

Neuropeptide Y and glucocorticoid secretion from guinea pig adrenal gland: An *in vivo* and *in vitro* study

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Abstract. The effects of neuropeptide Y (NPY) on adrenal glucocorticoid secretion are controversial, and we have investigated this issue in guinea pigs, where, like in humans and cows, the main glucocorticoid hormone is cortisol. *In vivo* experiments showed that prolonged NPY administration markedly lowered cortisol plasma concentration not only in normal guinea pigs, but also in animals whose hypothalamic-pituitary-adrenal axis and renin-angiotensin system had been pharmacologically interrupted by the simultaneous administration of dexamethasone and captopril. *In vitro* experiments ruled out the possibility that *in vivo* glucocorticoid anti-secretagogue action of NPY can ensue from a direct effect on the adrenal gland. In fact, NPY did not affect cortisol secretion from dispersed guinea pig inner adrenocortical cells. In contrast, NPY raised cortisol production from adrenal slices containing medullary tissue, and this effect was blocked by the β -adrenoceptor antagonist *l*-alprenolol. This finding, coupled with the demonstration that NPY enhanced catecholamine release from guinea pig adrenomedullary tissue, strongly suggests that NPY may stimulate glucocorticoid secretion in this species through an indirect mechanism involving catecholamines, that in a paracrine manner promote the secretion of inner adrenocortical cells. In light of these observations, the conclusion is drawn that the *in vivo* effects of NPY are mediated by mechanism(s) independent of either the suppression of the main adrenal agonists ACTH and angiotensin-II or the direct inhibition of adrenals secretion. The possibility merits an investigation into whether NPY enhances the production of peptides, which, like leptin, inhibit adrenal glucocorticoid secretion acting as circulating hormones.

Introduction

Neuropeptide Y (NPY) is a 36-amino acid peptide, which is widely distributed in the central nervous system, where it exerts various relevant physiological functions, among which is the control of food intake (reviewed in refs. 1,2). Like other orexigenic and anti-orexigenic peptides (3-6 and refs. therein), NPY has been found to control the hypothalamic-pituitary-adrenal axis, especially acting on its peripheral branch (2,7-10).

The action of NPY on the adrenal gland seems to be mainly concerned with zona glomerulosa (ZG) and mineralocorticoid secretion. The *bolus* administration of NPY was found to raise the aldosterone blood level in rats (11,12). Although previous *in vitro* studies reported an inhibitory action of NPY on basal aldosterone secretion (13), subsequent investigations demonstrated that this peptide magnified the aldosterone response of dispersed ZG cells to their main agonists [ACTH, angiotensin-II (Ang-II) and potassium] (14,15). Moreover, evidence has been provided that NPY is also able to affect ZG indirectly, via a mechanism involving the local release of catecholamines, that in turn enhance aldosterone secretion acting in a paracrine manner (16-19).

In contrast, the effects of NPY on zona fasciculata-reticularis (inner) adrenocortical cells are doubtful. *In vivo* studies revealed no acute effect of NPY on glucocorticoid blood levels (11,12). A clearcut inhibitory action of NPY on basal and ACTH-stimulated corticosterone secretion from dispersed inner adrenocortical cells has been described (20), but further studies have not confirmed this finding (13-15).

All the above mentioned studies were carried out in rodents. Hence, it seemed worthwhile to investigate the *in vivo* and *in vitro* effects of NPY on glucocorticoid and catecholamine secretion from the guinea pig adrenal gland. In fact, guinea pig adrenals, like those of humans, possess 17 α -hydroxylase activity, and therefore the main glucocorticoid hormone secreted is cortisol.

Materials and methods

Animals and reagents. Adult male guinea pigs, either bred in our laboratory facilities (*in vivo* experiments) or purchased from Charles-River (Como, Italy) (*in vitro* experiments), were kept under a 12:12 h light/dark cycle (illumination

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onset at 8:00 a.m.) at 23°C, and maintained on a standard diet and tap water *ad libitum*. The study protocol was approved by the local Ethics Committee for Animal Studies. The angiotensin-converting enzyme inhibitor captopril (Aceten) and dexamethasone (Dexona) were obtained from Worckhardt India and Cadilla India (New Delhi, India), respectively. NPY (human, rat, mouse), ACTH, angiotensin-II (Ang-II), *l*-alprenolol, bovine serum albumin (BSA), phosphate-buffered saline (PBS), and all other chemicals and laboratory reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO).

In vivo experiments. Animals were divided into 2 groups ($n=24$). One group was subcutaneously injected for 14 days with dexamethasone (2.5 mg/kg) and captopril (8.3 mg/kg). The other group was given daily injections of 0.9% NaCl. On the 7th day, half the animals in each group received NPY (0.1 mg/kg) for the next 7 days. At the end of the treatment, blood samples were collected from the retro-orbital vein (21), and stored at -20°C until cortisol assay.

In vitro experiments. Dispersed guinea pig inner adrenocortical cells, adrenal slices (containing both cortical and medullary tissue) and medullary tissue fragments were obtained as previously described (22). Dispersed cells and tissue fragments (10^4 cells and 5-6 mg of tissue) were put in Krebs-Ringer bicarbonate buffer with 3% glucose and 0.2% BSA and incubated with NPY (10^{-7} M) alone and in the presence of ACTH (10^{-9} M) or Ang-II (10^{-8} M). Tissue slices were also incubated with *l*-alprenolol (10^{-6} M) alone or in the presence of NPY (10^{-7} M). These concentrations of peptides were chosen because they were previously found to be the maximal effective ones in the rat adrenal gland (2). The incubation was carried out in a shaking bath at 37°C for 60 min (cortisol secretion) or 20 min (catecholamine secretion) in an atmosphere of 95% air-5% CO₂. Supernatants were stored at -80°C until hormonal assays, and the protein concentration of dispersed cells and tissue fragments was measured by the Sigma-Aldrich BCA protein assay kit.

Hormonal assays. Cortisol blood concentration was measured by enzyme immunoassay (EIA), as previously detailed (21). Cortisol was extracted from supernatants and purified by HPLC (23,24), and its concentration was estimated by radioimmunoassay (RIA), using a commercial kit purchased from IRE-Sorin (Vercelli, Italy). The catecholamine (epinephrine, E; nor-epinephrine, NE) concentrations in the supernatants were measured by HPLC, using a reverse phase column and a glassy carbon electrochemical detector (23,25).

Statistics. Data were expressed as means \pm SD or SEM, and their statistical comparison was performed by the paired sample *t*-test (cortisol blood concentration) or by ANOVA, followed by Duncan's multiple range test.

Results

The prolonged dexamethasone/captopril administration lowered the blood concentration of cortisol in guinea pigs by ~60%. NPY treatment for 7 days strikingly decreased the

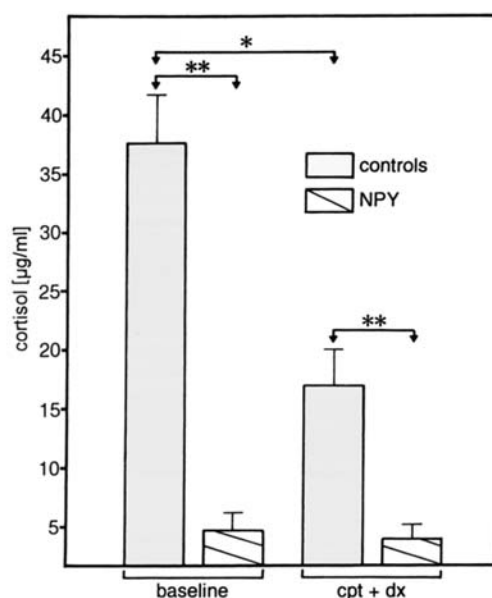


Figure 1. Effect of the prolonged administration of NPY on the blood concentration of cortisol in normal (baseline) and dexamethasone (dx)/captopril(cpt)-treated guinea pigs. Bars are the means \pm SD ($n=12$). * $P<0.05$ and ** $P<0.01$.

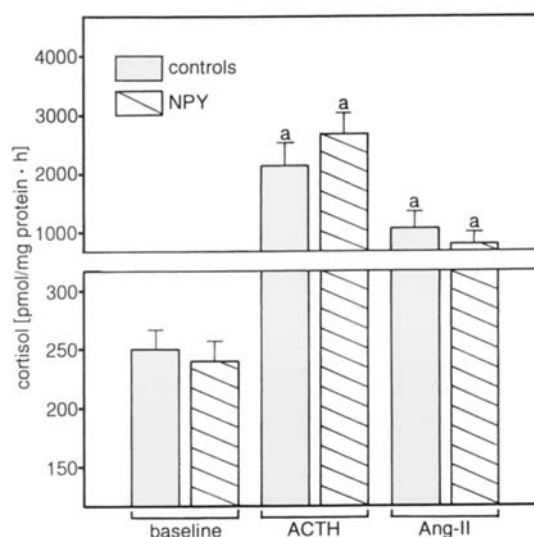


Figure 2. Lack of effects of NPY (10^{-7} M) on basal and agonist-stimulated cortisol secretion from dispersed guinea pig inner adrenocortical cells. Bars are the means \pm SEM ($n=6$). * $P<0.01$ from the respective baseline value.

plasma level of cortisol in both normal guinea pigs (approximately -90%) and dexamethasone/captopril-administered animals (approximately -80%) (Fig. 1).

NPY (10^{-7} M) did not affect either basal or ACTH (10^{-9} M)- and Ang-II (10^{-8} M)-stimulated cortisol secretion from dispersed guinea pig inner adrenocortical cells (Fig. 2). In contrast, NPY evoked a small but significant rise (~45%) in the basal cortisol production from guinea pig adrenal slices, without changing the agonist-stimulated one (Fig. 3). NPY also enhanced basal E and NE release from adrenomedullary tissue (by ~50 and 35%, respectively) (Fig. 4), and its secretagogue effect on basal cortisol production from adrenal slices was abrogated by 10^{-6} M *l*-alprenolol (Fig. 5).

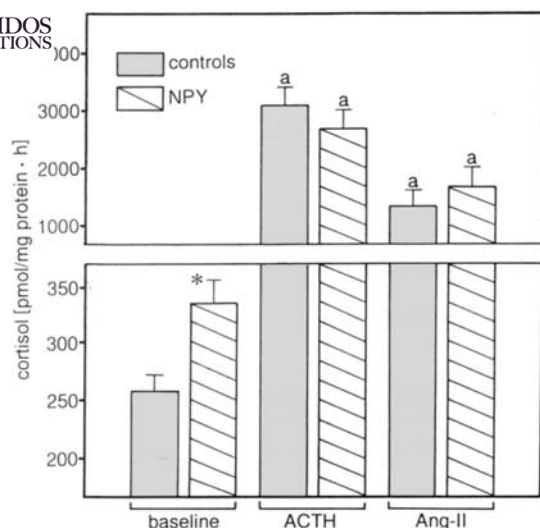


Figure 3. Effects of NPY (10^{-7} M) on basal and agonist-stimulated cortisol secretion from guinea pig adrenal slices containing medullary chromaffin tissue. Bars are the means \pm SEM (n=6). *P<0.05 from the respective control value; *P<0.01 from the respective baseline value.

Discussion

Our present *in vivo* experiments clearly showed that NPY inhibits cortisol secretion in guinea pigs. There is indication that NPY, when systemically administered, exerts an inhibitory effect on both pituitary ACTH secretion (7,26,27) and renin release (28-30). However, this mechanism does not positively underlie the *in vivo* glucocorticoid anti-secretagogue action of NPY because it occurred also in guinea pigs where cortisol production was dampened by the simultaneous pharmacological blockade of the hypothalamic-pituitary-adrenal axis and renin-angiotensin system.

A direct inhibitory effect of NPY on inner adrenocortical zones can be ruled out. In fact, this neuropeptide did not alter cortisol production from dispersed guinea-pig inner adrenocortical cells, nor did it suppress agonist-stimulated secretion from adrenal slices. Moreover, NPY enhanced basal cortisol yield from adrenal slices containing medullary tissue.

This last observation requires further discussion. As mentioned in the Introduction, NPY is included in that group of regulatory peptides (VIP and PACAP, tachykinins, endothelins, adrenomedullin and atrial natriuretic peptides) (22,31-34) which are able to enhance steroid secretion by eliciting the release of catecholamines, that in turn stimulate adrenocortical cells in a paracrine manner. The following evidence indicates that this mechanism may be involved in the mild *in vitro* glucocorticoid secretagogue action of NPY on guinea pig adrenal slices: i) in keeping with previous findings (19,22,35-37), NPY enhanced E and NE release from guinea pig adrenomedullary tissue; and ii) *l*-alprenolol, a specific β_1 -receptor antagonist, completely abolished cortisol response of guinea pig adrenal slices to NPY, without *per se* altering basal cortisol secretion. The majority of studies point out that such a catecholamine-mediated paracrine mechanism mainly concerns zona glomerulosa and aldosterone secretion (reviewed in ref. 16). However, it may be stressed that investigations were mainly carried out in the

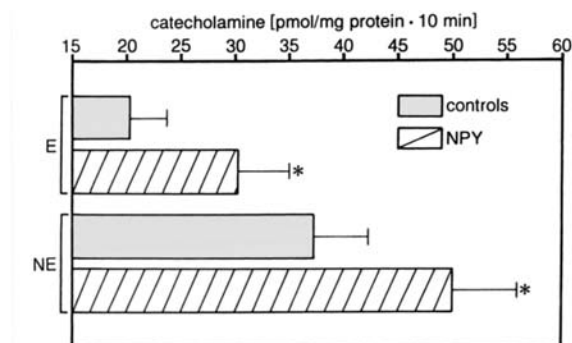


Figure 4. Effects of NPY (10^{-7} M) on basal catecholamine secretion from guinea pig adrenomedullary fragments. Bars are the means \pm SEM (n=6). *P<0.05 from the respective control value. E, epinephrine; NE, nor-epinephrine.

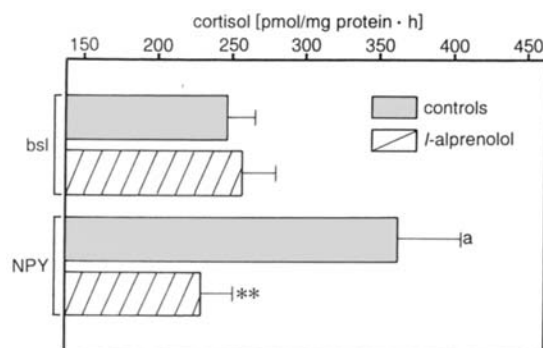


Figure 5. Effects of *l*-alprenolol (10^{-6} M) on basal (bsl) and NPY (10^{-7} M)-stimulated cortisol secretion from guinea pig adrenal slices containing medullary chromaffin tissue. Bars are the means \pm SEM (n=6). **P<0.01 from the respective control value; *P<0.01 from the respective baseline value.

rat, so that it is reasonable to conceive that, at variance with this species, guinea pigs possess not only zona glomerulosa, but also inner adrenocortical cells provided with β -adrenoceptors. Moreover, guinea pig inner adrenocortical cells, like those of cows and pigs, secrete cortisol, and catecholamines have been shown to raise steroid secretion from bovine and pig zona fasciculata cells cultured *in vitro* (38-41).

In light of the herein discussed evidence, it seems legitimate to conceive that the mechanism(s) underlying the *in vivo* glucocorticoid anti-secretagogue action of NPY involve(s) some indirect effect(s) of this peptide. Such effect(s) is (are) still unknown and thus only hypotheses can be advanced on this matter. However, we want to stress that NPY plays a role in the central control of food intake (1,42), and that to accomplish this function it interacts with other central regulatory peptides such as orexins and leptin exerting anti-orexineric or orexineric actions (43-45). While orexins stimulate the hypothalamic-pituitary-adrenal axis (4), the body of findings suggest that leptin, acting as a circulating hormone, inhibits glucocorticoid secretion in humans and cows (6,46-49). Hence, the possibility that the *in vitro* adrenocortical inhibitory effects of NPY in the guinea pig are mediated by leptin merits further investigative effort.

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