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COMPARISON OF THE EFFECTS OF NEUROTENSIN AND ACTH ON THE PITUITARY–ADRENOCORTICAL AXIS OF DEXAMETHASONE-SUPPRESSED RATS

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Summary—Neurotensin (NT) (12–48 $\mu g/kg^{-1}/day^{-1}$, for 2 days, s.c.), like ACTH (60 $\mu g/kg^{-1}/day^{-1}$, for 2 days, s.c.), counteracted the dexamethasone (Dx)-induced (120 $\mu g/kg^{-1}/day^{-1}$, for 4 days, s.c.) adrenal zona-fasciculata cell atrophy. NT notably raised, in Dx-suppressed rats, the plasma concentration of ACTH, which reached about that found after exogenous ACTH administration. However, at variance with ACTH, NT did not enhance either plasma corticosterone (B) level or B production by adrenal quarters *in vitro*. The conclusion is drawn that NT modulates the function of the rat pituitary–adrenocortical axis, by simultaneously stimulating hypophyseal ACTH release and inhibiting steroidogenesis at the adrenal level.

INTRODUCTION

The acute central administration of neurotensin (NT) raises blood levels of ACTH and corticosterone (B) in the rat [1, 2]. The increase in serum B concentration does not depend on the activation of tuberoinfundibular dopamine neurons, since it is not prevented by haloperidol [3]. On the other hand, prolonged systemic administration of very high doses of NT, despite a marked increase in the blood level of ACTH, had no effect on B secretion by adrenal glands of intact or dexamethasone (Dx)-suppressed rats, and evidence is available of a direct inhibitory action of this neuropeptide on basal B production by isolated rat adrenocortical cells [4]. The aim of the present investigation was to compare the effects of NT and ACTH on the pituitary–adrenocortical axis of Dx-suppressed rats.

EXPERIMENTAL

Adult female Wistar rats (120–150 g body wt) were given daily s.c. injections of 120 $\mu g/kg^{-1}$ Dx (Decadron; Merck, Milan, Italy) for 4 days. For the last 2 days, groups of rats also received s.c. injections of NT (Sigma, St Louis, MI, U.S.A.) at a dose of 12, 24 or 48 $\mu g/kg^{-1}$, or ACTH ($ACTH_{1-24}$ depot; Ciba, Milan, Italy) at a dose of 60 $\mu g/kg^{-1}$. The rats were decapitated between 9 and 10 a.m., 24 h after the last injection, and their trunk blood was collected and stored at −20°C until hormonal assays. Pituitary and adrenals were promptly removed. The posterior and intermediate lobes were separated and the weight of adenohypophysis was recorded. Adrenals were freed of adherent fat, and immediately processed for morphometry or biochemical assay.

Morphometry

The left adrenal gland was fixed in Bouin's solution, embedded in paraffin and serially sectioned at 6 μm. Sections were stained with haematoxylin–eosin, and on them the average volume of zona fasciculata (ZF) cells was estimated by stereology [5], as described elsewhere [6].

B secretion by adrenal slices

The right adrenal gland was quartered and preincubated for 30 min at 37°C in 1 ml Krebs–Ringer bicarbonate buffer with 0.3% glucose (KRBG). The incubation medium was discarded and new KRBG with 0.3% bovine serum albumin (Fraction V; Sigma) was added. After 30 min of incubation at 37°C, medium was

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Table 1. Effect of NT and ACTH on body, adenohypophysis and adrenal weights, and average volume of ZF cells

	Body wt (g)	Adenohypophysis wt (g)	Adrenal wt (mg)	ZF-cell vol. (μm^3)
Controls	127 ± 4	6.9 ± 0.4	30.9 ± 1.7	1445 ± 71
NT (12 $\mu g/kg^{-1}$)	133 ± 6	6.3 ± 0.6	37.6 ± 4.1	1478 ± 66
NT (24 $\mu g/kg^{-1}$)	143 ± 8	7.7 ± 0.8	37.3 ± 3.5	1686 ± 62[a]
NT (48 $\mu g/kg^{-1}$)	134 ± 5	7.6 ± 0.4	35.2 ± 2.1	2065 ± 144[b]
ACTH (60 $\mu g/kg^{-1}$)	125 ± 6	6.3 ± 0.6	43.0 ± 2.8[c]	2230 ± 98[d]

Data are group means ± SE ($n = 6$).
[a] $P < 0.05$, [b] $P < 0.02$, [c] $P < 0.01$ and [d] $P < 0.001$ vs controls.
Values of Dx-not-injected rats were: body wt 138 ± 6; adenohypophysis wt 7.0 ± 0.6; adrenal wt 42.1 ± 3.0 and ZF-cell vol. 2318 ± 87.

collected and stored at −20°C until B assay. Details of the procedure were published elsewhere [7].

Hormonal assays

ACTH was quantitated in unextracted plasma by RIA using a commercial kit (RIA-mat ACTH; Malinckrodt Diagnostica; St Louis, MI, U.S.A.). Cross-reactions with α-MSH, β-endorphin, β-lipotropin and other hypophyseal hormones were < 0.001%; detection limit: 18 pg/ml^{-1}; intra- and inter-assay variations: 6 and 8%, respectively. B was measured in plasma and incubation medium by competitive protein-binding, using Dx-pretreated dog plasma as a source of binding globulin [8]. Intra- and inter-assay variations: 5 and 7%, respectively.

Statistics

Results were averaged per experimental group and SE was calculated. The statistical comparison of the data was done by ANOVA followed by the unpaired Student's t-test.

RESULTS AND DISCUSSION

After NT administration the adrenal weight was higher than in Dx-only treated rats, however differences were not statistically significant. ACTH markedly increased the weight of the gland (40%). None of the treatments had an effect on the weight of the adenohypophysis. NT at a dose of 24 or 48 $\mu g/kg^{-1}$ notably reduced (36 and 84%, respectively) and ACTH almost completely annulled Dx-induced ZF-cell atrophy (Table 1). NT strikingly enhanced plasma ACTH level (from 2- to 4-fold), and this effect was observed with all doses of neuropeptide applied. Conversely, NT did not affect either plasma B level or B production by adrenal quarters. The latter parameters were increased by ACTH (3- and 2-fold, respectively) (Table 2).

These findings indicate that NT increases plasma ACTH concentration in Dx-pretreated rats, and partly prevents Dx-induced ZF-cell atrophy, without stimulating B secretion by adrenal cortex. Thus NT effects on adrenal cortex differ markedly from those of exogenous ACTH, which in Dx-pretreated animals increases both the volume of ZF cells and their steroidogenic capacity.

Dx is well known to inhibit CRH and POMC-derived peptide synthesis in the hypothalamus and pituitary gland, respectively [9–11], thus leading to atrophy of the adrenal cortex and reduction of its steroidogenic machinery, see [12], for review. Since corticosteroids and Dx are known to inhibit compensatory or stress-induced increase in ACTH secretion [13–15], the stimulatory effect of NT on ACTH secretion must depend on the central activation of a Dx-independent pathway or on a "break through" the incompletely established blockade.

Table 2. Effect of NT and ACTH on plasma concentrations of ACTH and B, and B secretion by adrenal quarters

	Plasma ACTH concn (pM/ml^{-1})	Plasma B concn (ng/ml^{-1})	B secretion by adrenal quarters ($ng/mg^{-1}/h^{-1}$)
Controls	24.8 ± 4.1	27.0 ± 9.1	9.2 ± 1.4
NT (12 $\mu g/kg^{-1}$)	70.4 ± 6.2[a]	35.6 ± 12.1	10.6 ± 1.2
NT (24 $\mu g/kg^{-1}$)	52.5 ± 9.3[b]	34.5 ± 5.3	12.0 ± 1.6
NT (48 $\mu g/kg^{-1}$)	97.9 ± 22.0[c]	29.1 ± 4.3	11.8 ± 0.5
ACTH (60 $\mu g/kg^{-1}$)	71.2 ± 18.7[b]	89.8 ± 22.0[b]	22.6 ± 3.2[c]

Values of Dx-not-injected rats were: plasma ACTH concn 49.5 ± 7.3; plasma B concn 180.5 ± 17.2 and B secretion by adrenal quarters 48.5 ± 6.1.
[a] $P < 0.001$; [b] $P < 0.05$; [c] $P < 0.01$.

We observed conspicuous differences as far as NT and ACTH effects on adrenal weight, average volume of ZF cells and B secretion. As mentioned above, NT is able to prevent Dx-induced adrenal atrophy, but, at variance with ACTH, it is not able to counteract the reduction in their steroidogenic capacity. It is difficult to explain this finding, inasmuch as it is generally accepted that the volume of adrenocortical cells parallels their secretory capacity, see [12] for review. Searching the possible causes of this discrepancy, a direct effect of NT on rat adrenal cortex must be considered. Indeed, high levels of specific binding sites for NT have been demonstrated in the inner layers of the rat adrenal cortex [16], and a clear-cut inhibitory effect of NT on the basal B secretion by isolated rat adrenocortical cells has also been reported [4]. Our findings, showing that in NT-treated rats, despite a high level of circulating ACTH, adrenal B secretion remains constant, are in keeping with a direct inhibitory effect of NT on adrenal steroid synthesis. In light of these considerations, it seems conceivable to assume that elevated, due to NT administration, plasma ACTH levels in Dx-treated rats, though being able to prevent adrenocortical atrophy, are not sufficient to overcome the Dx-NT-induced blockade of steroidogenic capacity of the cortex.

In conclusion, our study suggests a 2-fold opposite action of NT on the two main components of the rat hypophyseal–adrenocortical axis: simultaneous central stimulation of ACTH release and direct inhibition of steroidogenesis at the adrenal level.

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