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ORIGINAL ARTICLE

Effects of neuropeptide Y (NPY), galanin and vasoactive intestinal peptide (VIP) on pituitary hormone release and on ovarian steroidogenesis

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Abstract Ovarian folliculogenesis is regulated by the gonadotrophins, but in recent years other peptides have been found to serve as local regulators of ovarian function. The aim of this study is to evaluate the effects of NPY, galanin and VIP on pituitary and gonadal hormone release. Effects of NPY, galanin and VIP on progesterone, estradiol production by cultured rat granulosa and effects of these peptides on pituitary hormone release by cultured pituitary cells were examined according to methods previously described. Maximal effects of NPY, galanin and VIP on pituitary hormone release and on gonadal steroids were observed after administration of 10 nM of these peptides during 60 mins incubation. VIP and NPY, but not galanin, stimulated PRL release from cultured pituitary cells. VIP increased also LH release whenever NPY and galanin did not change LH release from pituitary cells. Galanin, but not NPY and VIP, leads to an increase of GH production. VIP, NPY and galanin did not change TSH and FSH release. NPY, galanin and VIP markedly stimulated progesterone release from cultured granulosa cells. NPY, galanin and VIP did not change estradiol and testosterone release. Conclusions. Direct effects of NPY, galanin and VIP on pituitary hormone release may indicate their role in the mechanism of pituitary hormone release. A marked increase of progesterone from cultured granulosa cells after VIP, NPY and galanin suggests that these peptides may be involved in the local ovarian steroidogenesis.

Introduction

It has been commonly accepted that some neuropeptides play an important role in the mechanism of hormone release.

Neuropeptide Y (NPY) and galanin are widely distributed not only in the central and peripheral nervous systems but also in various peripheral organs.

NPY and galanin-like activity have been discovered in hypothalamo-pituitary system, adrenal medulla, gastrointestinal, genitourinary and respiratory tracts [1, 2, 3, 4, 5, 6].

Although some data were presented that NPY and galanin may influence hypothalamo-pituitary regulation, the effects of these peptides on pituitary hormone release remain controversial [7, 8, 9, 10, 11, 12, 13].

Ovarian folliculogenesis is regulated by the gonadotrophins, but in recent years other peptides were found to serve as local regulators of ovarian function [14, 15, 16, 17].

Our previous studies indicated that VIP (vasoactive intestinal peptide) and PACAP 38 (pituitary adenylate cyclase activating polypeptide) may play a role in the local ovarian steroidogenesis [18].

Th aim of this study is to evaluate the effects of NPY, galanin and VIP on pituitary and gonadal hormone release.

Material and Methods

Effects of NPY, galanin and VIP on progesterone, estradiol production by cultured rat granulosa.

Effects of NPY, galanin and VIP on progesterone and estradiol production by cultured granulosa cells were examined.

The ovaries from WKY rats in diestrus were collected under aseptic conditions. Isolated ovaries were washed with PBS, supplemented with a mixture of antibiotics and then they were rubbed through a sieve (mech 50). Granulosa cells were treated with 0.15 collagenase and 0.1% hyaluronidase in Hank's buffer, at 37°C, for 30 min and digested in a buffer containing: 0,02% EDTA, 0.1% glucose, 0.1% NaCl, 0.19% $NaHCO_3$ and 0.1% trypsin at 37° C for the next 30 mins. Dispersed cells were washed with culture medium (RPMI, containing 0.5% BSA and 10% fetal calf serum) and seeded in culture medium in 96-well culture plates. The cells were then cultured for three days in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. After that period the medium was removed and the cells were cultured under serum-free conditions with NPY, galanin and VIP in varying concentrations: 1, 10, 100 nM. The control culture group was cultured in physiological solution. Cell cultures were maintained for three hours. Culture supernatants were then decanted and stored until a hormone analysis. That method was conducted according to details described previously [19, 20, 21, 22, 23]. The experiments were repeated 4 times, 2 x 10^5/l ml cells were present in each culture. For the statistical analysis, the unpaired Student test and the analysis of variance were used, as appropiate.

Effects of NPY, galanin and VIP on LH, FSH, PRL, GH, TSH release by cultured pituitary cells.

The procedure of pituitary tissue dissociation, cell preparation and cell culture were based on methods described previously [24, 25, 26]. Briefly, pituitary glands were obtained from three-month-old (weight app. 200 g) female WKY rats, anesthetized by pentobarbitalum vetbutal injection and decapitated. They were washed twice with DMEM pH 7.3 with 0.2% glucose, 2 mmol glutamine/l, 0.3% bovine serum albumin (BSA), penicillin (50 U/ml) and streptomycin (50 μg/ml) and processed for culture immediately. They were enzymatically dispersed during 20 min incubation at 37°C in 0.1% trypsin in PBS buffer (without Ca^{2+} and Mg^{2+}) followed by 20 mins of incubation in 0.1% Dnase I (deoxyribonuclease I from bovine pancreas, type IV) in DMEM pH 7.3 with, 0.3% BSA, penicillin (50 U/ml) and streptomycin (50 μg/ml). The glands were finally mechanically dispersed on a sieve (50 mesh) and washed twice by centrifugation for 10 min at 50 g with culture medium (DMEM pH 7.3 with 0.2% glucose, 2 mmol glutamine/l, 0.3% BSA and 10% fetal calf serum (FCS). The pituitary cells were counted in a hemocytometer and assessed for viability by exclusion of trypan blue (>85%).

The pituitary cells (0.2 x 10^6/ml) were incubated in 24-well culture plates for up to 48 hrs in a humidified atmosphere of 95% air and 55 CO_2 at 37°C. The culture plates were washed with twice the volume of the serum-free medium with 30 μg ascorbic acid/l 30 mins before every experiment. The neuropeptides were dissolved in saline at concentrations 1 mmol/l. They were diluted with serum-free medium with 30 μg ascorbic acid /l to final nanomolar concentrations.

For short-term effects, NPY, galanin and VIP were added after 48 hrs of culture and the medium was collected 30, 60, 120, 240 and 480 mins thereafter. The collected medium was stored at -20°C until assayed for LH, FSH, PRL, GH and TSH. All media and chemicals were purchased from Sigma (Sigma-

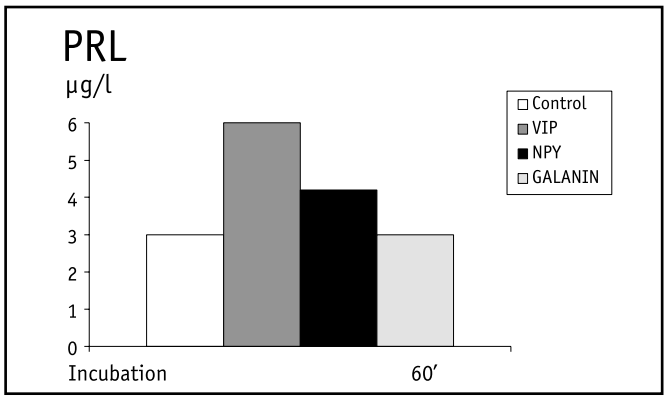

Fig. 1. Effects of NPY, galanin and VIP on prolactin release from cultured pituitary cells.

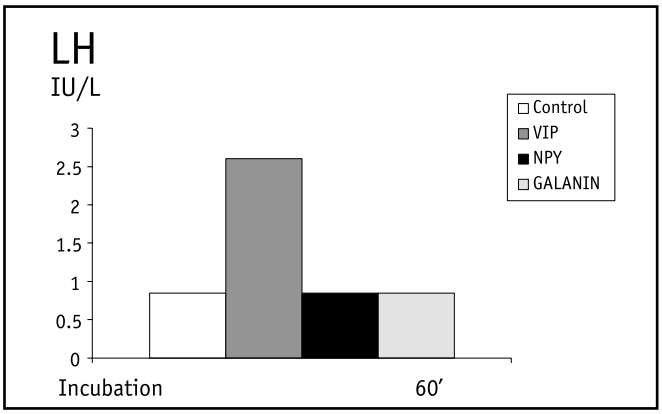

Fig. 2. Effects of NPY, galanin and VIP on LH release from cultured pituitary cells.

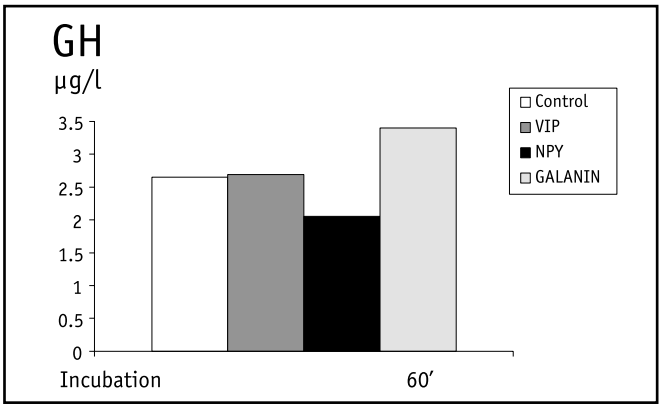

Fig. 3. Effects of NPY, galanin and VIP on GH release from cultured pituitary cells.

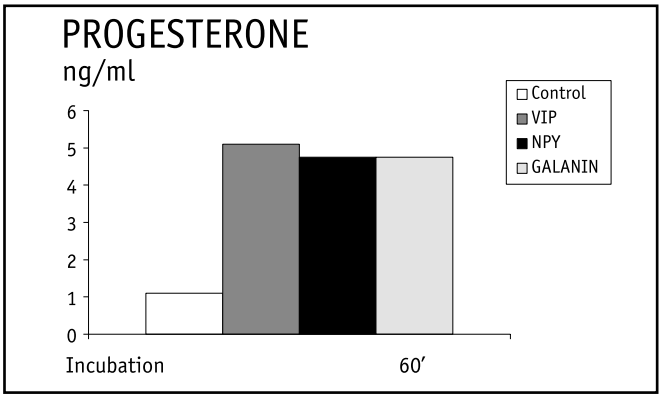

Fig. 4. Effects of NPY, galanin and VIP on progesterone release from cultured granulosa cells.

Aldrich Chemie GmbH, Deisenhofen, Germany) and culture dishes from Corning (Bibby Sterlin Ltd, Staffordshire, UK). The experiments were repeated 3 times, 2 x 10^5/l ml cells were present in each culture.

For the statistical analysis, the unpaired Student test and the analysis of variance were used, as appropriate.

Results

Maximal effects of NPY, galanin and VIP on pituitary hormone release and on gonadal steroids were observed after administration of 10 nM of these peptides during 60 mins incubation.

VIP and NPY but not galanin stimulated PRL release from cultured pituitary cells (Fig. 1). VIP increased also LH release whenever NPY and galanin did not change LH release from pituitary cells (Fig. 2).

Galanin but not NPY and VIP leads to an increase of GH production (Fig. 3).

VIP, NPY and galanin did not change TSH and FSH release (data not shown).

NPY, galanin and VIP markedly stimulated progesterone release from cultured granulosa cells (Fig. 4).

NPY, galanin and VIP did not change estradiol and testosterone release (data not shown).

Discussion

Our present and previous results [18] have demonstrated that VIP markedly increases progesterone production by cultured rat granulosa cells. The findings of Davoren et al. [27] confirmed the hypothesis that VIP was able to stimulate ovarian steroidogenesis. Previously we have published data about stimulation of adrenal progesterone release in response to VIP administration [28].

The effect of VIP on gonadotropin release remains controversial. It was reported that an intracerebroventricular injection of VIP led to an increase of LH-RH release, however, an intravenous administration of VIP did not change LH secretion [29, 30]. Our studies in vitro demonstrate that VIP stimulated PRL and LH release from pituitary cells. Some studies indicated that the orexigenic peptide NPY may play an important role not only in the control of appetite but also in the mechanism of hormone secretion [31, 32]. NPY neurons regulate the activity of the reproductive axis at hypothalamic and adenohypophysial levels [33]. NPY can stimulate LH-RH release in vivo through a direct Y1-like receptor-mediated action on

the LH-RH neuron itself [9].

Some authors demonstrated that NPY neurons participate in the generation of LH surge through increased production of NPY and subsequent potentialization of the release and/or action of LH-RH [7, 8]. On the other hand, NPY is one of the strongest orexigenic factors in the hypothalamic control of feeding behavior [31, 32, 33, 34].

NPY may have a role in mediating nutritional effects on the reproductive system and GH release [35].

It has been reported that leptin—a protein secreted by adipocytes—may modulate the NPY activity in the central nervous system [12].

NPY can inhibit GH secretion in the male and female rats [36, 37, 38] possibly by stimulating hypothalamic somatostatin release.

Our studies "in vitro" have demonstrated that NPY can directly stimulate PRL release from pituitary cells. We did not observe any effects on LH and GH release. However, NPY strongly stimulated progesterone release from cultured granulosa cells. Galanin-like immunoreactivity was discovered in the highest concentrations in the median eminence and this finding may suggest that galanin may have a role in the regulation of the anterior pituitary function [35, 39].

Galanin is produced in the hypothalamus and anterior pituitary and its synthesis is highly stimulated by estrogens [40]. It was also reported that galanin controlled the preovulatory surges of LH and PRL [10] and regulated steroidogenesis in ovarian tissue as an intraovarian regulatory peptide [14]. Galanin is cosecreted with GnRH and can modulate the secretion of GnRH [11].

Galanin increased GH release in rats after central (icv) and peripheral administration [41, 42, 43]. Immunoneutralization of endogenous galanin within the CNS significantly disrupts a normal GH-secretory pattern in male rats [44]. These results suggest an important physiological role for endogenous galanin in the control of spontaneous GH secretion [35].

Our results showed that galanin directly stimulated GH release from cultured pituitary cells. These findings are in accordance with "in vivo" studies [41, 42, 43]. We observed that galanin markedly increased progesterone production by cultured granulosa cells. These results confirmed the thesis that galanin takes part in the intraovarian regulation of progesterone release.

Conclusions

1. Direct effects of NPY, galanin and VIP on pituitary hormone release may indicate their role in the mechanism of pituitary hormone release.

2. A marked increase of progesterone from cultured granulosa cells after VIP, NPY and galanin suggest that these peptides may be involved in the local ovarian steroidogenesis.

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