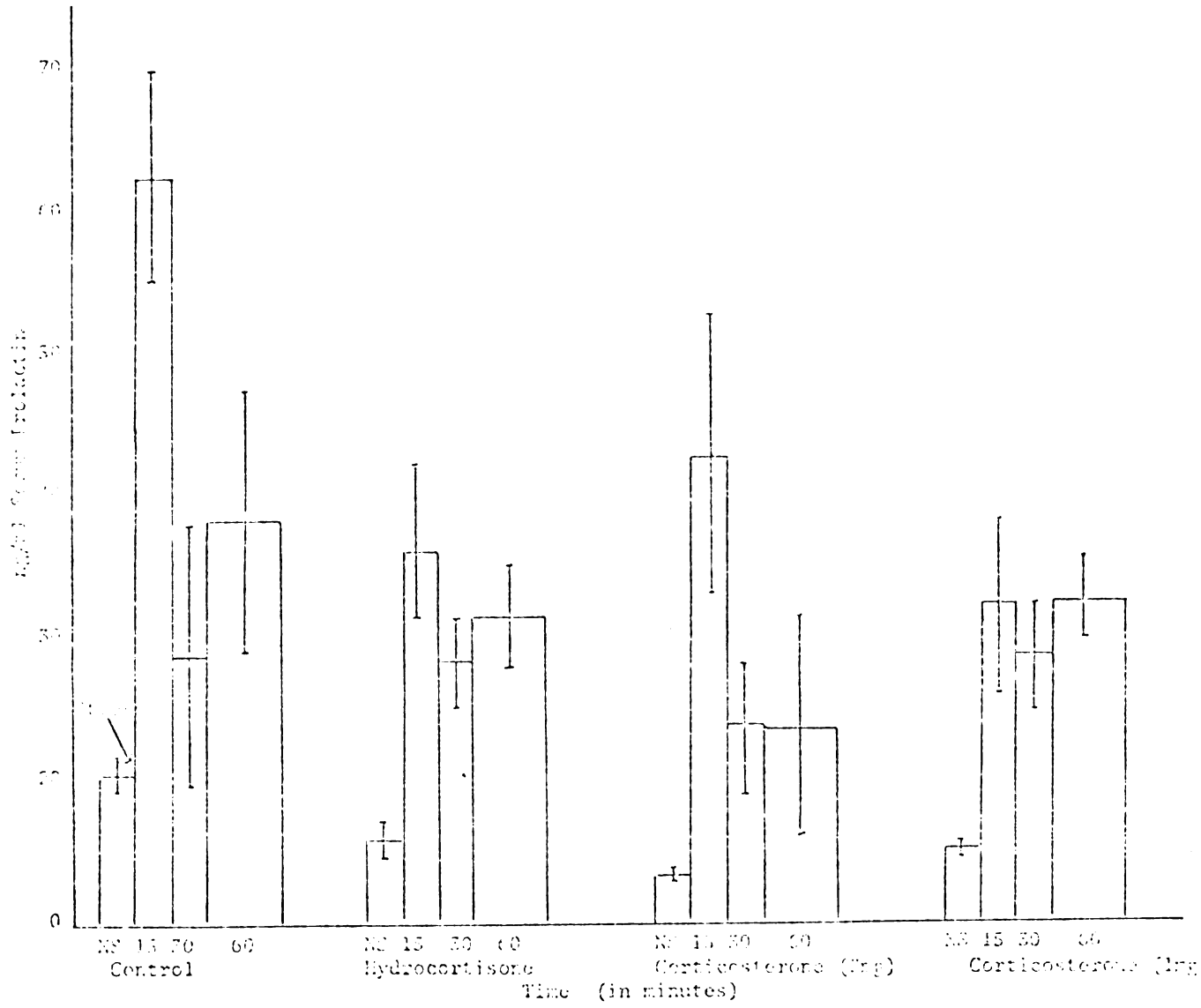

70
0
Control
Hydrocortisone
Corticosterone (1mg)
Time (in minutes)

Table 7. Effect of Corticosteroid Administration on Pituitary Prolactin Content Before and After 5-Minute Restraint Stress in Male Rats.

Treatment	Non-Stress	Pituitary Prolactin Content ng./Anterior Pituitary Minutes After Stress 15	30	60
Controls (PBS.1%Gelatin)	21,695.4 ± ** (5) 3235.4	15,527.8 ± (6) 1256.4	15,209.5 ± (6) 597.6	15,345.5 ± (6) 875.8
Hydrocortisone Acetate (1mg/rat)	15,333.1 ± (5) 499.7	14,201.2 ± (6) 805.3	14,420.6 ± (6) 781.6	16,086.7 ± (6) 1093.3
Corticosterone (5mg/rat)	20,849.4 ± (5) 155.1	22,459.1 ± (5) 3059.7	21,918.0 ± * (5) 1865.9	23,682.0 ± * (5) 642.9

( ) number of animals in each group
ng/Anterior Pituitary ± Standard Error of the Mean**
Significantly different at P ≤.05*

immobilization stress. However, there was a significant difference in pituitary prolactin content between animals receiving 3 mg of corticosterone and control animals in this time interval (Duncan's $P<.05$). The amount of pituitary prolactin in these animals was increased over that of animals receiving hydrocortisone acetate or the vehicle. Several factors could account for the apparent difference between the corticosterone group and the other treatment groups. One is that this group demonstrated a large standard error between samples. The mean of pituitary prolactin in each group represented an average among the individual animals tested. It was noticed that individual animals showed a variable response to stress. In addition, the weight and the amount of pituitary prolactin has been seen to vary with different shipments of rats and among rats of the same shipment. Consequently, the group given 1 mg of corticosterone was not included in the statistical analysis. These results are similar to the findings of Nicoll and Meites (1965). They observed that a high dose of cortisone or cortisol over a 10 day period elicited only a slight rise in the pituitary prolactin content of female rats. They also found that corticosterone had no effect on the amount of prolactin released from pituitary explants when cultured in vitro (Nicoll and Meites, 1964).

2) EFFECT OF ADRENALECTOMY, ADRENALECTOMY PLUS CORTICOSTEROID REPLACEMENT, AND SHAM-ADRENALECTOMY BEFORE AND AFTER STRESS.

The effect of adrenalectomy, adrenalectomy plus corticosteroid replacement, or sham-adrenalectomy on serum prolactin in response to stress is summarized in Table 8 and Figure 10. Analysis of variance showed that

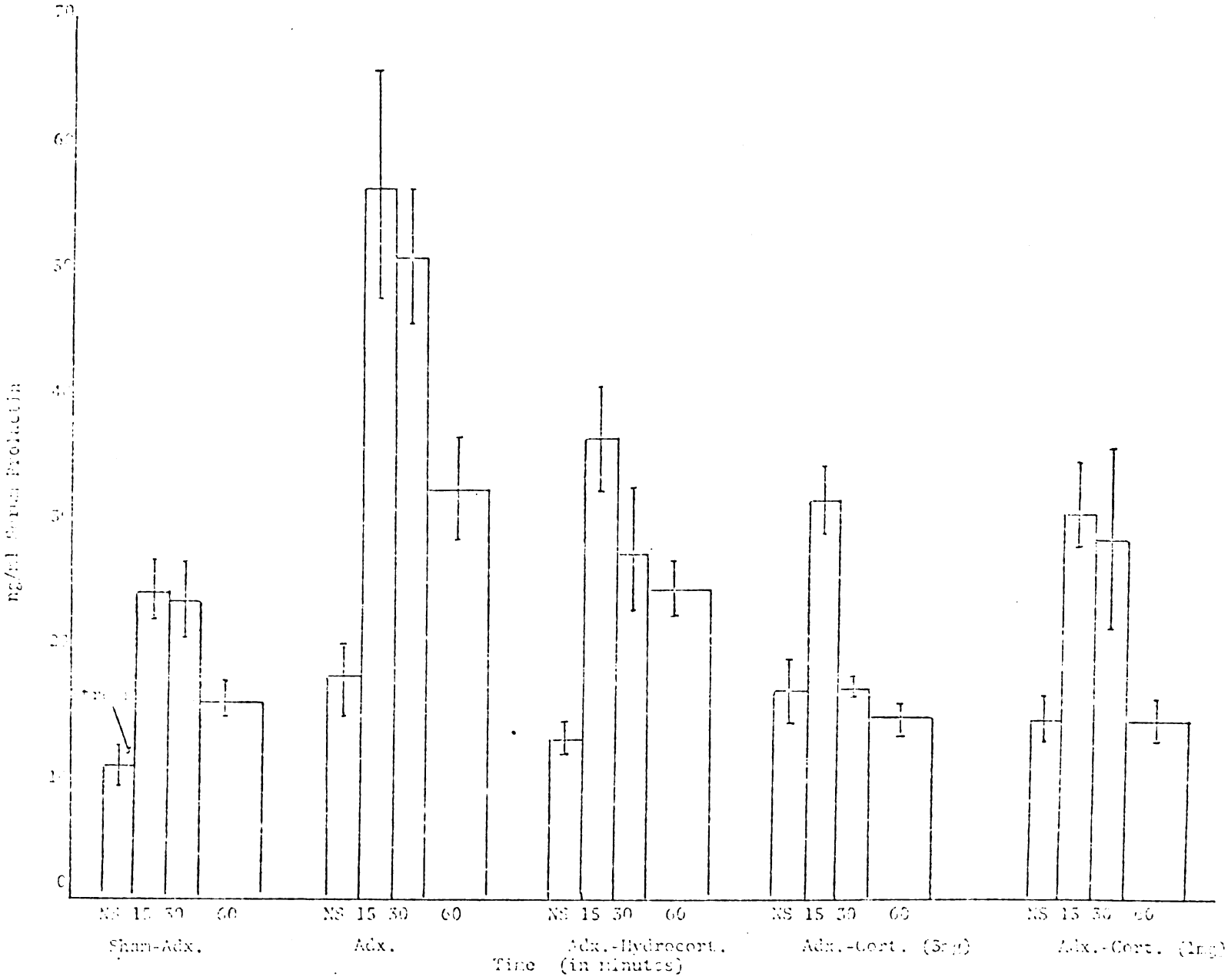
Table 8. Effect of Adrenalectomy, Adrenalectomy plus Corticosteroid Replacement, or Sham-Adrenalectomy on Serum Prolactin in Response to Restraint Stress.

Treatment	ng./ml Serum Prolactin							
	Non-Stress		Minutes After Stress					
			15		30		60	
Sham-Adrenalectomy (No Manipulation)	10.4 ± 1.9	(6)	24.2 ± 2.4	(8)	23.5 ± 3.2	(8)	15.6 ± 1.6	(8)
Adrenalectomy (No Manipulation)	17.8 ± 5.4	(10)	56.7 ± 10.7	(13)*	51.9 ± 6.5	(12)*	32.5 ± 5.3	(8)*
Adrenalectomy plus Hydrocortisone Acetate (1mg/rat)	13.8 ± 1.2	(5)	36.5 ± 5.2	(6)	27.2 ± 5.6	(5)	21.6 ± 2.2	(5)
Corticosterone (0.5 mg/rat)	16.4 ± 2.6	(5)	31.9 ± 2.6	(6)	16.9 ± 0.6	(6)	11.5 ± 1.2	(6)
(1mg/rat)	14.2 ± 2.4	(5)	30.8 ± 3.8	(6)	28.8 ± 8.3	(5)	14.2 ± 1.8	(5)

( ) number of animals in each group
ng./ml serum prolactin ± standard error of the mean**
Significantly different at $P < .05$*

Figure 10.

Effect of adrenalectomy, adrenalectomy plus corticosteroid replacement, or sham-adrenalectomy on serum prolactin in response to 3-minute restraint stress.


NS 15 30 60
Sham-Adx.
Adx.
Adx.-Hydrocort.
Adx.-Cort. (5mg)
Adx.-Cort. (1mg)
Time (in minutes)

there was no difference in prolactin levels prior to stress when all five groups were compared ($P>.025$). However, there was a significant difference in the amount of prolactin released among these groups 15, 30, and 60 minutes after restraint stress ($F=3.10$, $F=6.54$, $F=4.93$; $P<.05$, $P<.001$, $P<.005$ respectively). Further analysis revealed that at all three time periods following stress, prolactin levels in adrenalectomized animals were increased over those in sham-operated controls (Duncan's $P<.05$). Duncan's New Multiple Range test indicated that serum prolactin in the adrenalectomized was also increased above those animals receiving replacement therapy 15, 30, and 60 minutes after acute stress ($P<.05$). However, there was no difference in prolactin release among sham-operated control animals and adrenalectomized animals receiving either hydrocortisone acetate or corticosterone. The latter results are similar to the observations made in the normal rat after the administration of corticosteroids.

The pituitary prolactin content of adrenalectomized rats and adrenalectomized rats receiving either hydrocortisone acetate (1mg) or corticosterone (3mg and 1mg) in response to 3 minute immobilization stress is shown in Table 9. Comparison of the pituitary prolactin levels among these different treatment groups proved variable, yet there was a consistent trend. There was no statistically significant difference between the amount of pituitary prolactin of adrenalectomized animals and animals receiving corticosterone or hydrocortisone acetate as replacement three of the four time periods analyzed. Analysis of variance revealed that there was no difference between the levels of pituitary prolactin of all four groups at either 15 or 30 minutes after stress ($P>.025$). However, a

Table 9. Effect of Adrenalectomy and Adrenalectomy plus Corticosteroid Replacement on Pituitary Prolactin in Response to Restraint Stress.

Treatment	Non-Stress	Pituitary Prolactin Content ng./Anterior Pituitary Minutes After Stress 15		30		60	
Adrenalectomy (ADX.Prolactin)	13,455.6 ±** 1159.5	(8)	12,798.1 ± 1128.3	(12)	14,881.1 ± 982.6	(9)	13,707.2 ± 868.8	(10)
Adrenalectomy plus Hydrocortisone Acetate (1mg/rat)	18,140.2 ±** 600.9	(5)	13,836.4 ± 1492.3	(6)	14,776.9 ± 466.9	(6)	13,666.4 ± 569.1	(5)
Corticosterone (3mg./rat)	15,099.7 ± 362.7	(5)	13,345.9 ± 577.4	(6)	12,370.4 ± 511.3	(6)	12,114.0 ±* 322.3	(6)
(1mg/rat)	13,428.0 ± 535.6	(5)	9,226.2 ± 87.4	(5)	10,470.4 ± 762.2	(5)	12,559.0 ±* 251.6	(5)

( ) number of animals in each group
ng/Anterior Pituitary ±Standard Error of the Mean**
Significantly different at $P < .05$*

significant difference did appear between pituitary prolactin content of adrenalectomized animals and adrenalectomized animals given replacement treatment before and 60 minutes after restraint ($F=4.30$, $F=5.99$; $P<.05$, $P<.001$ respectively). Duncan's New Multiple Range test indicated that prior to stress there was no significant increase in the level of pituitary prolactin of adrenalectomized rats over adrenalectomized rats given corticosterone (1mg and 3mg) ($P>.05$). There was, however, a significant difference between adrenalectomized animals and animals receiving hydrocortisone acetate replacement (Duncan's $P<.05$). At 60 minutes after stress, further analysis showed that there was no difference ($P>.05$) in pituitary prolactin between adrenalectomy and adrenalectomy-hydrocortisone acetate treatment groups, even though there was a significant difference when the former group was compared to the adrenalectomy-corticosterone (1mg and 3mg) treatment groups (Duncan's $P<.05$). Some of the reasons which could account for the variation among the treatment groups at the different times before and after stress include the large standard error observed between samples, the variable response of individual animals to stress, and the difference in pituitary weight and prolactin content among individual animals. Despite this variation, there was no statistically significant difference in pituitary prolactin between adrenalectomized animals and animals receiving replacement treatment for the majority of times analyzed. These results are similar to those obtained by Ben-David et al. (1970). These workers observed that adrenalectomy alone increased serum prolactin levels 56% above intact controls, even though removing the influence of glucocorticoids did not significantly affect pituitary prolactin content.

DISCUSSION

The results of the present study indicate that both the removal or administration of glucocorticoids greatly influenced prolactin secretion. Administration of corticosteroids to male rats depressed the resting level of serum prolactin, but did not adversely affect this hormone's release in response to a stressful stimulus. In contrast, adrenalectomy greatly increased prolactin levels following restraint stress over sham-operated control animals and animals given replacement treatment. The effect of glucocorticoids or their removal on prolactin release does not appear to be the result of a direct action on the pituitary, since there was no change in pituitary prolactin content under either condition. Rather the influence of the pituitary-adrenal axis on prolactin secretion seems to be mediated via the hypothalamus. Therefore, the interpretation of these results lies in the effect adrenalectomy and corticosteroid administration may have on the balance of the biogenic amine systems in the hypothalamus. It has been shown that changes in brain monoamine neurotransmission produce marked changes in prolactin secretion (Meites et al., 1972).

Recent reports have presented evidence indicating that norepinephrine is capable of stimulating prolactin release. Workers using drugs which selectively alter norepinephrine levels have found significant changes in prolactin release. DL-DOPS, a drug which promotes norepinephrine synthesis, caused a significant rise in serum prolactin in ovarectomized rats

(Donoso et al., 1971). Meites and Clemens (1972) found similar results in that disulfram, a drug which blocks the synthesis of norepinephrine, led to a significant inhibition of prolactin release. Further, Koch et al. (1970) found small doses of norepinephrine increased the release of prolactin from pituitaries in vitro. These results support the idea that although the main influence of the adrenergic system on prolactin secretion is inhibitory, norepinephrine may stimulate its secretion under certain conditions. It is thus possible that an increased utilization of norepinephrine seen after adrenalectomy and stress could result in a facilitation of prolactin release in adrenalectomized animals subjected to restraint stress. Adrenalectomy has been associated with an increased synthesis of norepinephrine in brain tissue. Javoy et al. (1968) found that brain norepinephrine turnover was significantly increased 6 days after adrenalectomy, even though this increase was not present in animals adrenalectomized for 2 or 3 days. Similar results were obtained by Fuxe et al. (1970). These workers also found that an injection of cortisol (2.5mg/100g) partially blocked the increase in norepinephrine turnover found after adrenalectomy. Thus, there is an increase in the utilization of norepinephrine not only after various stresses, but also after adrenalectomy.

It is know that dopamine inhibits prolactin secretion both in vitro (Mac Leod, 1969 and Birge et al., 1970) and in vivo and acts partly by stimulating the release of PIF (Kamberi et al., 1970). Agents such as iproniazid and pargyline, which inhibit monoamine oxidase activity and thereby suppress the metabolism of dopamine, have been shown to elevate

PIF and reduce prolactin secretion (Lu and Meites, 1971). It is possible that a rise in monoamine oxidase activity, which would accelerate the degradation of dopamine, would lower the level of PIF and increase the amount of prolactin released in response to stress. Recent experiments have shown that adrenal steroids play a role in the degradation of catecholamines. Monoamine oxidase activity is significantly increased in the heart, brain, vas deferens, and kidney after adrenalectomy (Avakian and Callingham, 1968, Sampath and Clarke, 1972, and Parvez and Parvez, 1972). Parvez and Parvez (1973) showed that adrenalectomy produced a marked increase in monoamine oxidase activity in the hypophysis and a less marked but still significant rise in the hypothalamus. In addition, the inhibition of glucocorticoidgenesis by metopirone, a drug which blocks 11β-hydroxylation, was followed by a significant rise in monoamine oxidase (MAO) activity in the hypophysis, hypothalamus, and the rest of the brain. This increase in enzyme activity returned to normal following administration of hydrocortisone. These observations suggest that the presence of adrenal steroids might be a rate limiting factor for catecholamine degradation in normal rats. The absence of these hormones removes this limitation, resulting in higher levels of monoamine oxidase. Therefore, an increase in dopamine metabolism, the consequence of increased monoamine oxidase activity, would decrease the availability of dopamine, suppress stimulation of PIF and result in higher levels of prolactin.

It is not known which of these two effects predominates or whether it is a combination of these alterations in the balance of catecholamine neurotransmission that is responsible for the rise in prolactin seen after adrenalectomy and stress. Most likely, it is the combination of these two

effects, since a lowering of dopamine and thereby of the inhibitory influence of PIF would facilitate the action of a stimulatory neurotransmitter such as norepinephrine, which is elevated during adrenalectomy and stress.

It has also been shown that serotonin or the precursors of serotonin, tryptophan or 5-hydroxytryptophan elevate serum prolactin levels. There have been reports on the effects of adrenalectomy and administration of corticosteroids on the serotonergic system. The evidence is confusing and it is questionable whether changes in this amine system following adrenalectomy could account for the rise in prolactin of adrenalectomized animals after stress. Reports have shown that adrenalectomy can decrease (DeMaio, 1959), increase (Pleifer et al., 1963), or leave unchanged (Garattini et al., 1961) whole brain serotonin content. More recently, evidence has been presented indicating that serotonin turnover is reduced following adrenalectomy (Fuxe et al., 1970). However, this decrease in serotonin turnover varied in intensity depending on which area of the brain was studied. Thus, the brain stem appeared to have a greater decrease than did the telecephalon and diencephalon regions (Azimitia et al. 1970). As was seen in connection with the catecholamine system, administration of corticosteroids restored serotonin turnover to normal or even super-normal rates. Thus, alterations in the serotonergic system caused by the removal of adrenal steroids does not offer an explanation for the rise in prolactin found following adrenalectomy and stress. If the serotonin system were involved, there would be a decrease in prolactin secretion after adrenalectomy and stress, since a decrease in the utilization of serotonin was found following adrenalectomy. It is possible that the

effect of corticosteroid administration on the serotonergic system would have a greater effect in the normal rat.

In intact rats administration of corticosteroids appears to produce changes in the serotonergic system which are dissimilar from those found in the catecholamine system. Opposing alterations in these two systems of neurotransmission could affect prolactin secretion in different ways. The decrease in the resting level of prolactin following corticosteroid administration in male rats might be explained by the effect that adrenal steroids have on the degradation of catecholamines. It has been shown that hydrocortisone inhibits both MAO and COMT activities in vitro and that this inhibition is dose dependent (Parvez and Parvez, 1972). Thus, inhibition of these two enzymes, resulting in suppression of catecholamine degradation, could enhance PIF stimulation through dopamine and thereby cause a decrease in prolactin secretion. However, this inhibition of prolactin secretion was only transitory, since restraint stress was capable of stimulating prolactin release to levels comparable with those of control animals. Furthermore, my results and the findings of Krulich et al. (1973) indicate that after acute stress there is only a transient rise in serum prolactin followed by a gradual decline which continues until the levels return to normal 60 minutes after the initiation of stress. A possible explanation for the decline in prolactin seen at 30 minutes after stress is that adrenal steroids which also rise shortly after the initiation of stress might act indirectly to suppress prolactin secretion. The lack of inhibition of prolactin found after stress in animals receiving corticosteroid treatment argues against this explanation.

Reports concerning the effects of corticosteroid treatment on the

serotonergic system have shown that prolonged treatment with glucocorticoids caused an increase in serotonin turnover (Fuxe et al., 1970). In addition, administration of large doses of corticosterone or ACTH was found to enhance the conversion of radiolabeled tryptophan to serotonin 30 minutes after its administration, possibly by stimulating the enzyme tryptophan-5-hydroxylase (Millard et al., 1972). Thus in contrast, to the catecholamine system an increase in the utilization of serotonin could lead to an increase in the level of prolactin since it has been shown that serotonin stimulates prolactin secretion. This might explain why a small increase in the level of catecholamines caused by the inhibition of MAO and COMT was not successful in suppressing the stress-induced release of prolactin. However, it could also argue against finding a decrease in the non-stress level of prolactin. Some reasons for this seeming discrepance might lie in the different dose levels, duration of treatment, and experimental measures used by different workers. Thus, different parameters would cause different changes in monoamine neurotransmission depending upon the various experimental conditions used.

The consequence of adrenalectomy and corticosteroid administration on brain monoamine neurotransmission and their subsequent effects on prolactin secretion are both numerous and complex. The presence or absence of glucocorticoids alters the transport, synthesis, and degradation of catecholamines. In addition, adrenal steroids also effect the rate limiting enzyme on serotonin biosynthesis. Thus, shifts in the balance of biogenic amine neurotransmission brought on by alterations in the concentration of glucocorticoids have been shown to influence prolactin secretion. However, more work needs to be done to find out how the interaction between the

pituitary-adrenal axis and the biogenic amine systems affect the release of prolactin. It would be of interest to further determine the physiological significance of these interactions in states where both prolactin and ACTH are elevated, such as parturition and lactation. Perhaps by measuring PIF activity in the hypothalamus after adrenalectomy and corticosteroid administration a more definitive answer could be obtained to these questions.

GENERAL DISCUSSION

Each of the preceding studies demonstrated that central monoamines were intimately involved in regulating the patterns of ACTH and prolactin responses to restraint stress. The first study suggested that the rise and subsequent decline of norepinephrine, dopamine, and serotonin might be related to the fluctuations in prolactin and corticosterone levels after stress. The next study showed that modification of central amine transmission profoundly changed the pattern of hormone release in response to stress. Alterations in central neurotransmission produced by drugs, which modified the concentration of catecholamines, serotonin, or acetylcholine, changed peak levels, the time of the peak, and the rate of decline of these hormones after stress. The final study indicated that either the administration or removal of adrenal steroids not only modified the non-stress levels of prolactin, but also changed the secretion of prolactin in response to restraint stress by altering the turnover of catecholamines and serotonin. The question that remains unanswered is how central amines are involved in eliciting the release of ACTH and prolactin in response to stress.

The literature on the regulation of ACTH and prolactin indicated that there are excitatory and inhibitory amines controlling the secretion of each hormone. Based upon the results obtained from intraventricular injections of dopamine, this transmitter has been proposed to be inhibitory to prolactin release by stimulating PIF (Kamberi et al., 1971b).

Alterations in catecholamine activity produced by reserpine, chlorpromazine, dopa, and dopamine-ß-hydroxylase inhibitors indicated that norepinephrine may have an inhibitory role in the regulation of ACTH (Ganong, 1971). The neurotransmitter, serotonin, has been shown to be stimulatory to the release of both prolactin and ACTH (Kamberi et al., 1971a and Naumenko, 1968). However, all three present studies failed to find a consistent correlation between the proposed excitatory and inhibitory amines for ACTH and prolactin and the release of these hormones in response to stress. The evidence obtained from these studies suggested that the rise of these hormones subsequent to stress was the result of interaction between various amine systems and the hypothalamic neurons regulating PIF and CRF secretion. This raises the question as to how such interactions might occur.

Figure 11 illustrates a hypothetical model for a communication system employed by the brain that could modulate the release of prolactin and ACTH from the anterior pituitary. Various transmitter systems make up the components of this interneuronal system of communication (refer to 1 of the model). A list of these substances would include acetylcholine, norepinephrine, amino acids, peptides, and other biogenic amines such as dopamine, serotonin, and histamine. Whether the message conveyed by a transmitter is excitatory or inhibitory depends in part upon the chemical nature of the transmitter (see Control Boxes). For example, (control box A,6) dicarboxylic amino acids such as glutamate or aspartate excite, while monocarboxylic omega amino acids such as glycine and GABA generally inhibit. The transmitters norepinephrine, dopamine, and serotonin are

Figure 11.

Hypothetical model for a communication system in the brain that could modify the release of ACTH and prolactin in response to restraint stress.

\+ excitatory
\- inhibitory

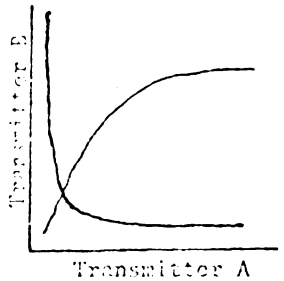
\- - - hypothesized

5-HT=serotonin
DA=dopamine
NE=norepinephrine
ACh=acetylcholine
PIF=prolactin inhibiting factor
PRF=prolactin releasing factor
CRF=corticotrophin releasing factor

Action &/or Interaction is a function of:

1. neuronal circuits
2. receptors
3. secondary messengers= C-AMP, PI
4. membrane properties
5. synaptic fatigue and false transmitters
6. types of transmitters
7. excitation or inhibition
8. time
9. feedback control

Control Box A



Release is a function of:

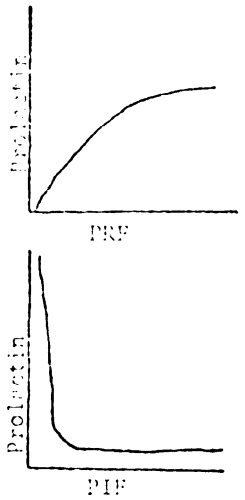
1. neuronal circuits
2. receptors
3. secondary messanges= C-AMP
4. membrane properties
5. time
6. feedback control

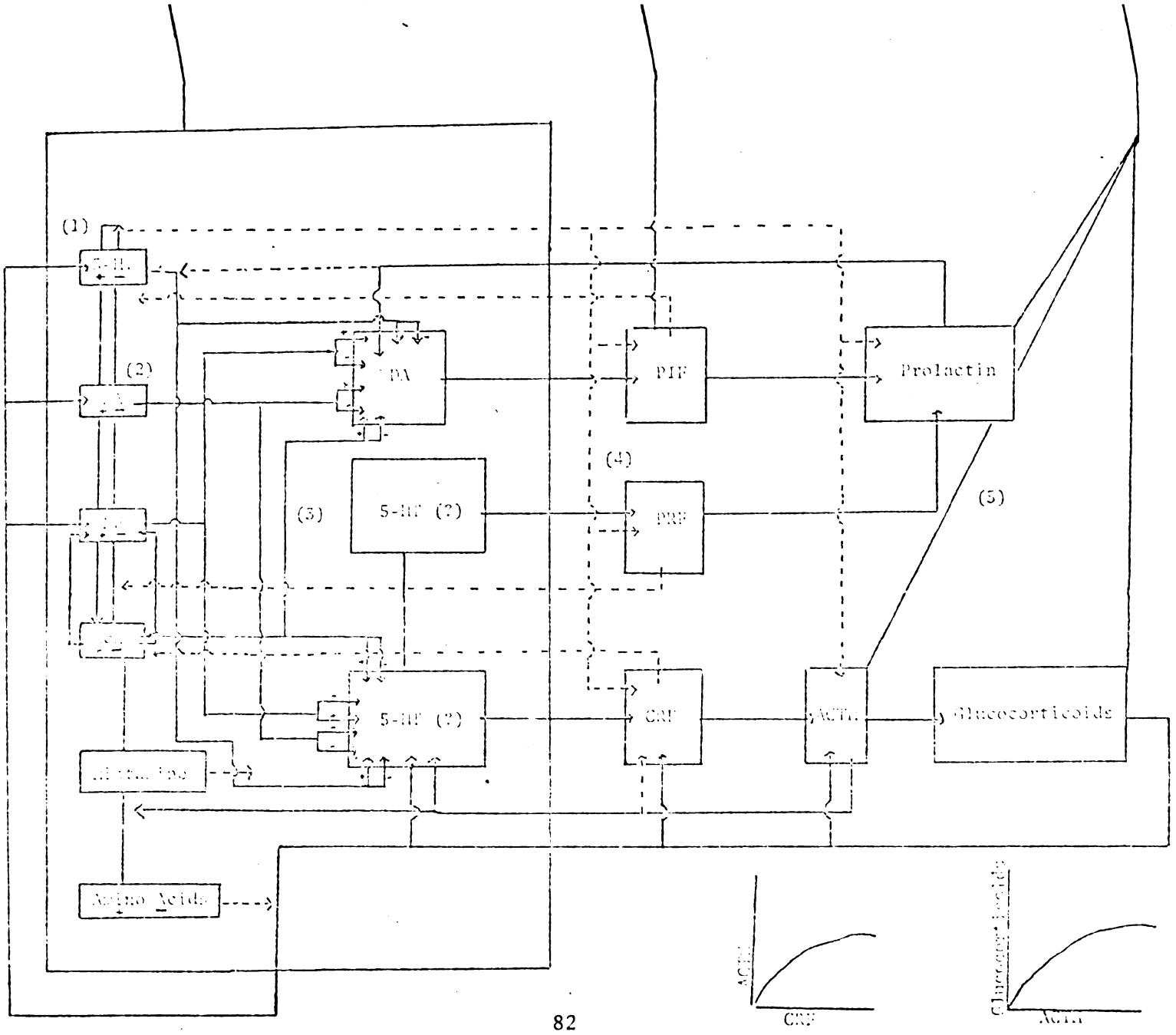
Control Box B

Release is a function of:

1. receptors
2. type of peptide
3. secondary messengers= C-AMP
4. time
5. feedback control

Control Box C




(1)
(2)
(3)
(4)
(5)
DA
PIF
Prolactin
5-HT (?)
PRF
5-HT (?)
CRF
ACTH
Glucocorticoids
Amino Acids
ACTH
CRF
Glucocorticoids
ACTH

generally capable of being either excitatory or inhibitory (Bloom, 1973). Discrimination between interneuronal transmitters may occur at synaptic receptors (control box A, 2; B; C). For example, the terminal system of sympathetic neurons contain cholinergic receptors which respond to acetylcholine in a variety of ways. Acetylcholine can excite rapidly via nicotinic receptors, excite more slowly and with longer duration at muscarinic receptors, and inhibit slowly and for longer duration at still other receptors (Smith, 1972). Thus, it becomes apparent that receptors in addition to differentiating between different types of transmitters also have the property of amplifying transmitter messages. Receptors for transmitter substances are an integral part of the synaptic membrane (control box A,4). Therefore, the coupling between the receptor site and its particular transmitter could change the electrophysiological properties of the synaptic membrane in favor of excitation or inhibition (control box A,7).

Another mechanism whereby transmitter messages may be amplified involves the activation of secondary messengers (control box A,3). Recent evidence reveals that the transmitters dopamine, serotonin, and norepinephrine all can activate adenyl cyclase. For example, application of norepinephrine or activation of noradrenergic pathways has been shown to lead to an increased content of cyclic AMP in Purkinje neurons of the cerebellar cortex (Siggins et al., 1973). In addition, while the mechanisms of peptide receptors in the pituitary are still unknown, action in the pituitary may involve activation of adenyl cyclase (control box C, 1, 2, 3). Borgeat et al. (1972) demonstrated that synthetic luteinizing hormone releasing factor (LRF) stimulated the accumulation of cyclic AMP in

the pituitary gland in vitro. Cyclic AMP and its dibutyryl derivative also have been shown to enhance the release of TSH, GH, ACTH, FSH, and LH from the pituitary gland (Zor et al., 1972). The activation of adenyl cyclase would therefore combine the precise stereochemical information of a transmitter with the unique biochemical consequences which attend initiation of cyclic AMP synthesis in the postsynaptic cells. Whether activation of adenyl cyclase reflects special properties of the receptor, transmitter molecule, or the postsynaptic nucleus is not known. However, mediation of synaptic messages by secondary messengers offers the possibility for longer terms changes in the neuronal membranes and in carbohydrate and protein metabolism. Thus, activation of secondary messengers may also represent a type of filtering device that distinguishes between significant and nonsignificant messages.

The activation of another group of secondary messengers, prostaglandins, (control box A,3&9) by the interaction of norepinephrine at its effector cell has been shown to inhibit the further release of this transmitter from its terminals (Smith, 1972). Such an action might represent a participant in the mechanism for interneuronal feedback control.

Another property of the actions of various transmitter substances is the duration of the response (control box A, B, C). As can be seen, this is an integrated function of the chemical nature of the transmitters, receptors, and activation of secondary messengers. Therefore, as revealed by electrophysiological and behavioral observations, certain nicotinic cholinergic actions and amino acis produce effects with rapid onset. Monoamines and muscarinic cholinergic actions proceed more slowly and for a

longer duration. By contrast, hypothalamic releasing factors and other substances with central action act over periods of hours-to-days in duration.

In summary, the transmitter systems comprising the CNS communication's network could be represented as control boxes where modulation within transmitter systems and between different transmitter systems may occur as functions of the properties discussed.

The interaction between different transmitter systems can take many forms (2). At the level of the membrane, the reaction between one transmitter and another could be translated into permeability changes for potassium-, sodium-, or chloride- ions that result in electric currents which either depolarize or hyperpolarize the neuronal membrane. The amino acid, GABA, has been shown to produce inhibition not only by post-synaptic inhibition through hyperpolarization, but also presynaptic inhibition via a depolarizing action (Bloom, 1973). Schade and Wilgenburg (1970) found that iontophorectic application of acetylcholine and dopamine to two different types of neurons of the snail (Helix ponatia) changed the firing pattern of these neurons. Whereas application of acetylcholine led to depolarization in one type of neuron, its application resulted in hyperpolarization of the other type of neuron tested. The same observation was made for dopamine. Thus, interaction between transmitter systems may be different depending upon which transmitter system is effected. Likewise, each transmitter system may employ different means for generating inhibition or excitation.

Reaction between transmitter systems may produce longer changes in

that the action of different transmitter systems may involve modifications in enzymatic profiles. Dopamine neurons have been shown to have a stimulatory influence on norepinephrine neurons. Depletion of dopamine at its synapses resulted in a decreased rate of norepinephrine disappearance as assessed through the use of dopamine and norepinephrine synthesis inhibitors and receptor stimulating agents (Persson and Waldeck, 1970). Furthermore, elevated levels of brain norepinephrine, dopamine, and serotonin have been found to cause a significant increase in brain choline acetylase activity, the enzyme mediating acetylcholine biosynthesis. By contrast, reduction of dopamine and norepinephrine induced by 6-OH-dopamine resulted in a significant though transient decrease in enzyme activity (Ho and Loh, 1972). Thus, the biosynthesis of the transmitter acetylcholine and cholinergic mechanisms may be modified by catecholamines and serotonin. Finally, destruction of the medial forebrain bundle has been shown to produce a marked reduction in the concentration of brain serotonin and norepinephrine secondary to a loss of enzymatic activities essential for the biosynthesis of these amines (Heller, 1972).

The third part of the model (3) represents an example of how different transmitter systems might react with either dopamine or serotonin, two transmitter systems in the hypothalamus hypothesized to be directly involved in regulating the secretion of ACTH and prolactin. The components that comprise part 4 are the peptide releasing factors which either elicit or inhibit the release of ACTH and prolactin. This transmitter system is located in the medial basal hypothalamus and functions similarly to the other transmitter systems, except that its postsynaptic cell is not

another neuron but the portal blood vessels and the anterior pituitary. As other transmitter systems possess multiple receptor sites for various chemical transmitters, so may this peptide transmitter system. This would allow this system to respond either to a sum of different transmitter interactions conveyed by a single transmitter or to integrate over multiple transmitter system input. Thus, the peptide transmitter system is the final integrator of messages conveyed by other transmitters from various parts of the CNS, ie. it decides upon what message should be sent to the pituitary. Part 5 of the model represents the final output of all the preceding interactions in terms of hormone release from the anterior pituitary gland.

Let us now consider in detail how interactions between various transmitter systems could modify the release of ACTH and prolactin. Our analysis will be limited to two transmitter systems in the hypothalamus, dopamine and serotonin, and the interaction of each system with other transmitter systems. One assumption will be made for simplication, ie. that both dopamine and serotonin are excitatory to its respective releasing factor. Norepinephrine has been shown to be both an excitatory and inhibitory transmitter (Bloom, 1973). Thus, inhibition of the serotonin system could be produced by an inhibitory action of norepinephrine. The resulting decrease in serotonin release from its terminals would be translated by CRF neurosecretory cells into insufficient membrane permeability changes to elicit the release of this peptide. Failure of CRF secretion would ultimately lead to inhibition of ACTH release. The exact opposite would occur if norepinephrine was stimulatory to the serotonin system. In this

case, an excitatory interaction between norepinephrine and the serotonin system would cause release of CRF and stimulate ACTH secretion. Further inhibition of the serotonin system could result from this system reacting with an inhibitory dopamine, acetylcholine, or even another serotonin transmitter molecule. Such an inhibition of serotonin, in turn, would cause inhibition of CRF release and suppression of ACTH secretion. Excitation of the serotonin system and finally stimulation of ACTH could be produced by an excitatory coupling of the serotonin system with an excitatory dopamine, acetylcholine, or serotonin molecule. Krieger and Krieger (1970) found that implantation of various transmitter substances into different areas of the cat brain resulted in a differential plasma corticosteroid response. For example, implantation of carbacol into the median eminence, posterior hypothalamus, and amygdala (anterior and central) resulted in stimulation of corticosteroid release. However, inhibition of corticosteroid secretion was obtained from lateral amygdalar, hippocampal, and septal implantations. Similar differential corticosteroid responses were seen with implantation of norepinephrine, serotonin, and GABA. However, the pattern of response for each transmitter substance was different. These findings provide indirect evidence that various transmitter systems in different areas of the brain do modify the secretion of ACTH possibly by a mechanism similar to that proposed.

A similar pattern of transmitter interaction might be proposed for the modification of prolactin secretion. In this case, reactions may occur for example between dopamine and the other transmitter systems. These

interactions would be interpreted further by PIF neurosecretory cells to either elicit or suppress the secretion of prolactin. Serotonin has been shown to be stimulatory to prolactin release. One possible explanation for this finding is the inhibition of the dopamine system via an inhibitory reaction with serotonin. The resulting decrease release of dopamine from its terminals would suppress the secretion of PIF and allow for stimulation of prolactin release. The transmitter norepinephrine has been found to both stimulatory and inhibitory to prolactin release. The seeminly dual role for norepinephrine might be explained in terms of transmitter systems interaction. The coupling of the dopamine system with an inhibitory norepinephrine transmitter would result in suppression of dopamine release from its terminals, depression of PIF secretion, and stimulation of prolactin release. The opposite effect would result if an excitatory interaction occurred between the dopamine system and norepinephrine. Further inhibition of the dopamine system and therefore stimulation of prolactin secretion could be produced by this system interacting with an inhibitory acetylcholine or another dopamine system. On the other hand, excitation of the dopamine system and thus inhibition of prolactin release could also be the result of the dopamine system reacting with an excitatory acetylcholine or serotonin system.

This hypothetical model therefore answers the question as to how interactions between various tansmitter systems might occur. It also offers a possible explanation whereby the fluctuations in the concentrations of dopamine, norepinephrine, and serotonin could cause the rise and subsequent decline of prolactin and corticosterone levels in response to

restraint stress. Thus, the common factor involved in the release of ACTH and prolactin and other anterior pituitary hormones in response to stress is the interaction of various transmitter systems during stress.

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