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The role of neuromedin B in the regulation of rat pituitary-adrenocortical function

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Summary. The effects of a 7-day administration of neuromedin B (NMB) and/or (Tyr[4], D-Phe[12])-bombesin, an NMB-receptor antagonist (NMB-A) on the function of pituitary-adrenocortical axis were investigated in the rat. NMB raised the plasma concentration of aldosterone, without affecting that of ACTH or corticosterone; the simultaneous administration of NMB-A prevented the effect of NMB. Neither NMB nor NMB-A treatments induced significant changes in adenohypophysis and adrenal weights, nor in the average volume of zona glomerulosa and zona reticularis cells. NMB-A administration lowered the volume of zona fasciculata cells, an effect annulled by the concomitant NMB administration. Our results suggest that NMB specifically stimulates aldosterone secretion, and that endogenous NMB or NMB-like peptides exert a tonic stimulating action on the growth of zona fasciculata cells.

Key words: Neuromedin B, Adrenal cortex, ACTH, Steroid secretion, Rat

Introduction

Gastrin-releasing peptide (GRP) and neuromedin B (NMB) are the only bombesin (BM)-like peptides so far identified in mammals. Three receptors of BM-like family have been discovered in mammals: GRP-preferring receptor, NMB-preferring receptor and orphan receptor (for review, see Kroog et al., 1995). Neuropeptides of BM family, among them GRP and NMB, are localized in all the components of the hypothalamo-pituitary-adrenal axis, and this suggests their involvement in the regulation of this system (for review, see Malendowicz and Markowska, 1994).

The aim of the present study was to investigate the role of endogenous NMB in the regulation of rat pituitary-adrenocortical axis. To achieve this goal rats were treated with both NMB and (Try[4], D-Phe[12])-BM, a NMB-receptor antagonist (NMB-A) (Kroog et al., 1995).

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Materials and methods

Experimental procedure

Adult female Wistar rats (200±20 g body weight) were kept under a 12:12 h light-dark cycle (illumination onset at 8:00 a.m.) at 23 °C, and maintained on a standard diet and tap water *ad libitum*. The rats were divided into equal groups (n=8), which were subcutaneously injected daily with NMB, NMB-A or NMB plus NMB-A (Bachem, Bubendorf, Switzerland) dissolved in 0.2 ml 0.9% NaCl, for 7 consecutive days. The dose was 1 nmol/100 g body weight. Control group received injection of the saline vehicle. The rats were decapitated 60 min after the last injection, the trunk blood was collected, plasma separated and stored at -30 °C. Adrenal and pituitary glands were promptly removed; under the dissecting microscope neural and intermediate lobes were separated from adenohypophysis. The gland weights were recorded.

Biochemical assays

ACTH plasma concentration was measured by RIA, using a commercial kit (ACTH-RIA kit; Cis Bio International, Gift-sur-Yvette, France). Aldosterone (ALDO) and corticosterone (CORTI) were extracted from plasma and purified, and their concentrations assayed as previously detailed (Malendowicz et al., 1993). Intra- and interassay variation coefficients were: ACTH, 6% and 8%; ALDO, 5% and 7%; and CORTI, 7% and 9% respectively.

Histology and morphometry

Adrenal glands were fixed in Bouin's solution, embedded in paraffin and serially cut at 5-6 μm. Sections were stained with hematoxylin-eosin, and the average volume of the zona glomerulosa (ZG), zona fasciculata (ZF) sand zona reticularis (ZR) cells was

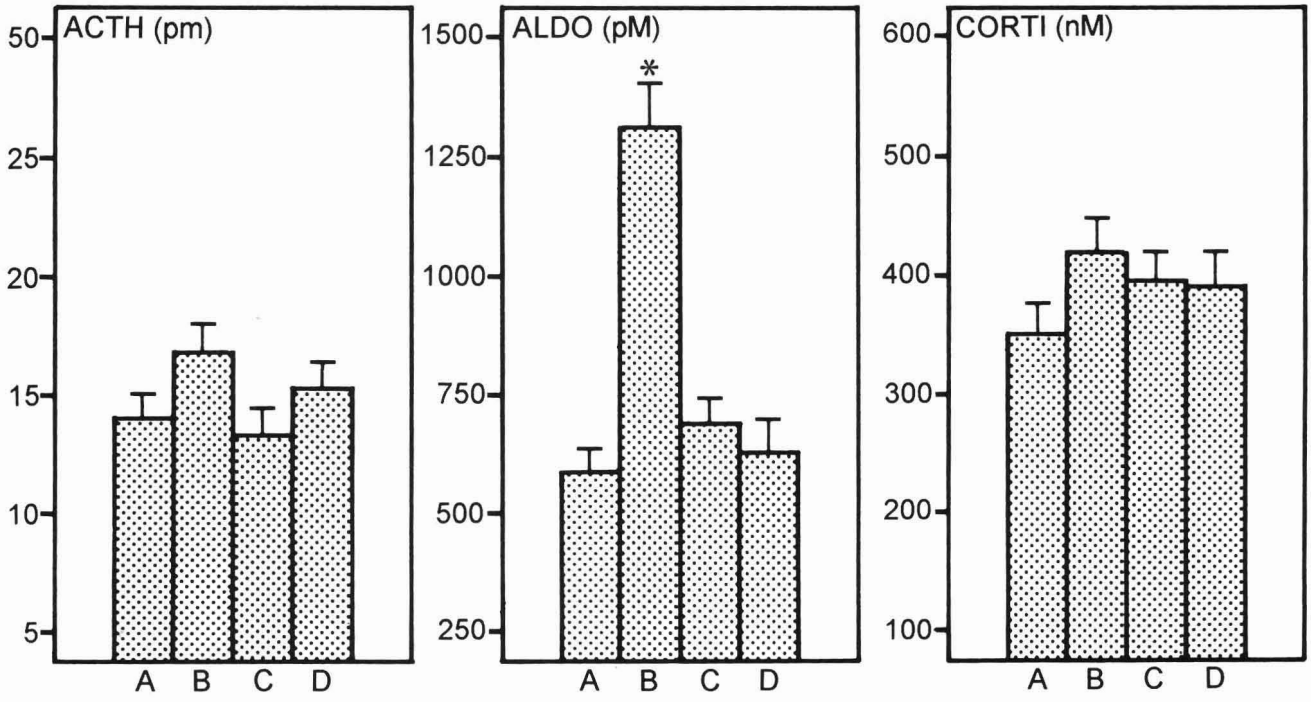

Fig. 1. Effect of 7-day NMB, NMB-A, and NMB plus NMB-A administration on the plasma concentration of ACTH, aldosterone (ALDO) and corticosterone (CORTI) in adult rat (means±SEM; n=8) A: Controls; B: NMB; C: NMB-A; D: NMB plus NMB-A. As compared with A group, *p<0.01.

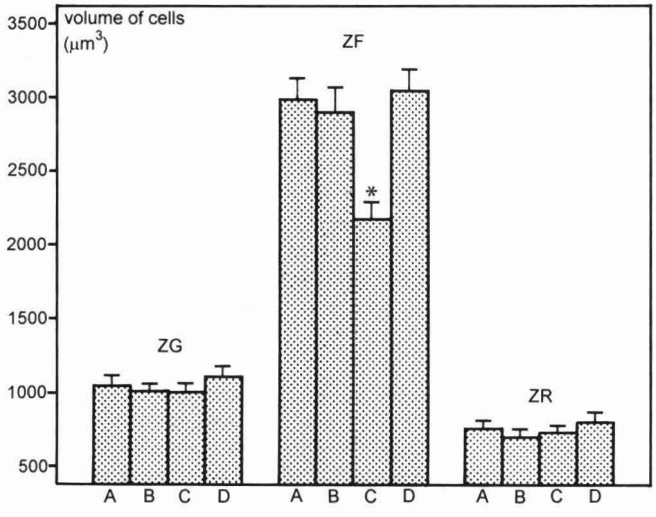

Fig. 2. Effect of 7-day NMB, NMB-A, and NMB plus NMB-A administration on the average volume of adrenocortical cells in adult rats (means±SEM; n=8) A: Controls; B: NMB; C: NMB-A; D: NMB plus NMB-A. As compared with A group, *p<0.01.

calculated according to Weibel (1979), as previously detailed (Malendowicz, 1987).

Statistical analysis

Individual results were averaged per experimental group, and SEM was calculated. The statistical comparison of the data was done by ANOVA, followed by the Multiple Range Test of Duncan.

Results

In all the experimental groups, the plasma levels of ACTH and corticosterone did not evidence any significant change. NMB markedly raised the blood concentration of aldosterone (2.2-fold), and this effect was annulled by the simultaneous injection of NMB-A (Fig. 1).

Neither NMB nor NMB-A treatments affected adenohypophyseal and adrenal weights (they ranged from 9.6 to 11.5, and 58.4 to 61.8 mg, respectively), nor the average volume of ZG and ZR cells (Fig. 2). NMB-A administration significantly lowered the volume of ZF cells (-27%), an effect prevented by the concomitant NMB injection (Fig. 2).

Discussion

Available data indicate that BM-like peptides exert manifold effects on the hypothalamo-pituitary-adrenal axis. They appear to potentiate CRH-stimulated ACTH release (Hale et al., 1984; Familiari et al., 1987), and to enhance or have no effect on blood ACTH concentration (Watanabe and Orth, 1988; Gunion et al., 1989; Olsen et al., 1992; Malendowicz and Nussdorfer, 1995). According to Malendowicz and Markowska (1994), the hypothalamo-pituitary effects of BM-like peptides may

depend upon the route of administration (intracerebroventricular *versus* systemic injection), and the dose and the time elapsed from the injection (acute *versus* chronic experiments).

Our present findings show that the long-term systemic administration of NMB and its receptor antagonist does not affect pituitary ACTH secretion in the rat, a finding consistent with our earlier studies (Malendowicz et al., 1994, 1995a,b) and in keeping with the lack of changes in the adrenal weight. Also, the lack of effect of the prolonged treatment with the two peptides on corticosterone secretion agrees well with the above mentioned results.

The two new findings of this study concern the effects of NMB on aldosterone secretion and ZF-cell growth. The prolonged NMB treatment increases the plasma concentration of aldosterone, and the specificity of this action seems to be proved by its prevention by the simultaneous administration of NMB-A. Since the administration of NMB-A alone does not evoke any appreciable change in aldosterone secretion, it appears reasonable to suppose that the aldosterone secretagogue effect of NMB may have only a pharmacological relevance.

Earlier morphometric studies showed that BM infusion for 1-4 days induced a transient rise in the average volume of ZF and ZR cells (Malendowicz et al., 1991, 1995b). Here, we observed that after a 7-day administration of NMB the volume of ZF cells remains unchanged. However, the treatment with NMB-A alone significantly decreases ZF-cell volume, and this effect is prevented by the concomitant administration of NMB. Hence, this finding strongly suggests that, under normal conditions, NMB exerts a tonic stimulating effect on the growth of ZF cells, which, however, is uncoupled with the maintenance of their glucocorticoid secretory capacity.

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