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Effects of pneumadin (PNM) on the adrenal glands. 5. Potent stimulating action of PNM on adrenocortical growth of dexamethasone-administered rats

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Summary. Pneumadin (PNM) is a biologically active decapeptide, originally isolated from mammalian lungs, that has been previously found to acutely stimulate pituitary-adrenocortical axis in rats. The effects of 2-day PNM administration on the atrophic adrenal cortices of rats treated for 8 days with dexamethasone (DX) were investigated. PNM significantly raised adrenal weight and the average volume of adrenocortical cells. The decapeptide strikingly increased ACTH plasma concentration; however, the blood levels of aldosterone and corticosterone, as well as steroid output by adrenal quarters were not apparently affected. In light of these findings the following conclusions can be drawn: (i) PNM enhances the growth of adrenal cortex in DX-administered rats by a mechanism involving the stimulation of ACTH release; and (ii) PNM treatment is probably too short to allow DX-atrophied adrenocortical cells to re-acquire all their differentiated secretory capacities.

Key words: Pneumadin, Adrenal cortex, Pituitary-adrenal axis, Steroidogenesis, Rat

Introduction

Pneumadin (PNM), a biologically active decapeptide originally isolated from mammalian lungs (Batra et al., 1990), was found to stimulate arginine-vasopressin (AVP) release in rats, thus evoking a potent antidiuretic effect (Batra et al., 1990; Watson et al., 1995). PNM does not affect basal steroid secretion of dispersed rat adrenocortical cells (Markowska et al., 1995a), but it enhances the blood levels of both aldosterone (ALDO) and corticosterone (B) in rats (Markowska et al., 1995b). Since these *in-vivo* effects are annulled by the simultaneous administration of an AVP receptor antagonist, it has been suggested that the adrenocortical secretagogue action of PNM is mediated by the enhanced release of AVP by neurohypophysis (Markowska et al., 1995c). Neurotensin, another biologically active neuropeptide which is known to enhance AVP release (for review, see Malendowicz, 1993), has been shown to stimulate pituitary-adrenal axis in rats and to prevent the dexamethasone (DX)-induced adrenocortical atrophy (Malendowicz et al., 1991a,b; Lesniewska et al., 1992b; Mazzocchi et al., 1993).

It therefore seemed worthwhile to investigate whether PNM was able to exert a stimulating effect on the growth and secretory capacity of the adrenal cortex of DX-suppressed rats.

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Materials and methods

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 animals (n=16) were given daily subcutaneous (s.c.) injections of 20 µg/100 g body weight of DX (Decadron; Merck, Miland, Italy) for 8 consecutive days. As expected, this treatment caused a complete atrophy of the adrenal cortex, due to the prolonged ACTH suppression (Lesniewska et al., 1992a): with respect to the saline-injected rats, adrenal weight and ACTH plasma concentration underwent 50% and 70% decreases, respectively. For the last 2 days of DX administration a group of rats (n=8) also received s.c. injections of 1.5 nmol/100 g body weight of PNM (rat; Bachem, Bubendorf, Switzerland). The rats were decapitated 12 h after the last injection, the trunk blood was collected, plasma was separated and stored at -30 °C. Adrenal glands were promptly removed and freed of adherent fat; their weights were recorded.

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

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

The right adrenal glands were 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 (Sigma, St. Louis, MO, USA) was added (Lesniewska et al., 1990). After 120 min of incubation at 37 °C, with continuous shaking, medium was collected and stored at -30 °C.

ACTH plasma concentration was measured by RIA, using a commercial kit (RIA-ACTH, Malinckrodt Diagnostica, St. Louis, MO, USA). ALDO and B were extracted from plasma and incubation medium and purified, and their concentrations assayed as previously detailed (Malendowicz et al., 1993). Intra- and interassay variations were: ACTH, 6% and 8%; ALDO, 5% and 7%; and B, 7% and 9%, respectively.

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

Results

PNM administration did not affect body weight, but it significantly increased adrenal weight (52%) (Fig. 1). This last effect was coupled with rises in the average volumes of ZG (13%), ZF (49%) and ZR cells (38%) (Fig. 2).

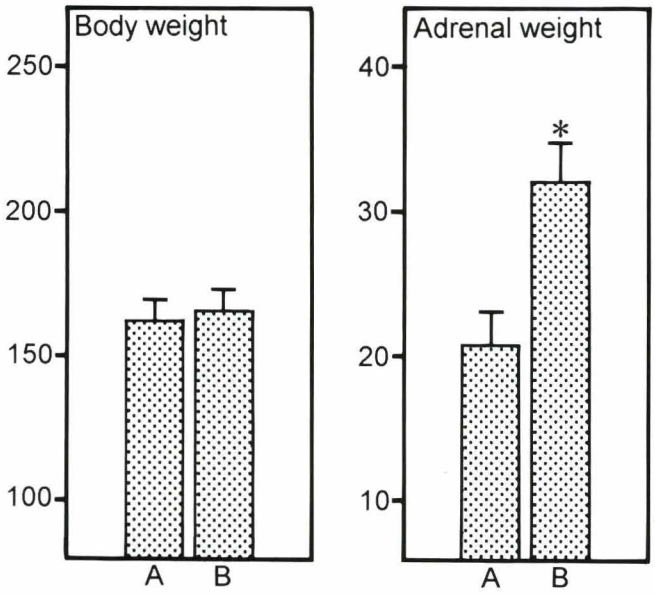

Fig. 1. Effect of PNM on body (g) and adrenal (mg/100 g body weight) weights of DX-administered rats. A: control rats; B: PNM-treated rats. Bars are means±SE (n=8). *: p<0.01 from control group.

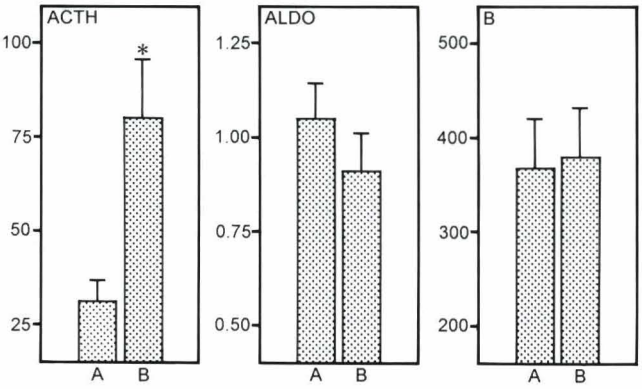

Fig. 3. Effect of PNM on the plasma concentration of ACTH (pM), ALDO (nM) and B (nM) of DX-administered rats. A: control rats; B: PNM-treated rats. Bars are means±SE (n= 8). *: p<0.01 from control group.

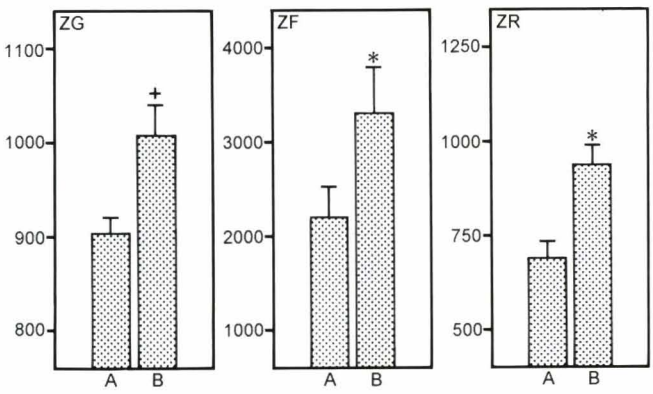

Fig. 2. Effect of PNM on the average volume (μm^3) of adrenocortical cells of DX-administered rats. A: control rats; B: PNM-treated rats. Bars are means±SE (n= 8). +: p<0.05; *: p<0.001 from control group.

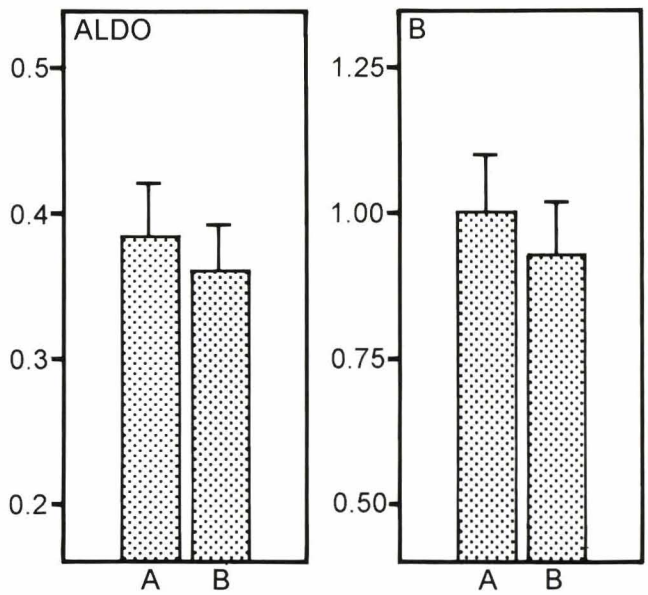

Fig. 4. Effect of PNM on steroid-hormone secretion (nmol/adrenal pair/120 min) of adrenal quarters from DX-administered rats. A: control rats; B: PNM-treated rats. Bars are means±SE.

PNM treatment markedly increased ACTH plasma concentration (2.7-fold); however, it had no apparent effect on plasma concentrations of both ALDO and B (Fig. 3), nor on ALDO and B secretions by adrenal quarters (Fig. 4).

Discussion

Our present findings clearly indicate that PNM exerts a potent stimulating effect on adrenocortical growth in DX-suppressed rats.

As mentioned in the introduction, the *in-vivo* effects of PNM on rat pituitary-adrenal axis are mediated by the peptide-induced increase in AVP release. However, this mechanism does not seem to underly the effect of PNM in DX-administered rats. In fact, AVP, though exerting a potent stimulating action on the growth and secretory activity of adrenal cortex in normal rats (Malendowicz, 1993; Mazzocchi et al., 1995), has no effect on adrenocortical weight and structure of atrophic adrenals of 7-day DX-treated rats (Lesniewska et al., 1992b), nor did it raise ACTH blood level in such animals (Lesniewska et al., 1991). It therefore seems legitimate to suggest that PNM-induced adrenocortical changes in DX-suppressed rats are mainly caused by stimulation of ACTH release.

The present results show that the PNM-induced stimulation of adrenal growth is not coupled with appreciable changes in the blood levels of ALDO and B, and in steroid output by adrenal quarters. This rather unexpected and puzzling finding is difficult to explain. It appears conceivable to admit that PNM treatment has been too short to allow DX-atrophied adrenocortical cells to re-acquire their well differentiated secretory capacity. Further studies are under way to confirm this hypothesis.

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