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Effects of Pargyline and Amphetamine upon Acute Stress Responses in Rats*† (33752)

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A number of independent studies (1–3) have shown that in rats a single dose of reserpine or chlorpromazine can cause a persistent hypersecretion of ACTH. This pituitary–adrenal hyperactivity is reflected by elevated plasma and adrenal corticosterone levels, depletion of pituitary ATCH and depletion of corticotropin-releasing factor (CRF) in the median eminence of the ventral hypothalamus. In order to understand the neuropharmacological basis of such endocrine responses to reserpine and chlorpromazine one may ask the question if there is any relationship between changes in the functional activity of biogenic amines in hypothalamus after these drugs and the hypersecretion of ACTH. Shore and Brodie (4) first showed that pretreatment of animals with a monoamine oxidase (MAO) inhibitor counteracts the central effects of reserpine and protects the released amines from metabolic biotransformation. The present paper describes the effect of pretreatment of rats with the MAO inhibitor pargyline or with amphetamine on the ACTH releasing property of reserpine or acute stress.

Materials and Methods. Adult female Wistar rats (150–175 g) were used in all experiments. The animals were fed commercial rat chow and water *ad libitum*. The rats were housed in a constant temperature and humidity room (Labline Inc., Chicago, Illinois) for at least 3 days before use and during this period they were adapted to the stress of handling and injection by giving saline (0.1 ml/100 g of body wt.) intraperitoneally for 3 consecutive days.

The doses of drugs are expressed as their salts unless otherwise specified. Pargyline hydrochloride (Eutonyl, Abbott Laboratories) was administered intraperitoneally in a dose of 50 mg/kg in saline. Amphetamine sulfate (Smith, Kline and French) was administered intraperitoneally in a dose of 5 mg/kg in saline. Control rats were given saline (0.2 ml/100 g of body wt.) by the same route. Drug-treated and control rats were sacrificed 24 hr after pargyline or 4 hr after amphetamine administration. The experiments were designed so as to sacrifice the animals between noon and 1:00 p.m. in all experiments.

Stress. The animals were subjected to an acute ether-laparotomy stress. The rats were exposed to ether vapor for 1 min by the end of which time they were completely anesthetized. The animals were then quickly subjected to laparotomy by making an incision of about 5 cm in the lumbrosacral region on one side of the animal at the adrenal region. This procedure required approximately 12–15 sec. The animals were sacrificed by decapitation 15 min from the onset of the stress. Mixed trunk blood was collected in heparinized tubes. The plasma and adrenal corticos-

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TABLE I. Effects of Pargyline and Amphetamine Pretreatment on Reserpine and Acute Stress-Induced Plasma and Adrenal Corticosterone Changes in Rats.

Treatment[a]	Adrenal corticosterone (μg/adrenal ± SE)		Plasma corticosterone (μg/100 ml ± SE)	
	Unstressed	Stressed	Unstressed	Stressed
Normal controls	0.43 ± 0.03 [10][b]	0.88 ± 0.13[c,d] [10]	12 ± 1.5 [10]	40 ± 3.6[c] [10]
Pargyline	0.12 ± 0.01 [4]	0.56 ± 0.05[d] [4]	9.0 ± 0.95 [4]	17 ± 4.1[c] [4]
Amphetamine	0.17 ± 0.01 [4]	0.51 ± 0.01[c] [4]	4.5 ± 0.89 [4]	28 ± 1.8[d] [4]
Pargyline + amphetamine	0.21 ± 0.03 [4]	0.68 ± 0.07[c] [4]	6.0 ± 1.6 [4]	23 ± 1.8[c] [4]
Reserpine	0.97 ± 0.07 [8]	0.82 ± 0.01 [8]	37 ± 3.7 [8]	38 ± 1.4 [8]
Pargyline + reserpine	0.42 ± 0.02 [4]	0.67 ± 0.06[d] [4]	7.5 ± 0.55 [4]	20 ± 1.9[c] [4]

[a] All drugs given intraperitoneally; amphetamine, 5 mg/kg, 4 hr; pargyline, 50 mg/kg, 24 hr; reserpine, 5 mg/kg, 8 hr.

[b] Number of animals used per observation.

[c] Difference unstressed compared to stressed significant at [d] $<.01$, [c] $<.001$.

terone concentrations were measured fluorimetrically, using the fluorescence reagent of Braunsberg and James (5) to modify the method of Guillemin *et al.* (6).

Anterior pituitary tissue and the tissue comprising the median eminence region of the ventral hypothalamus were collected from normal and stressed rats. The pituitary ACTH concentration was measured by a conventional 2 × 2 bioassay design, using the increase of adrenal corticosterone 5 min after injection into dexamethasone-blocked rats as the measurement of response (7). The CRF content of the median eminence tissue was measured by an *in vitro* method adapted from that of Saffran and Schally (8). This *in vitro* method depends on the measurement of the ACTH released into the medium, when pituitary halves were incubated for 1 hr with an extract of median eminence tissue. The ACTH released was measured as described above. Cerebral cortex tissue extracts were used as controls. These methods have been described in greater detail previously (1).

Results. Effect of pargyline and amphetamine pretreatment on acute stress-induced plasma corticosterone. Twenty-four hr after pargyline administration, or 4 hr after amphetamine administration, rats were sacrificed either resting or following an acute ether-laparotomy stress. Both pargyline and amphetamine reduced the resting plasma corticosterone level as compared to controls, and significantly lowered the adrenal corticosterone content in the resting state (Table I). Acute stress elevated the plasma corticosterone and adrenal corticosterone levels in the treated rats, but the response was significantly less in the treated rats than in the controls. When pargyline-treated animals were given amphetamine, the plasma and adrenal corticosterone of resting and stressed groups were not distinguishable from animals given pargyline alone. However, the responses were less than those of normal controls (Table I). Reserpine pretreatment produced a marked elevation of both the adrenal and plasma corticosterone, which did not respond further following acute stress. Pargyline treatment prior to reserpine eliminated completely the elevation of corticosterone in the resting state, and restored the ability to respond to the acute stress stimulus.

Effect of pargyline pretreatment on reserpine-induced changes in plasma and adrenal

TABLE II. Effect of Pargyline Pretreatment on the Reserpine-Induced Plasma, Adrenal Corticosterone, Pituitary ACTH, and CRF Content in Rats.

Treatment	Plasma corticosterone (μg/100 ml $\pm$ SE)	Adrenal corticosterone (μg/adrenal $\pm$ SE)	Pituitary ACTH	CRF[d] (mU/ME)
A. Controls	12 $\pm$ 0.44[a] [8][b]	0.52 $\pm$ 0.01[a] [8][b]	17 [12–20][c]	1.8 [1.5–2.1][c]
B. Reserpine (5 mg/kg, i.p. 8 hr)	37 $\pm$ 3.7 [8]	0.97 $\pm$ 0.07 [8]	10 [5.9–17]	0.50 [0.2–1.4]
C. Pargyline (50 mg/kg, i.p. 16 hr) + reserpine (5 mg/kg, i.p. 8 hr)	15 $\pm$ 0.67 [8]	0.55 $\pm$ 0.01 [8]	12 [10–13]	1.5 [1.3–1.8]

[a] Plasma and adrenal corticosterone values for groups A and C not significantly different.

[b] Number of animals used per observation.

[c] Fiducial limits of error, $p = 0.95$.

[d] CRF activity expressed as mU of ACTH released per mg of pituitary tissue per hour per median eminence extract.

corticosterone, pituitary ACTH and median eminence CRF content in rats. Sixteen hr after pargyline pretreatment, rats were injected with reserpine (5 mg/kg) intraperitoneally. Eight hr later the animals were sacrificed. Appropriate pargyline, reserpine, and saline controls were similarly prepared. Plasma and adrenal corticosterone, pituitary ACTH, and CRF content of the median emince were measured. The results of these measurements are shown in Table II. It is quite clear that pargyline pretreatment not only prevented the rise in adrenal and plasma corticosterone produced by reserpine, but it also prevented the marked release of CRF produced by the single injection of reserpine. The fall in pituitary ACTH content produced by reserpine was only partially reduced by pargyline pretreatment.

Discussion. Previous work from this laboratory and other laboratories showed that reserpine is capable of inducing a long-lasting ACTH hypersecretion (1, 2, 9). Reserpine depletes serotonin and catecholamine stores in the central nervous system. It was also shown that pretreatment of animals with a MAO inhibitor counteracts the central behavioral effects of reserpine and protects the released amines from metabolic transformation (4). If depletion of diencephalic monoamines is involved in the action of reserpine on the pituitary–adrenal system, then accordingly blockade of the metabolic transformation of the biogenic amines should prevent the effect of reserpine. The present report shows that this is indeed the case, since pargyline pretreatment blocked the reserpine-induced plasma and adrenal corticosterone elevation and CRF depletion.

Treatment with pargyline also reduced the resting adrenal corticosterone content and inhibited the stress-induced plasma and adrenal corticosterone elevation, suggesting that the set-point for the normal level of ACTH activity as well as the ability to respond to a surgical stress involve neural pathways which may be inhibited if endogenous brain monoamines are elevated. It is important to point out that some MAO inhibitors may have direct inhibitory effects on adrenal steroidogenesis (10). Pargyline does not share this property, hence the effects observed are more likely to be related to the MAO inhibition than to nonspecific effects.

Amphetamine administration in rats has been reported to cause ACTH discharge as assessed by adrenal ascorbic acid depletion (11, 12). In these studies, adrenal ascorbic acid measurement was done 1 hr after administration of amphetamine. By contrast, Harwood and Bigelow (13) reported that amphetamine reduced the resting levels of circulating corticoids in monkeys and Lorenzen (10) found that amphetamine reduced the secretion of 17-hydroxycorticosterone in the adrenal venous effluent of surgically stressed

dogs (10, 14). Naumenko (15) and Redgate (16) have pointed out that peripheral receptors (including cervical sympathetic and baroreceptor complex) may be involved in ACTH release with indirectly acting sympathomimetic amines and vasoactive agents. Amphetamine is primarily an indirect acting sympathomimetic amine. It has some intrinsic activity of its own (17, 18), but its action mostly depends on the release of norepinphrine from adrenergic nerve terminals. It is possible that earlier observations of adrenal ascorbic acid deplention observed by some investigators (11, 12) could be due to an indirect or direct effect of amphetamine upon peripheral receptors. This phase may be a transient one, associated with the pressor response to this drug. The more characteristic endocrine activity that we observed in this study was associated with the CNS behavioral alerting response. This consisted of an inhibition of both basal and the poststress ACTH secretion, which we interpret as being due to a reduced rate of CRF secretion, consistent with the view that there is a central adrenergic mechanism which tonically inhibits CRF secretion.

In an earlier communication (1) we postulated that reserpine-induced hypersecretion of ACTH may possibly be causally related to alteration in brain neurotransmitter function. The most characteristic action of reserpine is interference with the storage of catecholamines and serotonin in synaptic vesicles, reducing the synaptic transmission activity. The pattern of reserpine-induced neurotransmitter depletion and the time course of CRF depletion are similar. Likewise, the ACTH hypersecretion response to chlorpromazine and the depletion of cortisotropin-releasing factor which chlorpromazine produces may be thought of as a response to the central catecholamine blocking capability of this drug. The present study, demonstrating the reversal of the endocrine effects of reserpine by a MAO inhibitor, and demonstrating similar stress-inhibitory responses by amphetamine also seem to be consistent with the hypothesis that a catecholamine, either norepinephrine or dopamine, normally inhibits the output of CRF by hypothalamic neurones which have their endings in the median eminence. Reducing the activity of the catecholamine, either by depletion or by receptor blockade, allows CRF to escape into the hypothamamo–hypophyseal portal vascular system, by which route it stimulates pituitary ACTH release. Agumenting hypothalamic catecholamine activity either by MAO inhibitors or with amphetamine serves to inhibit the spontaneous liberation of CRF as well as to reduce the elevated output following a surgical stress. Thus we believe that a catecholamine may be an inhibitory neurotransmitter for CRF release. Considering the massive accumulation of dopamine terminals which Fuxe (19) describes in the neurovascular region of the median eminence, one is tempted to speculate that dopamine may be the inhibitory catechomaine in this region.

Summary. Pargyline and amphetamine lowered the resting levels of corticoids in the adrenals and plasma, and reduced acute stress induced rise in plasma and adrenal corticosterone. Pretreatment of rats with monoamine oxidase inhibitor (pargyline) blocked the depletion of the corticotropin-releasing factor in the median eminence and the hypersecretion of ACTH induced by reserpine. It is proposed that monoamines (probably catecholamines) are important neurotransmitters inhibiting the release of CRF from the nerve terminals in the median eminence of the hypothalamus.

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The Mitogenic Action of Bradykinin on Thymic Lymphocytes and its Dependence on Calcium* (33753)

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The mitotic activity of bone marrow and thymus tissue is stimulated by increasing the level of ionized calcium in the plasma of the rat (1–3), and is in fact directly proportional to the ambient calcium level (4). It has also been found that changes in the mitotic activity of bone marrow and thymus parallel changes in the growth rate of the rat, and are accompanied by marked and parallel shifts in the level of ionized plasma calcium (5). Thus there appears to be a direct involvement of the calcium ion, and the hormones which govern its concentration, in the control of overall growth and mitotic activity in the rat.

Although generalized changes in mitotic activity may be controlled by shifts in plasma calcium concentrations, it is unlikely that localized increases in cell division are mediated in this way. In the proliferative response to injury, for example, a general increase in plasma calcium level would affect uninjured as well as the injured tissue. Nevertheless the calcium ion can still play a role in this response. The demonstration that detergents and polyamines increased mitosis in thymocyte suspensions by sensitizing the cells to the stimulatory action of calcium (6), led us to speculate that a similar calcium-dependent mechanism might operate in the mitotic response to injury. If some substance were released in damaged tissue which could act upon the cell membrane either to displace bound calcium or to increase its permeability to calcium, this ion might then initiate the mitotic stimulation needed for repair.

To test this hypothesis the nonapeptide bradykinin was selected as a possible sensitizing agent, since it is one of several kinins responsible for the vascular dilatation and permeability changes of the inflammatory response to injury (7–11). Suspensions of rat thymocytes maintained *in vitro* were used as the test system as they provide a well-controlled, highly reproducible, and rapid assay procedure for mitogenic activity. These cell populations are physiologically realistic since they exhibit the same degree of mitotic competence over a 6-hr period as they do *in vivo* (5, 6).

Methods. Thymuses were removed under ether anesthesia from male Sprague-Dawley rats (weighing between 120 and 170 g) and minced with fine scissors in a balanced glucose–salts medium containing 5.5 m*M* glucose, 120 m*M* NaCl, 5.0 m*M* KCl, 5.0 m*M*

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