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Brain Biogenic Amines and Pituitary-Adrenocortical Function in the Rat

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MAICKEL, R. P. AND R. R. MARTEL. *Brain biogenic amines and pituitary-adrenocortical function in the rat.* PHARMACOL BIOCHEM BEHAV **19**(2) 321–325, 1983.—Treatment of rats with various doses of the monoamine oxidase inhibitor (MAOI) pargyline failed to alter plasma levels of corticosterone at 18 hours post-dosage even though brain levels of serotonin and norepinephrine were increased by 51 to 95 percent. Single pargyline doses of 17.5 or 25 mg/kg blocked the increased plasma corticosterone response to reserpine. Animals pretreated with two doses of the MAOI showed a time-dependent sedative response to reserpine. In these animals, the plasma corticosterone response to reserpine was blocked, while the responses to cold exposure or chlorpromazine were unaffected.

Biogenic amines Brain 5HT and NE Adrenal Cortex Pituitary-adrenal axis

THE control of pituitary-adrenocortical secretion at the level of the mammalian hypothalamus has been the subject of considerable research effort for more than twenty years. For example, Maickel *et al.* [15] and Westermann *et al.* [25] reported that administration to rats of single doses of reserpine and other agents capable of altering brain stores of biogenic amines such as serotonin (5HT) and norepinephrine (NE) produces an activation of pituitary-adrenocortical response similar to that of cold exposure. Subsequent studies demonstrated that administration of 5-hydroxytryptophan (5HTP) to rats results in an elevation of plasma corticosterone [18,19] and that this effect of 5HTP shows the expected stereospecificity [8].

Several other drugs have been shown to be capable of elevating plasma corticosterone levels, including the 5HT agonists quipazine [9], 1-(m-trifluoromethylphenyl)-piperazine [5], and p-chloroamphetamine [6]. In addition, the action of 5HTP can be enhanced by pretreatment of the animals with fluoxetine, a compound known to inhibit the reuptake of 5HT [7], and can be blocked by mianserin, but not by benserazide or atropine [23].

Oxotremorine is also capable of elevating plasma corticosterone levels, an action that can be blocked by atropine but not by methylatropine or mianserin [23]. Chlorpromazine elevates plasma corticosterone in a dose-related manner paralleling depression of motor activity [22], while α-methyltyrosine causes a delayed (14–16 hr) elevation of plasma corticosterone that can be blocked by clonidine [23].

Finally, Jones *et al.* [12] and Buckingham and Hodges [4] have shown that the release of corticotrophin-releasing factor (CRF) from rat hypothalamus *in vitro* is stimulated by addition of acetylcholine (ACh) or 5HT, and inhibited by addition of NE or γ-aminobutyric acid (GABA) to the incubation medium.

The present paper describes studies in which the monoamine oxidase (MAO) inhibitor, pargyline, has been used to alter brain levels of 5HT and NE in the rat prior to administration of doses of reserpine or chlorpromazine, or exposure to low environmental temperature. The results support a role for association of serotonergic and noradrenergic functions in the control of basal pituitary-adrenocortical function in the rat, presumably through control of ACTH release. An association of ACTH release, brain 5HT/NE levels, and reserpine-induced sedation is also reported; this may bear some similarity to the neuroendocrine dysfunctions reported in depressed patients [20].

METHOD

Experimental procedures were performed using unanesthetized Sprague-Dawley rats maintained in an animal care facility on a 14:10 light/dark cycle on an ad lib diet of Wayne Lab Blox and tap water for 7–10 days prior to experimental use. Control animals were dosed with distilled water. Reserpine, as the phosphate salt, was dissolved in distilled water and injected into the tail vein. Chlorpromazine and pargyline, as the hydrochlorides, were dissolved in distilled water and injected intraperitoneally. All injections were given in a volume of 1.0 ml per kg of body weight.

Animals were stunned, then immediately decapitated, and blood was collected into heparinized beakers. After transfer to tubes, the blood was centrifuged and plasma was stored at −40°C until assayed for corticosterone by the method of Guillemin *et al.* [11]. Brains were removed and stored at −40°C until 5HT and NE levels were assayed by the method of Maickel *et al.* [16]. Statistical comparisons were performed by appropriate ANOVA treatments.

TABLE 1

EFFECTS OF PARGYLINE ON PLASMA CORTICOSTERONE AND BRAIN 5HT AND NE

Treatment	N	Plasma Corticosterone μg/ml	Brain 5HT μg/g	Brain NE μg/g
None	22	0.13 ± 0.02	0.43 ± 0.04	0.47 ± 0.03
Pargyline, 10 mg/kg	10	0.13 ± 0.02	0.55 ± 0.02*	0.53 ± 0.01
Pargyline, 17.5 mg/kg	10	0.14 ± 0.02	0.64 ± 0.05*	0.57 ± 0.03*
Pargyline, 25 mg/kg	10	0.14 ± 0.02	0.71 ± 0.03*	0.61 ± 0.04*
Pargyline, 25 mg/kg (doses 18 hr apart)	20	0.13 ± 0.02	0.84 ± 0.06*	0.71 ± 0.05*

Each value is the mean ± SEM of values obtained from N rats. Measurements were made 18 hours after the last dose of pargyline. Values significantly different from controls ($p<0.05$) are indicated by *.

TABLE 2

EFFECTS OF PARGYLINE PRETREATMENT ON RESERPINE EFFECTS

Treatment: Pargyline mg/kg, IV	Treatment: Reserpine mg/kg, IV	N	Plasma Corticosterone μg/ml	Brain 5HT μg/g	Brain NE μg/g
—	—	8	0.13 ± 0.02	0.48 ± 0.04	0.46 ± 0.03
—	1.0	8	0.41 ± 0.03*	0.06 ± 0.01*	0.07 ± 0.02*
10	—	6	0.14 ± 0.03	0.57 ± 0.03	0.51 ± 0.04
10	1.0	6	0.37 ± 0.02*‡	0.24 ± 0.02*†‡	0.18 ± 0.02*†‡
17.5	—	6	0.15 ± 0.02	0.63 ± 0.03*	0.58 ± 0.03*
17.5	1.0	6	0.25 ± 0.07†	0.39 ± 0.06†‡	0.37 ± 0.06†‡
25	—	8	0.14 ± 0.02	0.70 ± 0.04*	0.61 ± 0.02*
25	1.0	8	0.15 ± 0.02†	0.57 ± 0.03†‡	0.50 ± 0.02†‡

Each value is the mean ± SEM of values obtained from N rats. Reserpine (1 mg/kg, IV) was administered at 18 hours after pargyline pretreatment, and rats were killed 6 hours after reserpine. Values significantly different from controls ($p<0.05$) are indicated by (*); those significantly different ($p<0.05$) from reserpine alone by (†); and those significantly different ($p<0.05$) from the corresponding pargyline pretreated by (‡).

RESULTS

Effects of Pargyline

The effects of various dosage regimens of pargyline on levels of plasma corticosterone and brain 5HT and NE are presented in Table 1. As can be seen, none of the pargyline treatments elevated plasma corticosterone, although resulting in elevations of 25% (10 mg/kg) to 95% (2 doses, 25 mg/kg each) in brain 5HT, and 13% (10 mg/kg) to 51% (2 doses, 25 mg/kg each) in brain NE.

Interactions of Pargyline with Reserpine

Table 2 presents the data obtained when rats pretreated with various doses of pargyline were given single doses of reserpine (1 mg/kg, IV). Reserpine alone produced the expected elevation of plasma corticosterone and decrements in brain 5HT and NE. Pretreatment with pargyline at the lowest dose (10 mg/kg) caused slight (but non-significant) elevations in both 5HT and NE, partially reversed with reserpine-induced lowering of brain amines, but had no significant effect on the elevation of plasma corticosterone produced by reserpine. Pretreatment with the largest dose of pargyline (25 mg/kg) completely blocked the reserpine-induced plasma corticosterone elevation; brain levels of 5HT and NE in these rats were lowered somewhat by reserpine, although they remained at levels slightly above those of untreated controls. The intermediate dose of pargyline (17.5 mg/kg)

TABLE 3

DIFFERENTIAL ASPECTS OF PARGYLINE-RESERPINE INTERACTION

Treatment	N	Plasma Corticosterone μg/ml	Brain 5HT μg/g	Brain NE μg/g	Degree of Sedation 3 hr	Degree of Sedation 6 hr
—	6	0.14 ± 0.02	0.44 ± 0.04	0.45 ± 0.03	None	None
Pargyline, 17.5 mg/kg	6	0.15 ± 0.03	0.65 ± 0.05	0.57 ± 0.04	None	None
Reserpine, 1.0 mg/kg	6	0.44 ± 0.04*	0.07 ± 0.02*	0.05 ± 0.02*	Deep	Deep
Pargyline + Reserpine	11	0.37 ± 0.03*‡	0.19 ± 0.01*†‡	0.16 ± 0.01*†‡	Deep	Deep
Pargyline + Reserpine	25	0.17 ± 0.01†	0.44 ± 0.03†‡	0.35 ± 0.03*†‡	None	Slight

Each value is the mean ± SEM of values obtained from N rats. Groups were selected as described in the text. Values differing significantly ($p<0.05$) from controls are indicated by (*); those differing from reserpine alone by (†); and those differing from pargyline alone by (‡).

TABLE 4

EFFECTS OF PARGYLINE ON CHLORPROMAZINE, COLD, AND RESERPINE

Pretreatment	Treatment	N	Plasma Corticosterone μg/ml	Brain 5HT μg/g	Brain NE μg/g
—	—	18	0.15 ± 0.02	0.46 ± 0.04	0.47 ± 0.03
—	Chlorpromazine	12	0.39 ± 0.02*	0.42 ± 0.03	0.48 ± 0.05
—	Cold	12	0.45 ± 0.03*	0.44 ± 0.04	0.46 ± 0.02
—	Reserpine	12	0.46 ± 0.05*	0.05 ± 0.01*	0.07 ± 0.02*
Pargyline	—	16	0.14 ± 0.02	0.83 ± 0.06*	0.75 ± 0.02*
Pargyline	Chlorpromazine	12	0.38 ± 0.03†	0.85 ± 0.05	0.77 ± 0.03
Pargyline	Cold	12	0.41 ± 0.04†	0.79 ± 0.04	0.73 ± 0.05
Pargyline	Reserpine	12	0.14 ± 0.02	0.76 ± 0.04	0.59 ± 0.05†

Each value is the mean ± SEM of values obtained from N rats. Pargyline pretreatment consisted of two doses of 25 mg/kg, IP at 18 hour intervals, with animals tested at 18 hours after the second dose. Animals were exposed to cold (4°C) for 3 hours, or given chlorpromazine (15 mg/kg, IP) and killed after 3 hours, or given reserpine (1 mg/kg, IV) and killed after 6 hours. Values differing significantly from corresponding control ($p<0.05$) are indicated by (*), those differing from corresponding pargyline pretreatment are indicated by (†).

had, as expected, effects that were midway between the other doses. Interestingly, the plasma corticosterone elevation evoked by reserpine was significantly less than that of reserpine alone, but did not differ significantly from that of pargyline alone, presumably because of the large variance. In addition, rats given the small dose of pargyline showed classical signs of reserpine administration (sedation, hunchbacked posture, ptosis) as early as 3 hours post-reserpine, while those pretreated with the largest dose of pargyline showed virtually none of the classic reserpine effects. The intermediate dose of pargyline produced two animals that were deeply sedated and four others that showed minimal signs of reserpine dosage at 3 hours.

This observation, coupled with the large variances observed in the plasma corticosterone and brain amine values, suggested the possibility of a non-homogeneous effect. Accordingly, 12 rats were pretreated with the dose of 17.5 mg/kg of pargyline, then given 1.0 mg/kg of reserpine, IV, 18 hours later. Three hours after administration of the reserpine, the animals were observed in an open field by a naive observer who had seen control and reserpine-treated rats under similar situations. The observer separated the animals into two groups ("reserpinized" and "non-reserpinized"). They were maintained for an additional 3 hours, then killed and samples assayed as usual. This experiment was repeated 3 times; the number of "reserpinized" rats were 4/12, 4/12, and 3/12. The data, in Table 3, clearly indicate that different effects occurred in the two groups. The rats that were deeply sedated (clearly "reserpinized") at 3 hours remained that way for the next 3 hours and showed elevated levels of plasma corticosterone and markedly decreased levels of brain 5HT and NE. In contrast, the rats that were virtually non-sedated ("non-reserpinized") at 3 hours showed only a slight degree of sedation at 6 hours, and had levels of plasma

corticosterone and brain 5HT and NE that did not differ significantly from normal.

Interactions of Pargyline with Chlorpromazine, Cold, Reserpine

Finally, the ability of pargyline to alter other pituitary-adrenocortical activation procedures was tested. For this purpose, a two dosage pretreatment was used, and the activation in response to a standard dose of chlorpromazine or reserpine or a standard exposure to cold was determined. As can be seen from the data in Table 4, the pargyline pretreatment had no effect on the pituitary-adrenocortical response to chlorpromazine or cold exposure, but completely blocked the response to the reserpine dosage.

DISCUSSION

The role of brain serotonergic systems in the control of the pituitary-adrenocortical system has been an area of study during two distinct periods. Westermann *et al.* [25] concluded that reserpine and similar releases of brain biogenic amines were able to evoke ACTH hypersecretion when they lowered whole brain levels of 5HT below 50% of normal. This effect seemed to be independent of changes in brain levels of NE, although one cannot discount the possibility of alterations in the balance of serotonergic/dopaminergic or serotonergic/noradrenergic functional systems [17,25].

Several papers in the past ten years have confirmed the role of brain serotonergic functions in the control of ACTH release. Jones *et al.* [12] and Buckingham and Hodges [4] showed that addition of 5HT to rat hypothalamus *in vitro* stimulated the release of CRF, the first step in pituitary-adrenocortical activation. Administration of 5HT agonists [5,9], 5HT uptake inhibitors [8], 5HT-releasing agents [6], or 5HTP [7, 8, 23] have all been shown to elevate plasma corticosterone levels, presumably by enhancing ACTH secretion.

The possible role(s) of other neuronal system, such as those involving dopamine, NE, or acetylcholine cannot be ignored. Smelik reviewed the overall problem of neurotransmitter control of ACTH release [21]. Other reviews have specifically dealt with the ability of NE or NE agonists to act as inhibitors of stress-induced ACTH secretion [10] or with possible role(s) of brain 5HT systems in the control of ACTH secretion [13,24]. Two recent reports are of more than passing interest. Berkenbosch *et al.* [2] have reported that β-adrenergic stimulation (isoproterenol infusion) elicits increases in plasma corticosterone, while Benkert *et al.* [1] found that cholinergic blockade produced by a single dose of biperiden also increases plasma glucocorticoid levels. Thus, as emphasized in a recent conference, the pituitary-adrenocortical system appears to be regulated by a conglomerate of neuronal components and mechanisms [14].

Nevertheless, the data presented herein support the concept that central control of pituitary-adrenocortical function involves some serotonergic pathways; the possible mechanism of action appears to involve an excessive amount of postsynaptic activity. Thus, reserpine-induced release of stored 5HT (in the presence of an MAOI), administration of a 5HT agonist, increased postsynaptic potency of 5HT (by blockage of reuptake), or increased 5HT (by administration of 5HTP) all result in activation of the pituitary-adrenocortical system. Of particular interest in this regard is a recent report demonstrating that brain 5HT systems are definitively involved in release of both ACTH and β-endorphin [3]. Whether other biogenic amines are also involved in these release processes remains a problem for further study.

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