were important regulatory components in the maintenance of the ecdysteroid titer, we have not yet been successful in showing such regulatory control.
Clearly the discovery of the prothoracic gland secretory product 3-dehydroecdysone raises more questions than it answers. The knowledge that prothoracic glands synthesize 3-dehydroecydsone from 7-dehydrocholesterol[4,5] is a first step in elucidating the ecdysone biosynthetic pathway. The putative roles of dehydroecdysone in the morphogenetic changes associated with metamorphic commitment and molting are undefined, but are deserving of future study.

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* Author, to whom all correspondence should be sent.

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Neuropeptide Y inhibits corticosterone secretion by isolated rat adrenocortical cells[1]

L. K. Malendowicz, B. Leśniewska and B. Miśkowiak

Department of Histology and Embryology, Poznań Academy of Medicine, 6 Święcicki Str., PL-60-781 Poznań (Poland)
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Summary. Studies with isolated rat adrenocortical cells have shown that neuropeptide Y (NPY) inhibits both basal and ACTH-stimulated corticosterone secretion. These results suggest the regulatory role of NPY in corticosterone secretion from the adrenal gland, especially during stress.
Key words. Neuropeptide Y; adrenal cortex; isolated adrenocortical cells; corticosterone secretion; inhibitory effect; rat.

Neuropeptide Y is widely distributed in neurons of the central and peripheral nervous system. NPY is contained in adrenal chromaffin cells and in nerve fibers passing through the adrenal cortex and medulla of various animal species, and it is co-released with catecholamines during sympathetic activation[2-4]. Evidence is available for the participation of this peptide in the systemic and/or paracrine adrenomedullary mechanism regulating aldosterone secretion, whereas the action of NPY on corticosterone secretion remains an open question[5,6]. Therefore we examined the effect of NPY on basal and ACTH-stimulated corticosterone secretion by isolated rat adrenocortical cells.

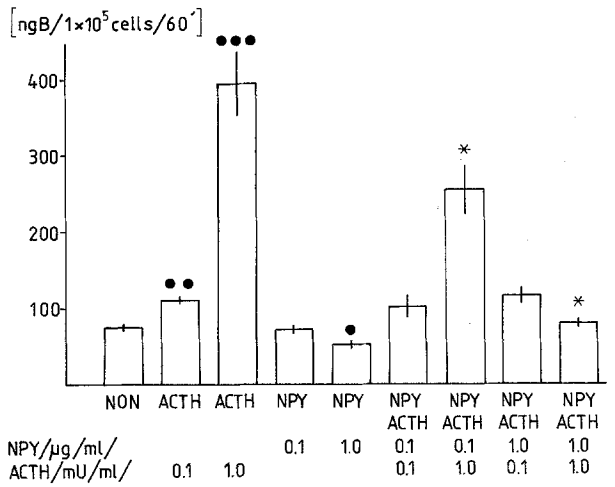
Materials and methods

Isolated rat adrenal cells were prepared by the method of Sayers et al.[7] with modifications described previously[8]. Adrenal glands from adult female Wistar rats (140–160 g) were removed, decapsulated, dissected into small pieces and preincubated for 5 min in Krebs-Ringer bicarbonate buffer (pH 7.3) enriched with 0.3% glucose (KRBG). The medium was discarded and adrenal tissue digested by 0.5% collagenase (type I, Sigma) in KRBG. The collagenase digestion was carried out by gentle stirring with a magnetic stirrer for 15 min at 37 °C and was repeated. Collected medium was centrifuged at 300 × g for 10 min and the sediment resuspended in KRBG containing 0.4% bovine serum albumin (Cohn's V fraction, Sigma) KRBGA). Cell viability was tested with the trypan blue test (0.5% solution) and the percentage of stained cells was always less than 5–6%. Adrenocortical cells were counted in a Burker chamber.
Cells were incubated for 60 min at 37 °C in a Dubnoff incubator (shaking at 50 rpm). Depending upon the experiment the number of cells used varied from 90,000 to 150,000 per ml. NPY (porcine, Sigma) was added in 0.9% NaCl and ACTH (1–24 ACTH, Sigma) in

0.1 N HCl. The final volume of the incubates was 1 ml. All glassware used for isolation and incubation was siliconized with Sigmacoat A (Sigma). After the incubation period, the tubes were rapidly frozen and stored at − 20 °C until corticosterone estimation. Corticosterone was quantified in unextracted medium by competitive protein binding[9]. A corticosterone-binding plasma-isotope solution (8 % plasma of a dexamethasone pretreated dog containing sufficient (1,2-^{3}H) corticosterone (Amersham International Ltd. Bucks) to saturate available binding sites) was added (0.5 ml) and the tubes were vortexed. The tubes were incubated for 10 min at 45 °C, followed by 30 min at 4 °C. Unbound steroid was removed by the addition of 40 mg of florosil (60–100 mesh; Florosil for Chromatography, Fluka AG, CH-9470 Buchs). Radioactivity of the supernatant was counted in a LKB 1211 Rackbeta scintillation counter. The intra-assay variation was 4 %. In this assay, interference by other steroids is insignificant owing to their low cross-reactivity[10].

Results and discussion

As follows from the figure, corticosterone secretion by isolated rat adrenocortical cells increased to nearly 150 % of that in the control in the presence of 0.1 mU ACTH, and to approximately 400 % at 1.0 mU. The lower dose of NPY used (0.1 µg/ml) had no effect on corticosterone secretion, whereas 1.0 µg/ml notably reduced basal corticosterone output to approximately 75 %. In the presence of 0.1 mU ACTH neither of the doses of NPY changed corticosterone output; however, the response of adrenocortical cells to 1.0 mU of ACTH was markedly suppressed by NPY (0.1 µg/ml to 64 % and 1.0 µg/ml to 20 %). Thus the present findings clearly demonstrate the inhibitory action of NPY on basal and ACTH-stimulated corticosterone secretion in isolated rat adrenocortical cells. It is of interest that at the lower ACTH concentration an inhibitory effect of NPY on corticosterone output was not observed. Earlier experiments revealed that NPY acutely stimulates aldosterone secretion, and in long-term experiments, the peptide was also able to enhance the growth and steroidogenic capacity of rat zona glomerulosa[5,6]. Since the peptide did not affect the zona fasciculata cells and production of corticosterone (prolonged s. c. infusion), the hypothesis was advanced that NPY effect on the rat adrenal cortex is specifically directed to the zona glomerulosa[6]. However, the present findings do not support the contention that there is a specific adrenoglomerulotrophic effect of NPY. Our data show that the potent inhibitory effect of NPY on ACTH stimulated corticosterone output may have physiological relevance in the regulation by NPY of corticosteroid secretion from the adrenal gland, especially during stress. The mechanism of the inhibitory action of NPY on corticosterone output remains an open question and deserves further investigation.



Corticosterone secretion by isolated rat adrenocortical cells (decapsulated glands) as affected by ACTH and NPY (ng/1 × 10^5 cells/60 min). Each bar represents the mean of 4 incubations, and vertical lines demonstrate ± SE. Statistical evaluation of the means by Student's t-test: differ from control (non) •, $p < 0.05$; ••, $p < 0.01$; •••, $p < 0.001$; differ from 1.0 mU ACTH group: *, $p < 0.001$.

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