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Effects of pneumadin (PNM) on the adrenal glands. 6. Further studies on the inhibitory effect of PNM on dexamethasone-induced atrophy of the rat adrenal cortex

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Summary. Pneumadin (PNM) is a biologically active decapeptide, which has previously been found to enhance rat adrenal growth; the mechanism is indirect and probably involves the stimulation of both arginine-vasopressin (AVP) and ACTH release. The effects of 2- and 6-day PNM administration on the atrophic adrenal cortices of rats treated for 8 and 12 days, respectively, with daily subcutaneous injections of 15 or 40 g/100 g body weight of dexamethasone (Dx) were investigated. Morphometry showed that PNM counteracted Dx-induced adrenal atrophy, by preventing the decrease in volume and number of the parenchymal cells. PNM raised aldosterone and corticosterone production of adrenal quarters from Dx-treated rats, but it did not evoke significant changes in the plasma concentrations of the two hormones. The preventive effect of PNM was only partial and almost exclusively evident in rats administered the lower dose of Dx. In light of these findings the following conclusions are drawn: (i) PNM is able to partially overcome the Dx-induced inhibition of the rat hypothalamo-pituitary-adrenal axis, probably by stimulating the pituitary release of AVP and ACTH, that in turn enhance adrenocortical growth; (ii) the PNM-induced improvement of the secretory capacity of atrophic adrenocortical cells is not sufficient to raise the blood level of corticosteroid hormones; and (iii) Dx exerts a direct inhibitory action on adrenocortical cells, which is not counteracted by PNM.

Key words: Pneumadin, Adrenal cortex, Hypothalamo-pituitary-adrenal axis, Steroidogenesis, Morphometry, Rat

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Introduction

Pneumadin (PNM), a decapeptide isolated from mammalian lungs, exerts a potent stimulatory effect on arginine-vasopressin (AVP) release both *in vivo* and *in vitro*, thereby evoking a marked antidiuretic action (Batra et al., 1990; Watson et al., 1995). We have recently showed that PNM may be involved in the regulation of the hypothalamo-pituitary-adrenal (HPA) axis in the rat. This peptide does not affect steroid secretion of dispersed rat adrenocortical cells, even though it induces a sizable increase in the cytosolic Ca^{2+} concentration (Markowska et al., 1995d); however, when acutely or chronically administered to intact rats, it enhances the blood levels of ACTH, aldosterone and corticosterone (Markowska et al., 1995a,c), an effect prevented by the V1 AVP receptor antagonists (Markowska et al., 1995b). PNM also stimulates the growth of adrenal glands in dexamethasone (Dx)-suppressed rats (Markowska et al., 1996).

The aim of the present study was to gain insight into the mechanism whereby PNM is able to partially prevent the Dx-induced adrenocortical atrophy in rats. To this end we have investigated the effects of different durations of PNM administration on the secretion and growth of the adrenal cortex of rats administered different doses of Dx.

Materials and methods

Animal treatment

Adult female Wistar rats (180-190 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*. Groups of rats (n=12) were given daily subcutaneous (s.c.) injections of

15 or 40 g/100 g body weight of Dx (Decadron; Merck, Milan, Italy) for 8 (Dx 8) or 12 consecutive days (Dx 12). For the last 2 or 6 days of Dx-administration half of the animals in each group (n=6) also received daily s.c. injections of 1.5 nmol/100 g body weight of PNM (rat; Bachem, Bubendorf, Switzerland). A group of rats (n=12) was given daily s.c. injections of 0.2 ml 0.9% NaCl vehicle for 12 days (basal group, B). 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 being recorded.

Morphometry

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 volume of the zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR), as well as the average volume and number of their parenchymal cells were calculated according to Weibel (1979), as previously detailed (Malendowicz, 1987).

In vitro steroid secretion

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.

Steroid hormone assay

Aldosterone and corticosterone were extracted from plasma and incubation medium and purified, and their concentrations assayed by RIA, as previously detailed (Malendowicz et al., 1993). Intra- and interassay variations were: aldosterone, 5% and 7%; and corticosterone, 7% and 9%, respectively.

Statistics

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, or the Multiple Range Test of Duncan.

Results

Dx, independently of the dose and number of days of administration, induced a clear-cut decrease in the adrenal weight and volumes of ZF/ZR and their parenchymal cells, as well as a marked lowering in the blood level and secretion by adrenal quarters of corticosterone. The volume of ZG was not affected, but the volume of its cells was lowered; aldosterone plasma

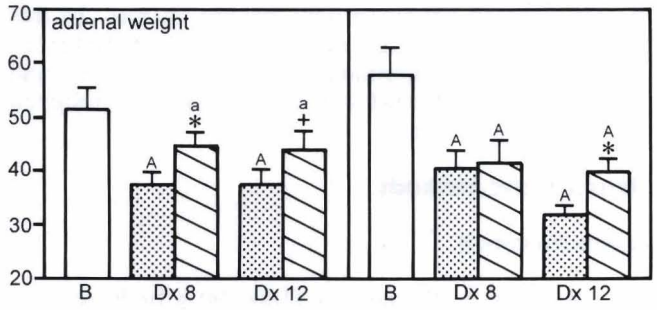

Fig. 1. Effects of PNM (hatched bars) on adrenal weight (mg) of rats administered 15 (left panel) or 40 g/100 g body weight of Dx (right panel). Bars are means ± SE (n=6). [a]: p<0.05 and [A]: p<0.01 from B; [+]: p<0.05 and *: p<0.01 from the respective control (Dx only administered) group (dotted bars).

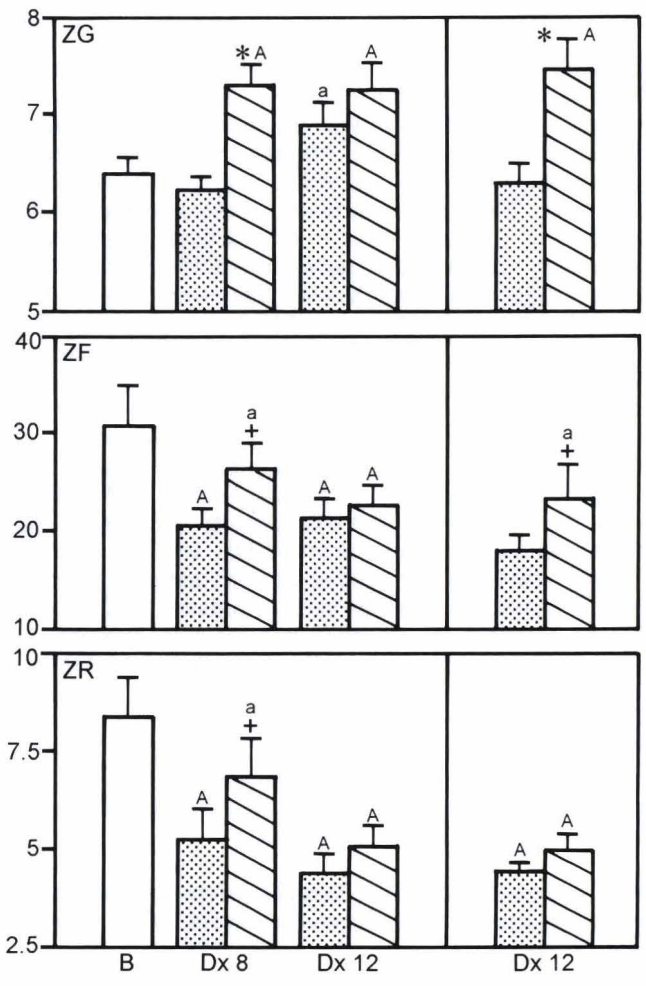

Fig. 2. Effect of PNM (hatched bars) on the volume of adrenocortical zones (mm^3) of rats administered 15 (left panels) or 40 g/100 g body weight of Dx (right panels). Bars are means ± SE (n=6). [a]: p<0.05 and [A]: p<0.01 from B; [+]: p<0.05 and *: p<0.01 from the respective control (Dx only administered) group (dotted bars).

concentration underwent a marked increase, and the same occurred for aldosterone production by adrenal quarters (Figs. 1-6).

As evidenced by the changes in the adrenal weight, PNM partially prevented the Dx-induced atrophy of the gland; however, in rats treated with the higher dose of Dx this effect was observed only when PNM was given for 6 days (Fig. 1).

Morphometry showed that in rats treated with the lower dose of Dx for 8 days, PNM abrogated the atrophy of all adrenocortical zones; this effect was apparently caused by the prevention of cell volume loss, since the number of parenchymal cells did not evidence significant changes. In rats administered the lower dose of Dx for 12 days, PNM did not change the volume of adrenocortical zones, but it decreased the average volume and increased the number of parenchymal cells. In animals treated with the higher dose of Dx for 12 days, PNM raised the volume of ZG and ZF, and their parenchymal cells, without changing their number; the volume of ZR and the average volume and number of its cells were not apparently affected (Figs. 2-4).

PNM did not significantly alter steroid blood concentrations, with the exception of a net lowering in aldosterone in the rats administered the lower dose of Dx for 12 days (Fig. 5). PNM enhanced aldosterone production by adrenal quarters obtained from rats treated for 8 days with the lower dose of Dx, while in quarters from animals administered for 12 days the higher dose of Dx PNM raised the yield of both aldosterone and corticosterone (Fig. 6).

Discussion

According to previous investigations (Lesniewska et al., 1992a; Malendowicz et al., 1992), Dx administration provokes a profound atrophy of inner adrenocortical

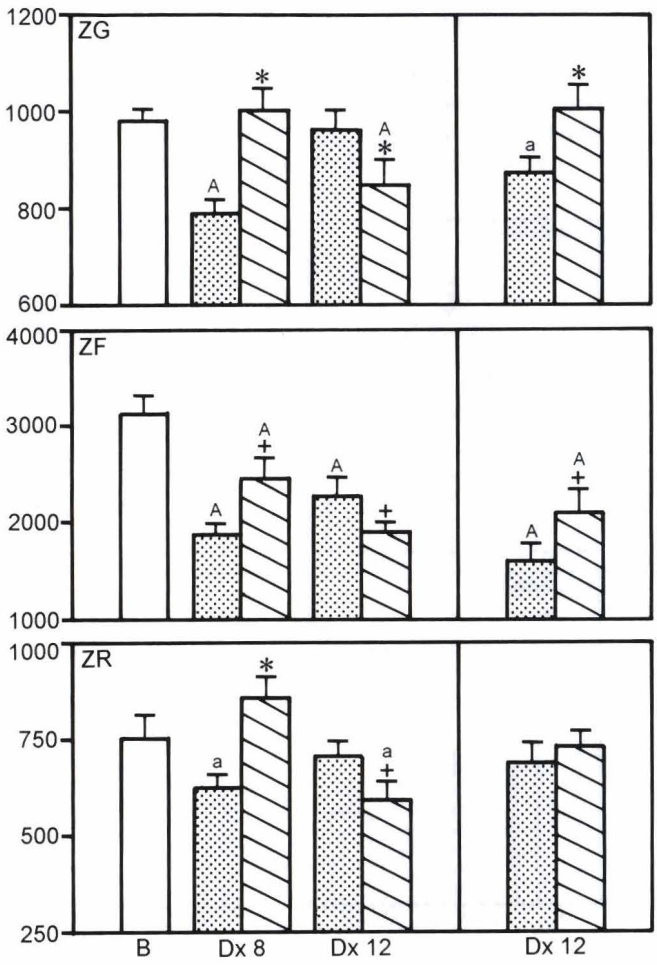

Fig. 3. Effect of PNM (hatched bars) on the average volume of adrenocortical cells (mm^3) of rats administered 15 (left panels) or 40 g/100 g body weight of Dx (right panels). Bars are means ± SE (n=6). [a]: $p<0.05$ and [A]: $p<0.01$ from B; [+]: $p<0.05$ and *: $p<0.01$ from the respective control (Dx only administered) group (dotted bars).

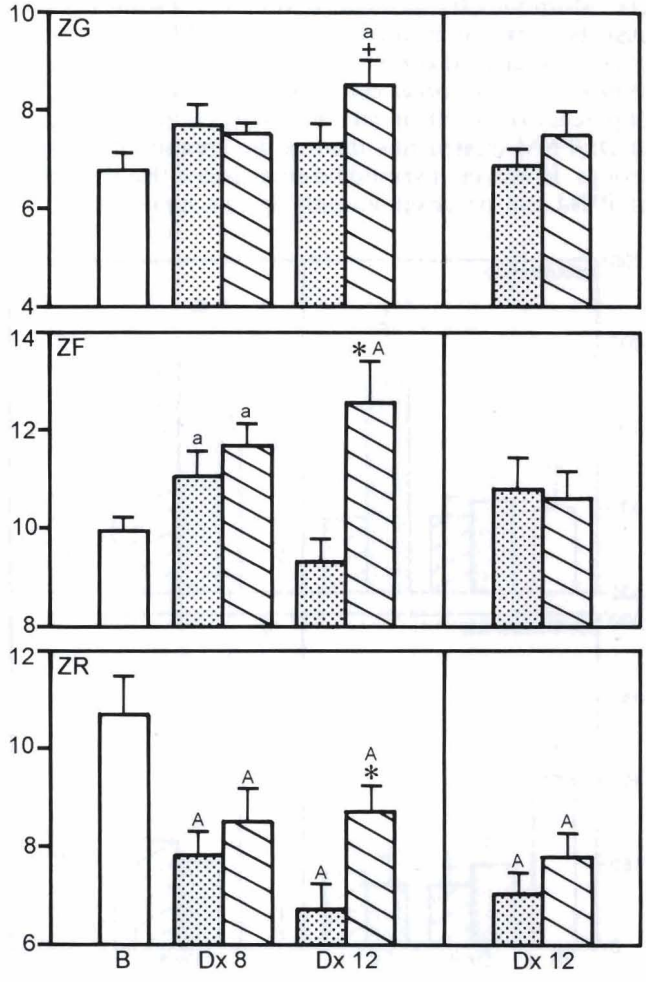

Fig. 4. Effect of PNM (hatched bars) on the parenchymal cell number in adrenocortical zones (N x 10^6) of rats administered 15 (left panels) or 40 μg/100 g body weight of Dx (right panels). Bars are means ± SE (n=6). [a]: $p<0.05$ and [A]: $p<0.01$ from B; [+]: $p<0.05$ and *: $p<0.01$ from the respective control (Dx only administered) group (dotted bars).

zones, connected with a marked impairment of their glucocorticoid secretory capacity. The intensity of this effect is not apparently related to either the dose of Dx or the duration of the treatment. The effects of Dx on the ZG and mineralocorticoid secretion are rather puzzling, since despite a net atrophy of ZG cells, the secretion of aldosterone appears to be raised. The mechanism underlying the Dx-induced marked increase in the level of circulating aldosterone is not known. Kenyon et al. (1990) have shown that Dx decreases the exchangeable body sodium in the rat, and suggested its natriuretic and kaliuretic properties. Hence, according to Lesniewska et al. (1991), this unexpected finding could reflect a compensatory hypersecretion of aldosterone due to the Dx-induced natriuresis.

In keeping with our previous findings (Markowska et al., 1996), PNM was found to partially prevent the Dx-induced adrenocortical atrophy, and this effect was more pronounced in the rats administered the lower dose of Dx. Morphometry evidenced very interesting cellular aspects of this preventive effect of PNM. Collectively, our data suggest that PNM primarily counteracts the Dx-induced adrenocortical cell loss; as a result, the number of parenchymal cells in all adrenocortical zones is higher in Dx/PNM-treated rats than in the appropriate control groups. However, it should be emphasized that this effect of PNM can be observed only in rats treated with the lower dose of Dx. Moreover, in the 8-day experiment, adrenocortical cells of PNM-administered rats are larger and in the 12-day experiment smaller than those of the respective control group.

Despite its marked trophic action, PNM does not evoke evident changes in the blood levels of corticosteroids, with the exception of the 12-day experiment where this peptide counteracts the rise in plasma aldosterone concentration evoked by the lower dose of Dx. In contrast, PNM appears to enhance aldosterone and corticosterone output by adrenal quarters, expecially in the group treated for 12 days with the higher dose of Dx. Taken together these findings suggest that PNM does not improve steroidogenic capacity of adrenocortical cells of Dx-suppressed rats to a level able to produce sizable changes in the blood levels of corticosteroids. Alternatively, the possibility cannot be ruled out that PNM enhances the metabolic clearance of adrenocortical hormones. Be that as it may, our results indicate that there is no obvious relation between the blood concentration of corticosteroids and their output by adrenal slices.

As mentioned above (see Introduction), PNM stimulates AVP release by neurohypophysis. AVP exerts a powerful stimulating action on the growth and secretion of ZG and ZF/ZR of rat adrenal cortex, acting

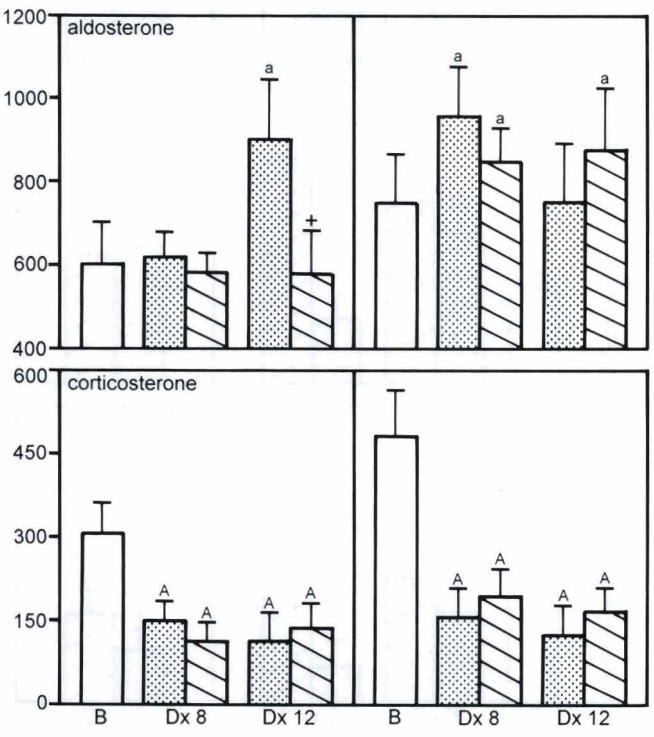

Fig. 5. Effect of PNM (hatched bars) on the plasma concentrations of aldosterone (pM) and corticosterone (nM) of rats administered 15 (left panels) or 40 μg/100 g body weight of Dx (right panels). Bars are means ± SE (n=6). [a]: p<0.05 and [A]: p<0.01 from B; [+]: p<0.05 from the respective control (Dx only administered) group (dotted bars).

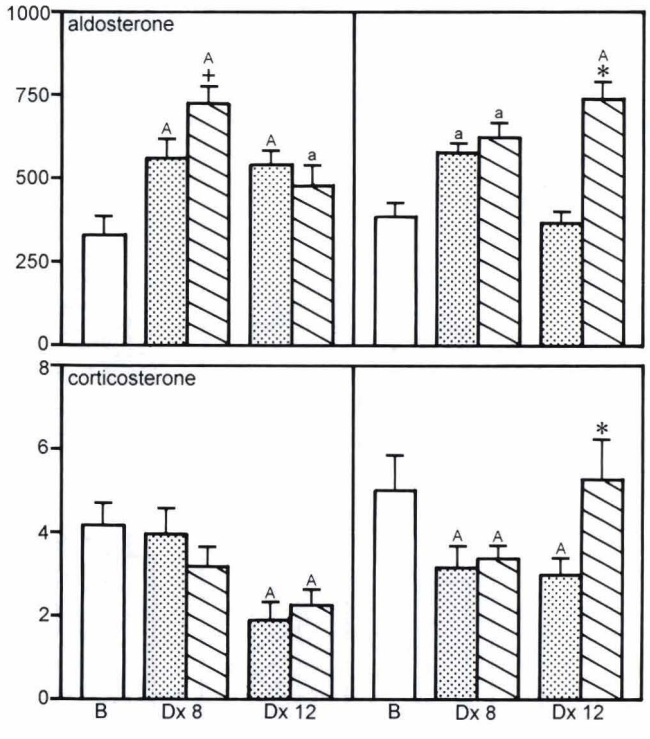

Fig. 6. Effect of PNM (hatched bars) on aldosterone and corticosterone secretion of adrenal quarters (pmol/100 mg/120 min) from rats administered 15 (left panels) or 40 μg/100 g body weight of Dx (right panels). Bars are means ± SE (n=6). [a]: p<0.05 and [A]: p<0.01 from B; [+]: p<0.05 and *: p<0.01 from the respective control (Dx only administered) group (dotted bars).

via the V1 receptor subtype (for review, see Guillon et al., 1995; Mazzocchi et al., 1995). Evidence is available that AVP reverses adrenocortical atrophy induced by short-term Dx administration, independently of any change in ACTH secretion (Lesniewska et al., 1991). However, this peptide has no effect on adrenals in which atrophy had been caused by a 7-day Dx pretreatment (Lesniewska et al., 1992b). Hence, it could appear conceivable that PNM-induced prevention of adrenal atrophy evoked by long-term Dx administration is exclusively mediated by ACTH, whose hypophyseal release is enhanced by PNM (Markowska et al., 1996). This contention conflicts with the demonstration that the stimulatory effect of PNM on the rat HPA axis is abolished by the antagonists of V1 AVP receptors (Markowska et al., 1995b). A possible clue to reconcile these conflicting data may be provided by the compelling evidence showing that AVP, like CRH, is a potent stimulator of hypophyseal ACTH release (Vale et al., 1981; Antoni, 1986; Buckingham et al., 1992; Bernardini et al., 1994). In light of these considerations, it seems legitimate to advance the hypothesis that a two-fold mechanism underlies the preventive effect of PNM on the Dx-induced inhibition of the HPA axis in rats: (i) direct stimulation of AVP secretion by the hypothalamic neurons, and (ii) indirect stimulation of ACTH release by pituitary corticotropes via AVP. Probably AVP and ACTH cooperate to enhance the growth of the adrenal cortex.

Before concluding, it must be recalled that PNM is able to only partially overcome the adrenocortical suppressive effect of Dx and almost exclusively when relatively low doses of the glucocorticoid are administered. This observation can be tentatively explained by taking into account that Dx exerts a potent direct inhibitory effect on the growth and secretion of rat adrenocortical cells (for review, see Nussdorfer, 1986). Probably, this last effect of Dx cannot be prevented by PNM, which does not seem to directly affect basal functions of adrenocortical cells (Markowska et al., 1995d). Further studies are under way to settle this hypothesis.

References

Antoni F.A. (1986). Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of a 41-residue corticotropin-releasing factor. Endocr. Rev. 7, 351-378.

Bernardini R., Chiarenza A., Kamilaris T.C., Renaud N., Lempreur L., Demitrak M., Gold P.W. and Chrousos G.P. (1994). *In vivo* and *in vitro* effects of arginine-vasopressin receptor antagonists on the hypothalamic-pituitary-adrenal axis in the rat. Neuroendocrinology 60, 503-508.

Batra V.K., Mathur M., Mir S.A., Kapoor R. and Kumar M.A. (1990). Pneumadin: a new lung peptide which triggers antidiuresis. Regul. Pept. 30, 77-87.

Buckingham J.C., Smith T. and Loxley H.D. (1992). The control of ACTH secretion. In: The adrenal gland. 2nd ed. James V.H.T. (ed). Raven Press. New York. pp 131-158.

Guillon G., Trueba M., Joubert D., Grazzini E., Chouinard L., Côte M., Payet M.D., Manzoni O., Barberis C., Robert M. and Gallo-Payet N. (1995). Vasopressin stimulates steroid secretion in human adrenal glands: comparison with angiotensin II effect. Endocrinology 136, 1285-1295.

Kenyon C.J., Brown W.B., Fraser R., Tonolo G., McPherson F. and Davies D.L. (1990). Effects of dexamethasone on body fluid and electrolyte composition of rats. Acta Endocrinol. (Copenh.) 122, 599-604.

Lesniewska B., Nowak M., Nussdorfer G.G. and Malendowicz L.K. (1990). Sex-dependent effect of melatonin on the secretory activity of rat and hamster adrenal gland *in vitro*. Life Sci. 47, 241-245.

Lesniewska B., Nowak M., Miskowiak B., Nussdorfer G.G. and Malendowicz L.K. (1991). Effects of arginine-vasopressin on the pituitary-adrenocortical axis of intact and dexamethasone-suppressed rats. Exp. Pathol. 43, 181-188.

Lesniewska B., Nowak K.W. and Malendowicz L.K. (1992a). Dexamethasone-induced adrenal cortex atrophy and recovery of the gland from partial steroid-induced atrophy. Exp. Clin. Endocrinol. 100, 133-139.

Lesniewska B., Nussdorfer G.G., Nowak M. and Malendowicz L.K. (1992b). Comparison of the effects of neurotensin and vasopressin on the adrenal cortex of dexamethasone-suppressed rats. Neuropeptides 23, 9-13.

Malendowicz L.K. (1987). Sex differences in adrenocortical structure and function. XXIV. Comparative morphometric studies on adrenal cortex of intact mature male and female rats of different strains. Cell Tissue Res. 249, 443-450.

Malendowicz L.K., Nussdorfer G.G., Markowska A. and Nowak K.W. (1992). Analysis of the preventive action of ACTH on dexamethasone-induced adrenocortical atrophy in the rat. Cytobios 71, 191-199.

Malendowicz L.K., Nussdorfer G.G., Markowska A., Nowak K.W. and Torlinski L. (1993). Effects of neuromedin-N on the pituitary-adrenal axis of dexamethasone-suppressed rats. Neuropeptides 24, 1-4.

Markowska A., Nussdorfer G.G., Belloni A.S., Nowak M. and Malendowicz L.K. (1995a). Effects of pneumadin (PNM) on the adrenal gland. 2. Evidence that PNM exerts an acute stimulating action on the rat pituitary-adrenal axis. Med. Sci. Res. 23, 825-826.

Markowska A., Nussdorfer G.G., Rebuffat P., Nowak M., Majchrzak M. and Malendowicz L.K. (1995b). Effects of pneumadin (PNM) on the adrenal gland. 3. Evidence that an antagonist of vasopressin receptors prevents the stimulating action of PNM on steroid secretion of rat adrenal gland. Neuroendocrinol. Lett. 17, 295-300.

Markowska A., Rebuffat P., Nussdorfer G.G. and Malendowicz L.K. (1995c). Effects of pneumadin (PNM) on the adrenal gland. 4. The response of rat pituitary-adrenal axis to the prolonged PNM infusion. Neuroendocrinol. Lett. 17, 301-306.

Markowska A., Warchol J.B., Neri G., Nussdorfer G.G. and Malendowicz L.K. (1995d). Effects of pneumadin (PNM) on the adrenal gland. 1. Evidence that PNM increases cytosolic free calcium concentration in dispersed rat adrenocortical cells, without evoking a secretory response. Med. Sci. Res. 23, 749-750.

Markowska A., Macchi C., Nussdorfer G.G. and Malendowicz L.K. (1996). Effect of pneumadin (PNM) on the adrenal gland. 5. Potent stimulating action of PNM on adrenocortical growth of dexamethasone-administered rats. Histol. Histopathol. 11, 583-585.

Mazzocchi G., Malendowicz L.K., Meneghelli V., Gottardo G. and Nussdorfer G.G. (1995). *In vitro* and *in vivo* studies on the effects of

arginine-vasopressin on the secretion and growth of rat adrenal cortex. Histol. Histopathol. 10, 359-370.

Nussdorfer G.G. (1986). Cytophysiology of the adrenal cortex. Int. Rev. Cytol. 98, 1-405.

Vale W., Spiess J., Rivier C. and Rivier J. (1981). Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and ß-endorphin. Science 233, 1394-1397.

Watson J.D., Jennings B., Sarda R., Pang S.C., Lawson B., Wigle D.A. and Flynn T.G. (1995). The antidiuretic effect of pneumadin requires a functional arginine-vasopressin system. Regul. Pept. 57, 105-114.

Weibel E.R. (1979). Stereological methods. 1. Practical methods for biological morphometry. Academic Press. London.

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