THE PHARMACOLOGICAL APPROACH TO THE STUDY OF THE MECHANISMS REGULATING ACTH SECRETION

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The common association of sympathoadrenal and pituitary adrenocortical activity in stress has suggested that catecholamines play a special role in regulating ACTH secretion. Several theories have been proposed through the years to explain this relationship. These included (a) Long's concept (1952) that emotional stimuli activate the hypothalamus which in turn stimulates the adrenal medulla via the spinal cord and splanchnic nerves, and the subsequently released epinephrine stimulates the release of ACTH; (b) Fortier et al.'s (1957) classification of emotional (epinephrine mediated) versus systemic stresses; (c) Smelik's (1959) suggestion that "neurotropic" stresses result in the release of epinephrine from the adrenal medulla which would in turn activate "hypothalamic nervous pathways leading to the neurohypophysis; and (d) involvement of hypothalamic norepinephrine in the regulation of pituitary function (Vogt, 1954; Carlsson et al., 1962; Vernikos-Danellis, 1965). There is almost as much evidence for, as against any one of these theories and the vast number of reports in the literature using indiscriminately various psychodepressants and psychic energizers to "elucidate" the involvement of catecholamines in pituitary ACTH secretion have merely added to the confusion. This is brought out nicely in the review by De Wied (1967) on the effects of chlorpromazine on endocrine function.

In a recent series of experiments (Vernikos-Danellis, 1966) it was observed that pretreatment of rats with one of the methyl xanthines, caffeine or theophylline, enhanced the stress-induced secretion of ACTH and antagonized the ability of steroids to inhibit hypothalamic-pituitary ACTH secretion. Since these methyl xanthines have been shown in a variety of tissues to inhibit in

vitro the 3'5' nucleotide phosphodiesterase that breaks down cyclic 3'5' adenosine monophosphate (AMP) and to potentiate the cyclic AMP-mediated effects of various hormones both in vitro and in vivo (Sutherland and Rall, 1958; Butcher and Sutherland, 1962; Hynie et al., 1966; Hess et al., 1963), the possibility existed that these drugs exerted their effects on the hypothalamic-pituitary unit by a similar mechanism. Studies on the distribution of the phosphodiesterase in various tissues of the rat (Vernikos-Danellis and Harris, 1966, unpublished) indicated high activity in both the median eminence and the anterior pituitary gland as compared to other organs. Furthermore, this enzyme was markedly depressed following incubation with one of the methyl xanthines in relatively large concentrations but also showed a 30% decrease in activity in the pituitary in in vivo experiments under the conditions that showed enhancement of the pituitary ACTH stress response. β-adrenergic blocking drugs are generally believed to exert their effects by inhibiting the activation of adenyl cyclase (Murad et al., 1962; Robison et al., 1967). Of these MJ-1999 [(2-Isopropylamino, 1-Hydroxyethyl) methanesulfonanilide)] offers advantages by being devoid of intrinsic adrenergic activity. Figure 1 shows the results of an experiment designed to determine whether MJ-1999 could prevent the increase in ACTH secretion following ether stress and the potentiation of this response by caffeine.

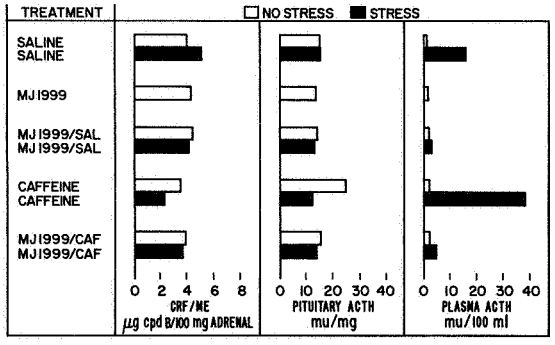

Figure 1. Changes in the concentration of corticotropin releasing activity, pituitary and plasma ACTH before and 2.5 minutes after stress (ether 1 min.) in rats pretreated with saline, caffeine, or MJ-1999 30 minutes previously. CRF content is expressed as µg corticosterone per 100 mg adrenal tissue in rats given a subcutaneous injection of 2.5 mg prednisolone 4 hours earlier, resulting from the intravenous injection of a crude acid extract of 1 rat median eminence. Pituitary and plasma ACTH was expressed as mU/mg tissue or mU/100 ml. plasma.

Throughout this work female Sprague-Dawley rats weighing 100 to 120 g. were used as the donors. Plasma and pituitary ACTH concentrations were determined in male rats of the same weight and strain 4 hours after hypophysectomy or 4 hours after a subcutaneous injection of 2.5 mg prednisolone per 100 g. body weight for CRF determinations. In these methods the increase in adrenal corticosterone concentration produced by one or more dilutions of the sample is compared to two doses of standard ACTH. (Vernikos-Danellis et al., 1966).

A dose of 4 mg/100 g. body weight of MJ-1999 was given subcutaneously 30 minutes prior to caffeine (2 mg/100 g. s.c.) or saline administration. Thirty minutes later half the animals in each group were stressed with ether (one minute) and decapitated 2.5 minutes after the beginning of the ether. The remaining animals served as unstressed controls. This dose of MJ-1999 is 4 times as great as that which is reported to prevent the rise in the isoproterenol-induced activation of myocardial phosphorylase in the rat (Kvam et al., 1965). The results show that it also prevented the stress-induced secretion of ACTH, and markedly depressed the increase in pituitary ACTH and the enhancement of the ether stress usually seen after caffeine.

Since catecholamines, vasopressin, and histamine share the property of activating adenyl cyclase in different tissues or exerting their effects through the mediation of cyclic AMP (Robison et al., 1967) and have also been implicated at one time or another in the mechanism regulating ACTH secretion, it became of interest to determine whether these substances could also potentiate the secretion of ACTH in response to a subsequent stress and whether a straightforward pharmacological study using α- and β-adrenergic blocking agents would yield any information about the mechanism of action of these substances, in the endocrine response to stress.

Female rats kept under controlled environmental conditions were given a single intraperitoneal injection of saline solutions of isoproterenol (80 μg), epinephrine (80 or 160 μg), norepinephrine (80 or 160 μg), dopamine (1 mg), vasopressin (500 mU), histamine (300 μg), or 0.9% normal saline (0.2 ml). Throughout this paper all doses are expressed as weight units of free base. Ten minutes after the injection they were decapitated and the plasma separated from the pooled heparinized blood, was frozen and stored at -12^{o} C until assayed for its ACTH content. The results as shown in Table 1, indicate that norepinephrine is more potent than epinephrine which is in turn more potent than isoproterenol in stimulating ACTH secretion, and that dopamine, histamine, and vasopressin in larger amounts have a similar effect. Since the potency of the catecholamines in this respect appeared to be

Table 1. Changes in plasma ACTH concentrations 10 minutes after the intraperitoneal injection of various substances in saline-, phentolamine-, or MJ-1999-pretreated rats. Doses are expressed as μg free base per rat. B.P. = blood pressure. Pooled samples from 10 rats were assayed in 5 to 20 assay animals. (Fiducial limits at P = 0.95 are given in parentheses.)

Treatment (i.p. in 0.2 ml)	Receptor type	Change in B.P.	Plasma ACTH mU/100 ml		
			Saline (0.2 ml/rat)	Phentolamine (1 mg/rat)	MJ-1999 (4 mg/rat)
Saline	-	-	1.7 (0.8-2.1)	1.5 (1.0-1.9)	1.8 (1.4-2.0)
Isoproterenol 80 μg	β	0 - ↓	1.9 (1.6-2.4)	2.0 (1.6-2.5)	1.4 (1.0-1.7)
Epinephrine 160 μg	α + β	↑	8.3 (6.5-9.8)	2.5 (2.0-2.9)	3.7 (3.1-4.4)
80 μg	α + β	↑	5.5 (4.9-6.4)	-	-
Norepinephrine 160 μg	α	↑	12.3 (10.3-14.0)	2.8 (2.0-3.4)	14.0 (-)
80 μg	α	↑	3.4 (2.6-4.1)	-	-
Dopamine 1 mg	Dopaminergic ?	0 - ↑	3.7 (3.1-4.1)	1.9 (0.9-2.7)	4.0 (3.2-4.8)
Vasopressin 500 mU	?	↑	5.6 (4.8-6.2)	5.8 (5.0-6.3)	3.9 (3.2-4.6)
Histamine 300 μg	?	↓	4.8 (4.1-5.6)	4.2 (3.8-4.8)	4.5 (4.0-5.1)
Ether 1 min.			4.2 (3.8-5.0)	5.0 (4.2-5.8)	1.2 (0.7-1.6)

related to their pressor activity, a similar series of experiments was performed in rats given i.p. 30 minutes earlier 1 mg of the α-adrenergic blocking agent phentolamine. The results are in agreement with previous observations (Tepperman and Bogardus, 1948; Guillemin, 1955; George and Way, 1957; Van Peenen and Way, 1957) that α-adrenergic blocking agents do not inhibit the response to stresses other than epinephrine and extend this observation to include norepinephrine and dopamine. The increase in circulating ACTH concentration caused by histamine or vasopressin administration or exposure to ether was not affected by pretreatment with phentolamine. In contrast, previous experiments (Figure 1) had shown that pretreatment of animals with the β-adrenergic blocking agent MJ-1999 effectively inhibited the stress-induced ACTH secretion following ether. It therefore appeared possible that this drug might inhibit other types of stresses and thus suggest a role for catecholamines common to all types of stress in the mechanism regulating ACTH secretion. The results in Table 1 show that β-adrenergic blocker injected subcutaneously 30 minutes earlier in a dose of 4 mg/100 g. body weight, markedly inhibits the acute pituitary response to epinephrine and ether and surprisingly reduces that to vasopressin.

Since those substances with greatest β-adrenergic activity were least effective in stimulating ACTH secretion and since the β-adrenergic blocker was previously shown to inhibit the ability of caffeine to enhance ether stress, experiments were designed to compare the ability of the substances studied to enhance a second stress and to determine whether α- or β-adrenergic blockade affected this property. Thirty minutes before exposure to one minute of ether stress rats were given the following substances: saline (0.2 ml), isoproterenol (80 μg), epinephrine (80 μg), norepinephrine (80 μg), dopamine (1 mg), vasopressin (250 or 500 mU), histamine (300 μg). All animals were decapitated 2.5 minutes after the beginning of the ether and their plasma ACTH content determined. Where α-blockade was required phentolamine was administered 30 minutes before the injection of the neurohumor or one hour before the ether stress. The time sequence was therefore: phentolamine followed 30 minutes later by the neurohumor, followed 30 minutes later by one minute ether and decapitation at 2.5 minutes after the beginning of the ether stress. Since MJ-1999 inhibited the ether stress per se, the timing was selected in such a way as to allow the response to ether to return yet maintain adequate β-adrenergic blockade at the time of the injection. It is of interest here to note that the inhibition of the ether stress was very transient as compared to the effect of the blocker on the enhancement of a second stress by epinephrine which lasted at least 4 hours, (unpublished observations). The time sequence in this series of experiments was therefore; MJ-1999 followed 2.5 hours later by the injection of the neurohumor, followed 30 minutes later by one

minute ether and decapitation at 2.5 minutes after the beginning of the ether stress. Table 2 shows that epinephrine, isoproterenol, and vasopressin were all effective in enhancing the ACTH secretion in response to ether stress. Histamine, dopamine, and 80 μg of norepinephrine were without effect; section C of Table 2 shows however, that increasing the dose of norepinephrine to 160 μg and the intensity of the stress to ether followed by laparotomy resulted in a significant enhancement of ACTH secretion. Section B of Table 2 shows that if histamine stress is substituted for the ether, epinephrine pretreatment still enhanced this stress response while vasopressin did not. In contrast to the ACTH stimulating activity of the catecholamines the order to potency of these substances in enhancing a second stress was: isoproterenol > epinephrine > norepinephrine. Pretreatment with phentolamine did not alter the potentiating ability of these substances and in fact increased that of epinephrine. Pretreatment with the β-blocker, MJ-1999, abolished the enhancing properties of all the substances tested including vasopressin.

In order to determine the site of action of these two differing effects of peripherally administered catecholamines, the corticotropin releasing activity (as measured by the increase in adrenal corticosterone) of a crude acid extract of rat median eminence (MEE) was compared in rats in which the endogenous secretion of CRF was effectively inhibited by a single subcutaneous injection of prednisolone (2.5 mg/100 g. 4 hours earlier) with animals given phentolamine only, steroid plus phentolamine, or normal saline only (see Table 3). Phentolamine injected 3-1/2 hours after the steroid and 30 minutes before use of the animals was without effect in preventing the stress of ether and intravenous saline and the response of the pituitary to MEE, nor did it affect the sensitivity of the adrenal cortex to injected ACTH.

Table 4 shows a similar series of experiments using MJ-1999 instead of phentolamine. Once more the blocker was administered 30 minutes before use of the animals. MJ-1999 markedly depressed not only the increase in adrenal corticosterone resulting from the stress of ether and intravenous saline but also that reflecting the corticotropin releasing activity of the median eminence extract both in the presence and in the absence of the steroid. In order to eliminate the possibility that this inhibition was exerted at the adrenal level, the increase in adrenal corticosterone in response to two doses of standard ACTH was compared in steroid blocked rats with and without MJ-1999. The adrenergic blocker had no direct effect on the responsiveness of the adrenal cortex to ACTH indicating that its site of action in depressing the corticotropin releasing activity of MEE was exerted primarily at the pituitary.

Table 2. ACTH concentration in the plasma of saline-, phentolamine-, or MJ-1999-pretreated rats, 2.5 minutes after ether (1 minute), ether/sham ULA (1 minute ether and sham unilateral adrenalectomy) or 10 minutes after an intraperitoneal injection of histamine. Doses are expressed as μg free base per rat. Each value represents pooled plasma samples from at least ten rats. (Fiducial limits at P = 0.95 are given in parentheses.)

	Treatment	Stress	Plasma ACTH mU/100 ml		
			Saline (0.2 ml/rat)	Phentolamine (1 mg/rat)	MJ-1999 (4 mg/rat)
A.	Saline	Ether (1 min)	4.2 (3.7-5.1)	5.0 (4.1-5.8)	4.3 (3.8-4.8)
	Isoproterenol 80 μg	Ether (1 min)	9.1 (8.0-10.2)	7.6 (6.9-8.1)	3.8 (3.0-4.3)
	Epinephrine 80 μg	Ether (1 min)	7.0 (6.2-8.1)	10.0 (9.6-10.9)	5.3 (4.6-6.0)
	Norepinephrine 80 μg	Ether (1 min)	3.7 (3.1-4.3)	3.4 (2.8-4.1)	4.0 (3.1-4.9)
	Dopamine 1 mg	Ether (1 min)	4.8 (4.0-5.4)	4.8 (4.1-5.6)	4.4 (3.8-5.0)
	Vasopressin 500 mU	Ether (1 min)	7.1 (6.9-7.8)	7.5 (6.5-8.3)	5.8 (4.9-6.3)
	250 mU	Ether (1 min)	5.8 (5.3-6.6)	6.0 (5.0-7.0)	4.5 (3.9-5.0)
	Histamine 300 μg	Ether (1 min)	4.0 (3.4-4.9)	4.7 (4.0-5.4)	4.3 (3.7-5.0)
B.	Saline	Histamine (300 μg)	3.9 (3.1-4.6)	-	-
	Epinephrine 80 μg	Histamine (300 μg)	5.7 (5.0-6.5)	-	-
	Vasopressin 500 mU	Histamine (300 μg)	3.1 (2.6-3.9)	-	-
C.	Saline	Ether/Sham ULA	10.1 (9.2-12.0)	-	-
	Norepinephrine 160 μg	Ether/Sham ULA	14.2 (12.9-15.9)	-	11.1 (9.9-12.0)

Table 3. Effect of ACTH, MEE or the stress of intravenous saline under ether anesthesia on the adrenal corticosterone concentration of rats pretreated with saline only, saline plus phentolamine (1 mg/rat), prednisolone (2.5 mg/100 g. body weight) plus saline or prednisolone plus phentolamine. Number of animals given in parentheses.

	Adrenal Corticosterone Concentration (μg/100 mg tissue ±S.E.)			
I.V. Injection	Saline 4 hr + saline 30 min	Saline 4 hr + phentolamine 30 min	Prednisolone 4 hr + saline 30 min	Prednisolone 4 hr + phentolamine 30 min
None	3.02±0.10(5)	4.88±0.22(5)	1.09±0.07(5)	1.21±0.08(5)
Saline	6.65±0.32(5)	5.80±0.31(5)	1.24±0.15(5)	1.14±0.11(5)
1 ME	-	6.72±0.48(5)	3.01±0.20(5)	3.51±0.28(5)
ACTH 33.3 μU	-	-	1.87±0.19(5)	1.99±0.09(5)
ACTH 100 μU	-	-	6.39±0.41(5)	6.12±9.38(5)

Table 4. Effect of ACTH, MEE, or the stress of intravenous saline under ether anesthesia on the adrenal corticosterone concentration of rats pretreated with saline only, saline plus MJ-1999 (4 mg/rat), prednisolone (2.5 mg/100 g. body weight) plus saline, or prednisolone plus MJ-1999. Number of animals given in parentheses.

	Adrenal Corticosterone Concentration (μg/100 mg tissue ±S.E.)			
I.V. Injection	Saline 4 hr + Saline 30 min	Saline 4 hr + MJ-1999 30 min	Prednisolone 4 hr + saline 30 min	Prednisolone 4 hr + MJ-1999 30 min
None	3.09±0.22(5)	3.16±0.24(5)	1.78±0.09(5)	1.86±0.13(5)
Saline	7.57±0.49(5)	4.38±0.38(5)	1.47±0.10(5)	1.57±0.08(5)
1 ME	-	4.83±0.42(5)	3.15±0.19(5)	2.11±0.09(5)
ACTH 33.3 μU	-	-	2.32±0.17(5)	2.10±0.09(5)
ACTH 100 μU	-	-	6.24±0.51(5)	6.25±0.45(5)

These findings suggested the hypothesis that catecholamines secreted peripherally during exposure to various stress situations affect the hypothalamic pituitary unit by at least two distinct mechanisms: direct stimulation of ACTH secretion related to their α-adrenergic hypertensive properties, mediated by the hypothalamus and possibly reflexly by higher centers in the central nervous system, and a β-adrenergic-receptor-mediated property of enhancing the ACTH secretion to a subsequent stress by an action primarily on the adenohypophysis. This latter property of the catecholamines appears to be shared by vasopressin; whether this is an intrinsic effect of this peptide or one mediated in some way by catecholamines remains to be determined. On the other hand the mechanism by which histamine stimulates pituitary ACTH secretion does not fall within either of these categories. It is of interest to point out in this respect the recent observation of Dallman (1967) that prior exposure to the stress of scalding enhances the increase in circulating corticosterone in response to histamine whereas prior administration of histamine has no effect on the response to scalding.

The presence and distribution of catecholamines in the central nervous system has been well documented (Vogt, 1954; Fuxe, 1963; Carlsson et al., 1962). However, studies with peripherally administered tritiated epinephrine and norepinephrine have shown that these substances do not cross the blood brain barrier although the anterior pituitary takes up large amounts of the labelled amines; to a lesser extent so does the median eminence and radioautographic studies demonstrate that they penetrate only a short distance into the hypothalamus (Axelrod et al., 1959; Weil-Malherbe et al., 1961; Samorajski and Marks, 1962). Attempts to study the role of brain amines on pituitary ACTH secretion have proved confusing largely because of the lack of specificity and knowledge of the site and mechanism of action of the drugs used to deplete or inhibit synthesis of these amines. Interest in α-methyl p-tyrosine (α-MT) stems from its ability to inhibit tyrosine hydroxylase and thereby to interfere with the synthesis of catecholamines (Spector et al., 1965). This agent depletes norepinephrine stores in peripheral sympathetic nerve endings and reduces the concentrations of norepinephrine and dopamine in the brain. It is especially useful for studying the role of brain catecholamines since (1) it is more effective in lowering the concentrations of these amines in the central nervous system than in the periphery (Torchiana et al., 1965), (2) it does not influence 5-hydroxytryptamine levels, and (3) unlike α-methyl-m-tyrosine and α-methyldihydroxyphenyl alanine, it is not converted into "false transmitters" (Spector et al., 1965). It therefore became of interest to use this drug as a tool in the study of the role of brain norepinephrine on pituitary ACTH secretion particularly since the long lag between maximal inhibition of brain and adrenal medullary amines because of the slower catecholamine turnover in the adrenal lent itself to the

investigation of the relative contribution of these two components to the functional integrity of the hypothalamic-pituitary unit.

In this series of experiments the animals were used six hours after the intraperitoneal injection of a saline suspension of α-MT in a dose of 200 mg/Kg. body weight, unless otherwise specified. A similar suspension of l-tyrosine was injected into those animals that served as controls. At this time brain amines are markedly depressed (Rech et al., 1966).

Figure 2 shows the plasma corticosterone concentrations before and 15 minutes following the stress of ether (one minute) in rats that were either uninjected or had received an intraperitoneal injection of l-tyrosine or α-MT. The results show that the stress-induced increase in plasma steroids was reduced in the α-MT treated rats. Figure 3 shows that this reduced steroid response reflects reduced ACTH secretion in response to stress. Approximately 50% inhibition of the ACTH secretion in response to the stress of ether and sham adrenalectomy was found and increasing the dose of α-MT did not inhibit this response further. Since this phenomenon could have been due to the availability to the median eminence and pituitary of catecholamines originating from the adrenal, a similar experiment was performed in rats 24 hours after adrenalectomy. At this time period after removal of the adrenal glands female rats show a marked increase in the sensitivity to stress, secreting greater amounts of ACTH in response to the relatively mild stress of one minute ether than do intact animals (Hodges and Vernikos, 1959). Figure 4 shows that under these conditions one-tenth of the dose of α-MT sufficed to cause a 50% inhibition of the stress response and the usual dose of

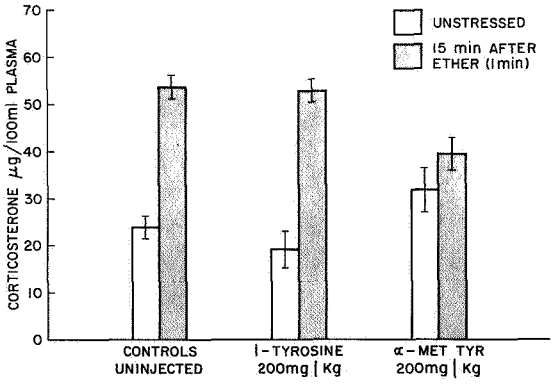

Figure 2. Plasma corticosterone concentrations before and 15 minutes following stress (ether 1 min) in uninjected rats or animals that had received six hours earlier an intraperitoneal injection of a saline suspension of l-tyrosine or α-methyl tyrosine in a dose of 200 mg/Kg. Number of animals given in parentheses.

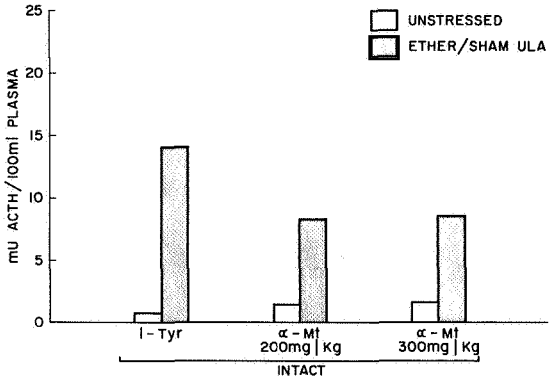

Figure 3. Plasma ACTH concentrations before and 2.5 minutes after stress (ether 1 min. and sham unilateral adrenalectomy) in rats given six hours earlier an intraperitoneal injection of a saline suspension of 200 mg/Kg. l-tyrosine or α-methyl tyrosine in dose of 200 mg or 300 mg/Kg.

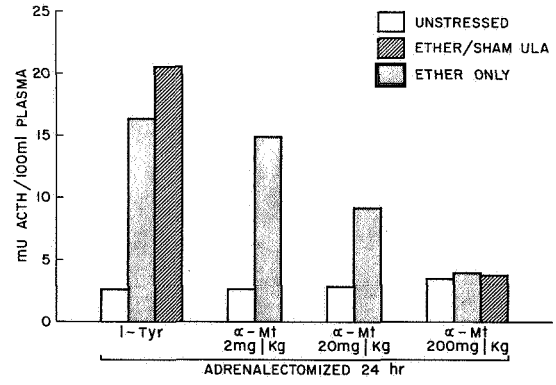

Figure 4. Plasma ACTH concentrations before and 2.5 minutes after stress (ether 1 min. or ether 1 min. and sham unilateral adrenalectomy) in rats 24 hours after the removal of their adrenal glands and six hours after an intraperitoneal injection of 200 mg/Kg. body weight l-tyrosine or 2, 20, or 200 mg/Kg. of α-methyl tyrosine.

200 mg/Kg. body weight now produced 96% inhibition of both ether stress and the more severe stress of ether and sham adrenalectomy. Figure 5 illustrates the relationship between percent inhibition of the stress-induced secretion of ACTH and percent inhibition of whole brain norepinephrine in intact, sham adrenalectomized and 24 hour adrenalectomized rats receiving different doses of α-MT. It would appear that there is a threshold in brain amine levels above which the stress response is not greatly affected and below which small changes in amine content markedly affect pituitary ACTH secretion.

Experiments in progress with Drs. Levine and Barchas of Stanford University studying the effects of this drug in adrenal demedullated and chronically adrenalectomized rats, as well as looking into the cause of the apparent increased sensitivity of the adrenalectomized rat to inhibition of brain amines by α-MT are too preliminary to discuss at the present time. Nevertheless, together with this rather elementary exercise in experimental pharmacological design presented here they help to point out certain well known but neglected facts, and suggest new cautions in the interpretation of results, the building of models and the art of hypothesizing, with regard to the role of catecholamines in regulating pituitary ACTH secretion.

The word catecholamines merely denotes structural relationship of those biogenic amines, possessing an o-dihydroxybenzene ring.

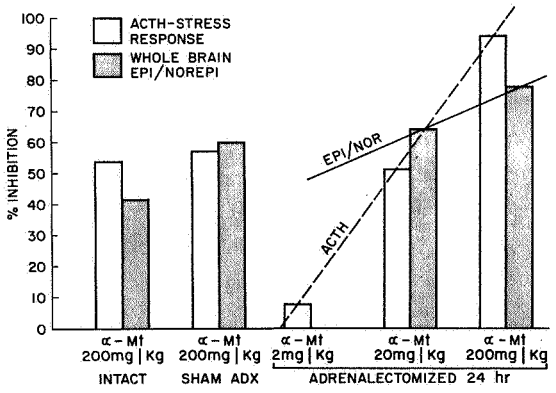

Figure 5. Comparison of the percent inhibition of the stress-induced secretion of ACTH and whole brain epinephrine/norepinephrine content in intact, sham adrenalectomized, and 24 hour adrenalectomized rats given six hours earlier an intraperitoneal injection of different doses of α-methyl tyrosine.

It does not denote any kind of uniformity of physiological or pharmacological mechanism or site of action. In fact the members of this group have little in common other than coming through the same biosynthetic pathway and are characterized by an extraordinary ability to exert similar or differing effects at the same site, similar effects at different sites, or differing effects at different sites. Yet in the study of their role on pituitary corticotropic secretion they have been used almost interchangeably and often their role as a group emphasized or dismissed on the results of experiments obtained with a single member. The experiments described in this paper perhaps help to emphasize that (a) catecholamines do play a role in regulating ACTH secretion, (b) that each member of the catecholamine group exerts distinct, varied and often overlapping effects, (c) that there is an intricate relationship and balance between the actions of circulating catecholamines exerted at pituitary and hypothalamic level and those exerted by centrally located amines on the various structures of the central nervous system, both stimulant and inhibitory that appear to be involved in maintaining the functional state of the hypothalamic-pituitary ACTH secreting system.

Finally the physiological significance of the close association between the glandular tissues of the adrenal cortex and medulla has been recently emphasized by the work of Wurtman (1966) and of Jost and his associates (Margolies et al., 1966) by the demonstration that the synthesis of the N-methyl-transferase enzyme, which forms epinephrine, is under the control of adrenal corticosteroid hormones. Together with the present findings, it is tempting to suggest in addition to the well known negative feedback loop between adrenal cortex and central CRF controlling centers, the existence of a positive feedback loop between adrenal cortex and anterior pituitary mediated by epinephrine from the adrenal medulla.

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