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BRIEF COMMUNICATION

Plasma Corticosterone Following Alterations of Hypothalamic Catecholamines in Rats

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SIGG, E. B. AND K. L. KEIM. *Plasma corticosterone following alterations of hypothalamic catecholamines in rats.* PHARMAC. BIOCHEM. BEHAV. 4(1) 95–97, 1976. – The role of central catecholamines in the regulation of resting ACTH secretion has been investigated by relating plasma corticosterone to changes in hypothalamic catecholamines after treating rats with various amine depleting agents. The hypothesis of a noradrenergic inhibitory control is not supported by the data since a correlation between hypothalamic catecholamine content and plasma corticosterone levels could not be established.

ACTH Central catecholamines Plasma corticosterone Catecholamine depleting agents

SEVERAL reports support the hypothesis that brain catecholamines (CA) inhibit ACTH secretion [12]. The evidence centers around the findings that pargyline and amphetamine, which increase central adrenergic activity, decrease resting plasma corticosterone (CS) levels [1]. On the other hand, alpha-methyl-para-tyrosine (AMPT) and centrally injected guanethidine deplete central CA and increase plasma CS [3, 11, 13]. Thus, the inverse relationship of central CA to plasma CS as revealed by such pharmacological procedures has been interpreted as evidence for a central adrenergic system which inhibits the secretion of ACTH.

However, with repeated administration to rats of a much smaller dose of AMPT, a decrease in brain CA without a concominant increase in ACTH secretion has been observed [9]. These findings have been confirmed [5], and it was concluded that a large dose of AMPT exerts a nonspecific stress that was not found with low, multiple dosing. Also, when hypothalamic norepinephrine (NE) is depleted after intraventricular administration of 6-hydroxydopamine (6-OHDA), plasma CS does not differ significantly from those of untreated controls [2].

This controversial role of catecholamines in the regulation of resting ACTH secretion has been further investigated by relating plasma CS concentrations to hypothalamic NE and dopamine (DA) content after treating rats with various amine depleting agents.

METHOD

Animals

Fifty day-old male Sprague-Dawley rats were caged individually for 2 weeks at 23°C in a room with controlled humidity and an automated light cycle. The lights were on from 0600 to 1800 hr. The animals had free access to food and water and weighed between 280–320 g at the time of the experiment. Each group consisted of 5 animals.

Procedure

All drugs were administered IP in saline at 1600 hr. The solubility was increased by the addition of minimal amounts of 1N HCl. The following drugs were injected: methyl ester of alpha-methyl-para-tyrosine (250 mg/kg, pH 2.3), alpha-methyl-meta-tyrosine (400 mg/kg, pH 2.0), alpha-methyldopa (150 mg/kg, pH 1.8), and guanethidine (30 mg/kg, pH 2.2). Control animals were injected with saline adjusted to the appropriate pH.

The next day between 0830 and 0930, the rats were singly transferred to an adjacent room and decapitated within 20 sec. Plasma was obtained from trunk blood through siliconized funnels into tubes which contained heparin and then frozen. At a later date, corticosterone was measured fluorometrically according to the method of Mattingly [8]. A block of brain tissue extending from behind the chiasma, the lateral borders being approximately 1.5 mm from the midline and the dorsal border just above the arcuate nucleus (109 ± 6 mg), was dissected from ice-cooled brains. This sample which contained the hypothalamus was processed for differential elution from Dowex columns according to the method of Horst *et al.* [4]. NE and DA were determined fluorometrically according to the method of Laverty and Starmon [7].

Statistical analysis was performed using Student's *t*-test; a level $p<0.02$ carried significance.

TABLE 1

THE EFFECT OF CATECHOLAMINE DEPLETING AGENTS ON HYPOTHALAMIC NE AND DA, AND PLASMA CORTICOSTERONE IN RATS

	N	Hypothalamic NE μg/g	Hypothalamic DA μg/g	Plasma CS μg%
Basal control	5	2.08 ± 0.20	0.48 ± 0.05	5.7 ± 0.6
Sham control	5	1.81 ± 0.05	0.41 ± 0.02	6.3 ± 0.6
Guanethidine	5	2.00 ± 0.11	0.42 ± 0.08	5.8 ± 0.3
αMDA	5	2.01 ± 0.21	0.53 ± 0.07	25.6 ± 4.6
αMPT	5	0.49 ± 0.11	0.12 ± 0.01	50.2 ± 1.6
αMMT	5	0.38 ± 0.04	0.54 ± 0.05	33.5 ± 4.9

RESULTS

The results, summarized in Table 1, indicate that plasma CS levels from sham-injected controls did not differ statistically from those of noninjected, basal controls. Hypothalamic NE and DA levels tended to be lower in sham-injected rats than those in the basal control group, though this difference did not reach statistical significance. The guanethidine pretreated rats did not differ from the sham-injected controls.

When compared to sham-injected animals, treatment with alpha-methyl-dopa (AMDA) increased the resting CS titers four fold ($p<0.01$), but did not statistically alter the hypothalamic content of either amine. In contrast, AMPT elevated CS levels almost ten-fold over sham-injected values ($p<0.001$) concomitant with a marked depletion of both hypothalamic NE and DA ($p<0.001$). AMPT caused a marked reduction in the content of hypothalamic DA ($p<0.001$). The administration of alpha-methyl-metatyrosine (AMMT), which increased resting CS considerably less than did AMPT ($p<0.02$), lowered hypothalamic NE even more while not affecting hypothalamic DA. However, the AMMT induced depletion of hypothalamic NE was not significantly different from that caused by AMPT.

DISCUSSION

The finding that AMPT administration depleted hypothalamic CA and elevated plasma CS is in accordance with the results of others [3, 10, 13]. However, our results treating rats with other agents known to lower brain biogenic amines are not consistent with the AMPT model which sustains the inverse relationship between hypothalamic CA and plasma CS. Thus, AMDA increases plasma corticosterone almost to the same extent as AMMT but, in contrast to the latter, does not deplete hypothalamic NE. Moreover, AMMT depletes hypothalamic NE by 79% from control, increasing plasma CS 5.3 times over control, while AMPT depletes hypothalamic NE by 73% from control increasing plasma CS 8.2 times over control.

The finding that, in contrast to AMMT and AMDA, AMPT depletes hypothalamic DA raises the question whether DA contributes to a possible inhibitory biogenic amine control of ACTH secretion.

In conclusion, there is no convincing evidence that low brain NE, per se, is functionally correlated with ACTH secretion when measured by an elevated resting plasma CS level.

The assumption of a noradrenergic inhibitory control is contradicted by these data as well as by the finding that intraventricular injection of 6-OHDA, which depletes both whole brain NE and DA, does not alter the resting concentration of plasma CS [2,6]. Also, it should be kept in mind that tissue concentrations of amines may not be indicative of their physiological role. The significance of the relationship between transmitter availability to the receptor site which controls ACTH secretion has yet to be evaluated.

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