

CENTRAL EPINERGIC* INHIBITION OF CORTICOSTERONE RELEASE IN RAT

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Summary

Drugs known to inhibit phenylethanolamine-N-methyltransferase (EC 2.1.1.28), the final enzyme in the epinephrine synthetic pathway were administered to rats and their effects on pituitary-adrenal function studied. 2,3-Dichloro- α -methylbenzylamine (DCMB) produced dose-related increases in plasma corticosterone in basal and stressed rats. Evidence for the central nature of this tonic inhibitory effect of epinephrine was its continued presence in adrenal demedullated rats. 2-Chloro-3-trifluoromethyl- α -benzylamine (CTFMB) administration elevated plasma corticosterone and this effect was highly correlated to the decrease in hypothalamic epinephrine concentration in both sham operated and adrenal demedullated rats. These findings argue for tonic epinephrine inhibition of pituitary-adrenal function.

Introduction

The mammalian central nervous system is known to possess cells containing phenylethanolamine-N-methyltransferase (PNMT; EC 2.1.1.28), the enzyme that converts norepinephrine to epinephrine, as well as epinephrine itself (1,2,3, 4,5). Several possible functions for epinephrine neurons in physiology and behavior have been suggested, including vasomotor control, sympathetic regulation, stress regulation (6,7) and neuroendocrine regulation of growth hormone release (8). Adrenergic neurons may possess more general neuroendocrine properties, however. One or more catecholamines normally inhibits the secretion of adrenocorticotrophic hormone in rats, dogs, and primates (9). The direct injection of norepinephrine or epinephrine into the third ventricle of pentobarbital-anesthetized dogs produced an inhibition of corticoid output in response to surgical stress (10). The existence of pharmacological

*The term "epinephrine neuron" is defined as a neuron containing the enzymes in the catecholamine pathway, including phenylethanolamine-N-methyltransferase as well as epinephrine itself. The term epinephrine is used to avoid the ambiguity of the term adrenergic neuron which has alternatively been defined as a neuron containing either catecholamines (i.e., norepinephrine or epinephrine) or a neuron containing epinephrine.

agents which are centrally active inhibitors of PNMT allows investigation of the role of epinephrine neurons in pituitary-adrenal function. Indeed, experiments with the PNMT inhibitor SKF 64139 have been suggested but no results have as yet been published (10). However, experiments with this particular PNMT inhibitor may be difficult to interpret since SKF 64139 is a potent monoamine oxidase inhibitor as well as possessing α_2 -receptor-blocking properties (11). The PNMT inhibitors 2,3-dichloro- α -methylbenzylamine (DCMB) and 2-chloro-3-trifluoro-methyl- α -benzylamine (CTFMB) do not show MAO inhibitor properties (12) and to our knowledge are not α_2 antagonists. These two compounds were therefore used to investigate the possible contributions of PNMT containing neurons to normal pituitary-adrenal regulation. Plasma corticosterone, and in some experiments hypothalamic catecholamines, were examined following PNMT inhibition.

Materials and Methods

Adult male Sprague-Dawley rats (Charles River Farms), each 70 days at the start of testing were group-housed with food (Teklad 4.0 percent fat rodent diet S-0836) and tap water available, and normal 12 h/12 h lighting (lights on 0800-2000h).

Experiments began between 0800 and 1000 h. Rats were briefly removed and injected intraperitoneally with 0.9 percent sodium chloride vehicle or 2,3-dichloro- α -methylbenzylamine (DCMB) or 2-chloro-3-trifluoromethyl- α -benzylamine (CTFMB). Additional experiments employed subgroups of adrenal demedullated or surgical control rats, prepared 3 weeks prior to the experiments by standard surgical procedures (13), and rats individually exposed to 95 dB of white noise for 1 h prior to sacrifice according to procedures developed within our laboratory (14).

All rats were sacrificed by decapitation 4 hours postinjection. Time between final cage removal and sacrifice was less than 30 sec. Trunk blood was collected in heparinized tubes and subsequently centrifuged at 2400 RPM for 30 min. Plasma was removed and frozen at -40° until assayed by competitive protein binding assay by the method of Murphy (15). Corticosterone was employed as the assay standard and determinations were made in triplicate. Within assay, coefficients of variance were less than 5 percent.

Brains were removed and immediately frozen on dry ice and stored in small containers at -40°C until dissected. Brains were allowed to partially thaw on a chilled glass plate resting on a bed of ice and hypothalami were removed and placed in 1.5 ml polypropylene tubes and stored on dry ice until assayed. The concentrations of hypothalamic epinephrine, norepinephrine, dopamine, and dihydroxyphenylacetic acid (DOPAC) were measured using ion pairing reverse phase HPLC with electrochemical detection as previously described (16).

Statistical comparisons utilized univariate analysis of variance (17).

Results

Both drugs produced a dose related increase in plasma corticosterone. At the higher dose, a 3-4-fold increase in circulating steroid above baseline levels was observed. A highly significant inverse correlation was found between hypothalamic epinephrine and plasma corticosterone concentrations. This effect was seen in both surgical control and adrenal demedullated rats. The hypothalamic concentrations of norepinephrine, dopamine, and DOPAC were not related to plasma corticosterone concentrations in either group.

Figure 1 displays the effects of DCMB ($F=8.9$; $df=2,27$; $p < .001$) and CTFMB (Part B) on plasma corticosterone. It may be seen that significant increases occur in both surgical control and adrenal demedullated rats ($F=2.9$; $df=5,28$; $p < .003$). CTFMB produced a dose related decrease in hypothalamic epinephrine in control ($F=6.1$; $df=2,14$; $p < .05$) and adrenal demedullated rats ($F=5.0$; $df=2,13$; $p < .05$). Hypothalamic norepinephrine, dopamine and DOPAC were not affected by CTFMB. These results are presented in Table 1. The relationship between hypothalamic epinephrine and plasma corticosterone can be seen in Figure 2.

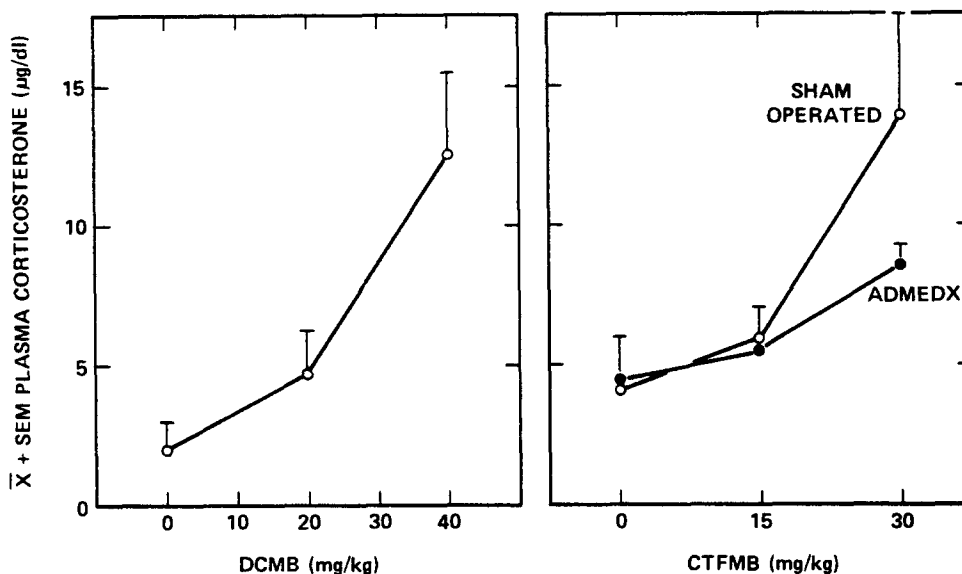


Figure 1.

DCMB or CTFMB was administered IP to control or adrenal demedullated rats four hours prior to blood collection. The PNMT inhibitors produced a dose related rise in plasma corticosterone.

To further examine the influence of PNMT inhibition upon corticosterone, rats were examined in a factorial study involving noise stress and an intermediate level of DCMB. In Table 2 it may be seen that both DCMB and stress increase plasma corticosterone and their combined effect was an additive one. Thus, two PNMT inhibitors increased corticosterone. A significant increase also occurred in the absence of the adrenal medulla, and it occurred in a manner that did not appear to depend upon stress.

Discussion

The present findings suggest that epinergic neurons are involved in the tonic inhibitory control of plasma corticosterone in the rat. These findings appear to depend upon central epinephrine, adrenal demedullated rats showed increased corticosterone after PNMT inhibition. The lack of effect of CTFMB, at the doses tested, on hypothalamic norepinephrine, dopamine, and DOPAC is evidence for the specificity of this compound in inhibiting epinephrine synthesis. We have reported that the PNMT inhibitor SKF 64139 is a potent *in vivo* MAO inhibitor (11), however CTFMB and DCMB do not appear to have this property (12).

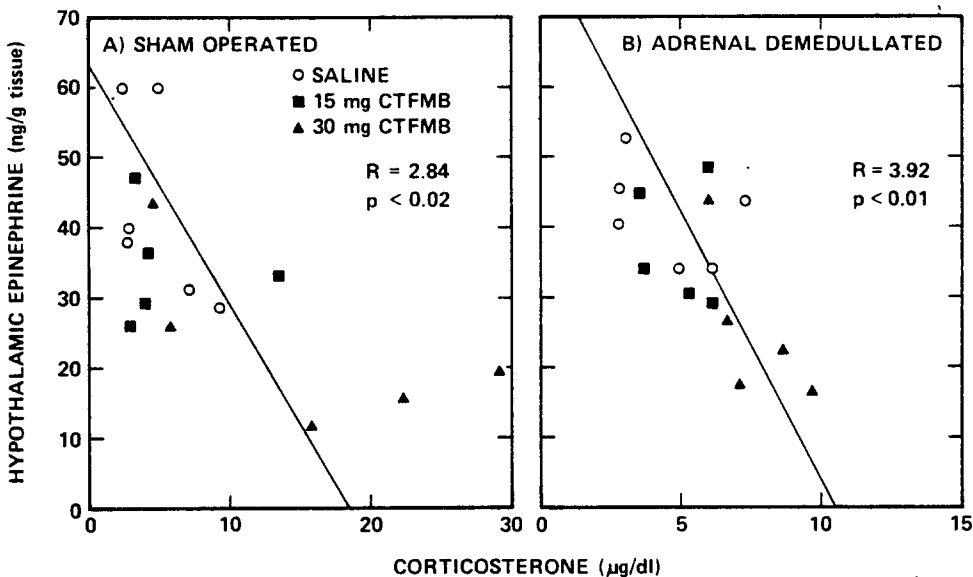


Figure 2.

Various doses of CTFMB were administered to sham operated (a) or adrenal demedullated (b) rats. In both groups the concentration of hypothalamic epinephrine was inversely related to plasma corticosterone concentration.

TABLE 1.

HYPOTHALAMIC CATECHOLS $\bar{X} \pm$ SEM
ng/g tissue

SHAM	EPINEPHRINE	NOREPINEPHRINE	DOPAMINE	DOPAC
0 mg/kg CTFMB (6)	48.6 \pm 6.9	2432 \pm 169	389 \pm 39	159 \pm 18
15 mg/kg CTFMB (6)	33.8 \pm 3.2	2374 \pm 110	392 \pm 25	120 \pm 12
30 mg/kg CTFMB (5)	25.3 \pm 6.2	1957 \pm 226	455 \pm 39	152 \pm 15
F =	6.10	2.71	0.32	2.67
df =	2,14	2,14	2,14	2,13
p <	.05	NS	NS	NS
ADMX	EPINEPHRINE	NOREPINEPHRINE	DOPAMINE	DOPAC
0 mg/kg CTFMB (6)	42.1 \pm 3.1	2282 \pm 130	462 \pm 29	209 \pm 11
15 mg/kg CTFMB (5)	37.6 \pm 4.4	2654 \pm 314	490 \pm 57	179 \pm 08
30 mg/kg CTFMB (5)	25.5 \pm 5.4	2263 \pm 235	474 \pm 43	169 \pm 23
F =	4.96	1.12	0.14	2.65
df =	2,13	2,13	2,13	2,13
p <	.05	NS	NS	NS

TABLE 2.

Mean Corticosterone (+ SEM) Following Stress
and Drug Treatment in the Rat

Drug:	Stress	
	Basal	Acute Stress
NACL	2.1 ± 0.5	35.3 ± 2.7 ^a
DCMB 20 mg/kg	3.7 ± 0.4 ^a	39.5 ± 2.4 ^{a,b}

a) $p < .05$ in comparison with saline control (t test for independent samples)

b) $p < .05$ in comparison with DCMB control (t test for independent samples).

The argument could be raised that CTFMB and DCMB produce an irritation which leads to an increased corticosterone concentration due to a stress response. This is very unlikely for several reasons: 1) stress produces a rise in hypothalamic DOPAC levels (18), this response was not found after PNMT inhibition; 2) stress may interact with PNMT inhibition to decrease the concentration of hypothalamic norepinephrine (19), this did not occur in the present experiment; 3) the "irritant" effect would need to be closely correlated to the ability of CTFMB to lower hypothalamic epinephrine as hypothalamic epinephrine concentrations were shown to be inversely related to plasma corticosterone concentrations; 4) the elevation in plasma corticosterone by DCMB was still present when rats were exposed to a 95 dB white noise stress, a procedure which also elevates corticosterone levels. On balance the possibility that these findings are due to a nonspecific irritant effect is unlikely.

Since elevated corticosteroids, and ACTH, are of physiological and psychiatric significance, the present findings may shed light upon the regulation of normal corticosterone release and also upon disease states characterized by elevated steroid levels. This final point of course assumes some degree of commonality in the endocrine system of rats and other species.

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