

# The Relationship Between Neuropeptides and Hormones in Starvation

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## **Abstract**

**OBJECTIVES:** Some hormonal disturbances were demonstrated in starvation. Leptin, NPY and galanin play an important role in the control of appetite and in the mechanism of hormone release.

**METHODS:** In order to evaluate the effect of starvation on the relationship between leptin, neuropeptide Y (NPY) galanin and pituitary and gonadal hormones release, plasma leptin, NPY and galanin as well as serum LH, FSH, prolactin (PRL), estradiol, progesterone levels in non-starved female rats (in diestrus) and after 72 hrs of starvation were measured with RIA methods. Effects of leptin, NPY and galanin administration on pituitary and gonadal hormones were investigated *in vivo* and *in vitro* experiments.

**RESULTS:** Plasma leptin, NPY and galanin as well as serum estradiol and progesterone concentrations were significantly lower in starved rats as compared with non-starved rats. However serum prolactin level was significantly higher in starved rats. Opposite effects after leptin and NPY administration on hormone release *in vivo* and *in vitro* experiments were observed in non-starved rats. However, in starved rats we did not find changes in pituitary and gonadal hormones release after leptin, NPY and galanin injection or the hormonal response was blunted.

**CONCLUSIONS:** 1) The disturbances in neuropeptides activity and in hormones release were observed in starvation. 2) Leptin, NPY and galanin have direct and indirect effects on pituitary and gonadal hormones release. 3) In starvation the hormonal response to leptin, NPY and galanin is impaired.

## Introduction

Some neuropeptides play an important role in the control of appetite and in the mechanism of hormone release [1, 2, 3, 4, 5, 6, 7, 8, 9]. Our previous results have demonstrated disturbed neuropeptides release in both obese and anorectic patients [10, 11, 12]. It has been reported that starvation activates the hypothalamic-pituitary adrenal axis and suppresses gonadal should this be gonadal, somatotropic and thyroid axes as a response for adaptation [13]. Neuropeptides such as a neuropeptide Y (NPY), galanin and leptin may affect hormones release and on other hand, the hormonal status may modulate neuropeptide activity [6, 7, 8, 14, 15]. The aim of this study is to evaluate the effect of starvation on the relationship between leptin, neuropeptide Y (NPY) and galanin and pituitary and gonadal hormones release.

## Material and Methods

Plasma leptin, galanin concentrations as well as serum LH, FSH, prolactin (PRL), estradiol, progesterone levels in non-starved female rats (in diestrus) and after 72 hrs of starvation were measured with RIA methods. Plasma NPY, galanin and leptin concentrations were measured using commercial Kits (Peninsula Lab., Belmont, CA). Sensitivity of NPY assay was 2 pg/tube and the inter-assay and intra-assay co-efficients of variation were 8,5% and 7,5%, respectively. Sensitivity of galanin was 13 pg/tube and the inter-assay and intra-assay co-efficients of variation were 7,3% and 6,1%, respectively. Sensitivity of the leptin assay was 0,5 ng/ml and inter-assay and intra-assay co-efficients of variation were 8,3% and 6,2% respectively.

Effects of leptin, NPY and galanin administration on pituitary and gonadal hormones were evaluated in experiments *in vivo* in non-starved and starved rats.

Leptin, NPY and galanin were injected intravenously (i.v.) in a doses 1, 5, 10 µg and the blood was withdrawn after 15 mins. Statistical analyses were performed with nonparametric tests using the program Statistics and Distribution Fitting (Statistica and Windows) \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Progesterone and estradiol release from cultured ovarian granulosa cells and LH, FSH, PRL from cultured pituitary cells were investigated in experiments *in vitro*.

### *Effects of NPY, galanin and leptin on progesterone, estradiol production by cultured rat granulosa cells.*

The method of culture of granulosa cells was conducted according to details described previously [18, 19, 20, 21, 22]. 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 (mesh 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<sub>3</sub> and 0.1% trypsin at 37°C for the next 30 minutes. 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<sub>2</sub> at 37°C. After that period the medium was removed and the cells were cultured under serum-free conditions with NPY, galanin and leptin in varying concentrations: 1, 10, 100 nM. The control culture group was cultured in a physiological solution. Cell cultures were maintained for three hours. Culture supernatants were then decanted at 30, 60, 120 mins and stored until a hormone analysis. The experiments were repeated 4 times, 2x10<sup>5</sup>/1 ml cells were present in each culture.

### *Effects of NPY, galanin and leptin on LH, FSH, PRL release by cultured pituitary cells.*

The procedure of pituitary tissue dissociation, cell preparation and cell culture were based on methods described previously. Briefly, pituitary glands were obtained from three-month-old (weight app. 200 mg) 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<sup>2+</sup> and Mg<sup>2+</sup>) 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<sup>6</sup>/ml) were incubated in 24-well culture plates for up to 48 hrs in a humidified atmosphere of 95% air and 5% CO<sub>2</sub> 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

$\mu\text{g}$  ascorbic acid/l to final nanomolar concentrations.

For short-term effects, NPY, galanin and leptin were added after 48 hrs of culture and the medium was collected 30, 60, 120 mins thereafter. The collected medium was stored at  $-20^{\circ}\text{C}$  until assayed for LH, FSH, PRL.

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

## Results

### *Leptin, NPY and galanin in non-starved and starved rats.*

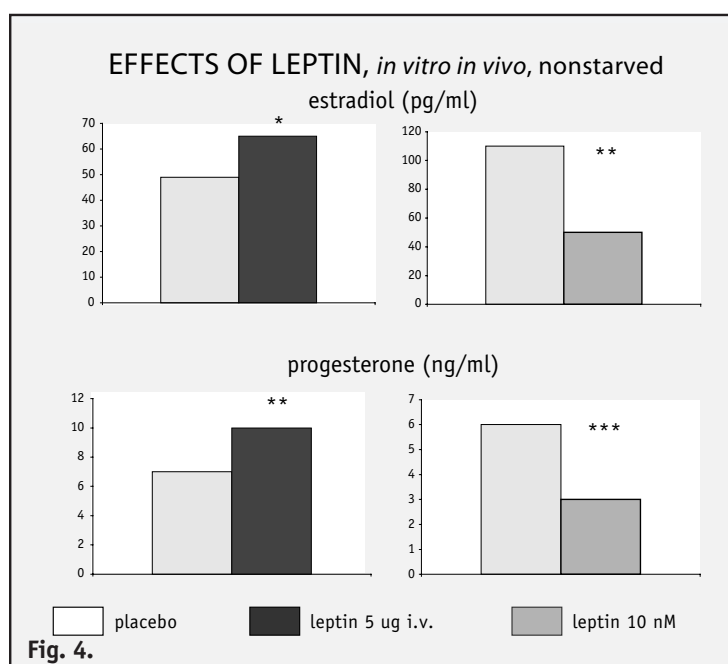
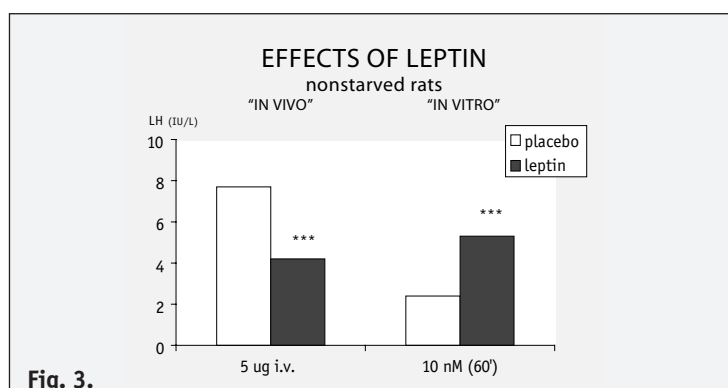
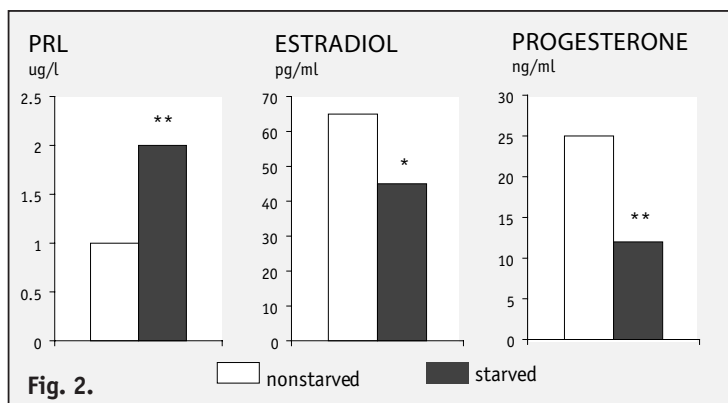
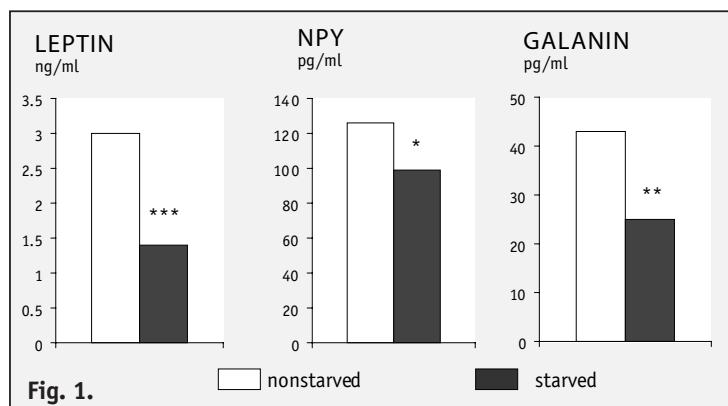
Plasma leptin, NPY and galanin concentrations were presented in Figure 1. Plasma leptin, NPY and galanin concentrations in starved rats were significantly lower as compared with non-starved rats ( $p < 0.001$ ,  $p < 0.05$ ,  $p < 0.01$ , respectively).

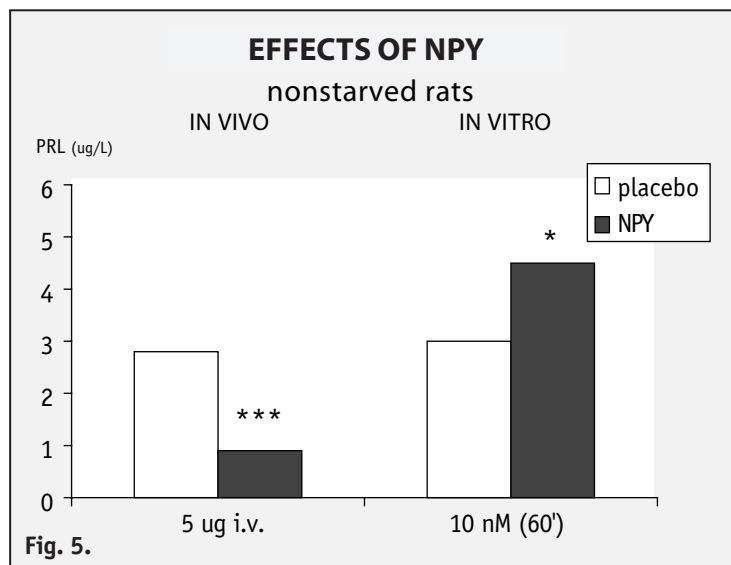
### *Pituitary and gonadal hormones in non-starved and starved rats.*

In starved rats serum progesterone and estradiol levels were significantly lower ( $p < 0.01$ ,  $p < 0.05$  respectively). However, the serum prolactin (PRL) level was significantly higher ( $p < 0.01$ ) than that in non-starved rats (Fig. 2). Serum LH and FSH did not differ significantly (data non shown).

### *Effects of leptin administration on pituitary and gonadal hormones release in vivo and in vitro experiments.*

Maximal effects of leptin, NPY and galanin were observed after administration of  $5 \mu\text{g}$  i.v. – *in vivo* experiments and after administration to cell culture of  $10 \text{ nM}$  leptin, NPY and galanin after 60 min incubation *in vitro* experiments. In non-starved rats, leptin decreased significantly LH release ( $p < 0.01$ ) *in vivo* experiment (Fig. 3). Opposite effects were observed *in vitro* experiment. Leptin at a dose  $10 \text{ nM}$  after 60 mins of incubation increased LH release from cultured pituitary cells in non-starved rats (Fig. 3). In non-starved rats, leptin injected i.v. led to the increase of estradiol ( $p < 0.05$ ) and progesterone ( $p < 0.01$ ) (Fig. 4) and *in vitro* it caused a decrease of estradiol ( $p < 0.01$ ) and progesterone release ( $p < 0.001$ ) from cultured granules cells (Fig. 4). In starved rats any effects of leptin

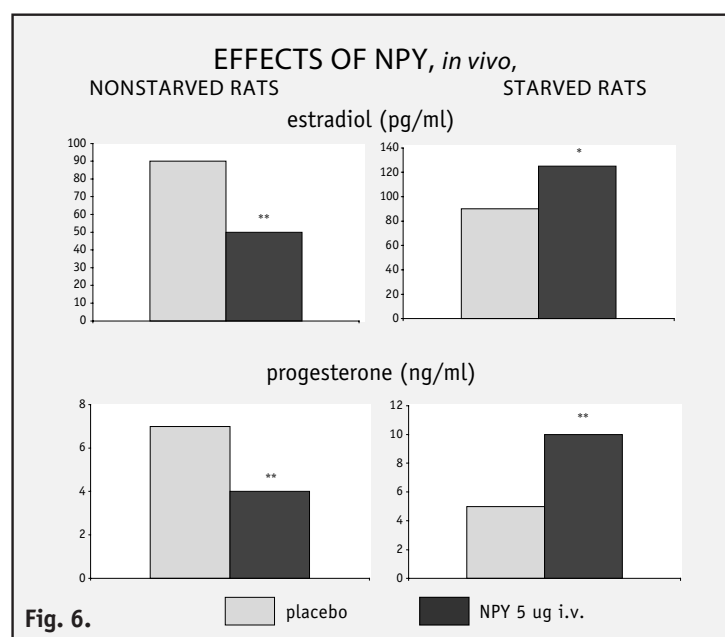




action were observed *in vivo* and *in vitro* experiments.

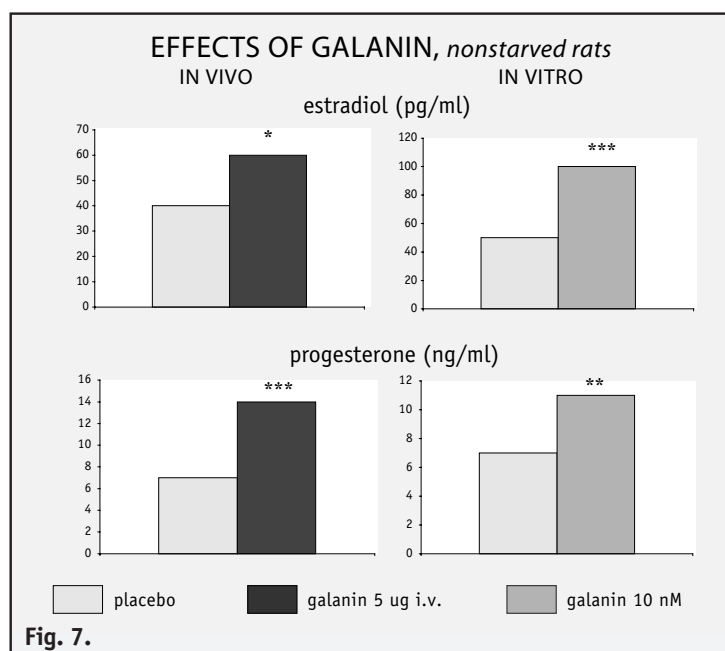
*Effects of NPY administration on pituitary and gonadal hormones release in vivo and in vitro experiments.*

In non-starved rats NPY administered i.v. (*in vivo*) decreased PRL release ( $p < 0.001$ ) and increased PRL release from cultured pituitary cells ( $p < 0.05$ ) (Fig. 5). NPY *in vivo* decreased estradiol and progesterone ( $p < 0.01$ ,  $p < 0.01$ , respectively) (Fig. 6). In vitro NPY did not produce significant changes in estradiol and progesterone release. In starved rats NPY injected i.v. led to an increase of estradiol and progesterone release ( $p < 0.05$ ,  $p < 0.01$ , respectively) (Fig. 6).



*Effects of galanin administration on pituitary and gonadal hormones release in vitro and in vivo experiments.*

In non-starved rats, galanin in experiments *in vivo* stimulated estradiol and progesterone release ( $p < 0.05$ ,  $p < 0.001$ ) (Fig. 7), as well as it stimulated estradiol and progesterone in experiments *in vitro* ( $p < 0.001$ ,  $p < 0.01$ ) (Fig. 7). In starved rats galanin did not cause significant changes in estradiol and progesterone release. We did not find significant changes in pituitary hormones release in either non-starved and starved rats.



**Discussion**

Our results showed a decrease of plasma NPY, galanin and leptin in starved rats.

It is known that NPY, galanin and leptin play an important role in the control of appetite, energy expenditure and in the neuroendocrine regulation of hormone release. NPY is widely distributed in the central (CNS) and peripheral (PNS) nervous system but also it is found in the adrenal medulla and in  $\beta$  cells of pancreas [26, 27, 28]. NPY neurons regulate the activity of the reproductive axis at hypothalamic and hypophyseal levels. NPY stimulates GnRH release *in vivo* through a direct  $Y_1$ -like receptor-mediated action on the LH-RH neuron itself [29]. NPY neurons participate in the generation of the LH surge through increased production of NPY and subsequent potentiation of the release and / or action of Gn-RH [30]. However, central infusion of NPY inhibits reproductive function in rats and delays sexual maturation [8].

Galanin has been discovered in the central nervous system, the adrenal medulla and the gastrointestinal, genitourinary and respiratory

tracts [31, 32]. Galanin is produced in the hypothalamus and anterior pituitary and its synthesis is highly stimulated by estrogen [33]. It has also been reported that galanin controls pre-ovulatory surges of LH and prolactin [34] and regulates steroidogenesis in ovarian tissue as an intra-ovarian regulatory peptide [35]. Galanin is co-secreted with gonadotropin-releasing hormone (GnRH) and can modulate the secretion GnRH [36]. NPY and galanin can regulate eating behavior and hormonal control via both central and peripheral mechanisms. The interaction between central and peripheral signals is due to leptin. Leptin – a hormone secreted by adipocytes, informs the brain of the amount of adipose tissue in the body [37]. The ob leptin receptors are expressed strongly in hypothalamus and leptin may modulate the activity of peptides controlling feeding behavior [38, 39]. Leptin injected into ob/ob mice, which are genetically deficient in leptin, not only produced body weight reduction but also restored fertility [40]. These findings suggest that normal leptin secretion is necessary for normal reproductive function [9, 41].

The work of Chehab et al. [9] suggests that leptin triggers puberty. In humans, during the 'juvenile obesity' phase, increased circulating leptin exerts an effect on the body fat and energy metabolism preceding onset of puberty [42].

Impairment of reproductive function has been known to occur under conditions of food restriction and/or increased energy expenditure [5, 43, 44]. The secretion of leptin is related to changes in food intake. At 22h of fasting leptin production rates in lean and obese patients were decreased [45]. Kelly et al [46] demonstrated that fall in leptin with fasting causes a rise in neuropeptide Y (NPY) messenger RNA (mRNA).

Our previous results indicated that in anorectic patients, low levels of leptin did not cause an increase in NPY and this finding confirms the existence of disturbances in the feedback mechanism of leptin-NPY [11]. It has been reported that falling leptin levels in response to starvation results in decreased estradiol levels and amenorrhoea in subjects with anorexia nervosa or strenuously exercising athletes [15]. The circulating leptin exert a stimulatory effect on the reproductive axis and 3-day starvation completely abolished both LH and PRL surges [16].

Ours results demonstrated a decrease of estradiol, progesterone release in starved rats, however serum PRL was increased. We also observed a marked decrease of plasma NPY, galanin and leptin in starved rats.

These findings may suggest the existence of a relationship between activity of neuropeptides and disturbed hormone secretion in starvation and may confirm the hypothesis of other authors, that hypo-

thalamic neuropeptides synthesis responds rapidly to the altered metabolic signals [47]. Eckert et al. showed that low leptin levels in untreated anorexia nervosa increased with refueling [48]. Barash et al [49] showed that administration of leptin led to an increase of ovaries weight and stimulation of LH release. In *in vitro* experiments leptin stimulated release of LH-RH in medial basal hypothalamus (MBH) and gonadotropin from the pituitary [50].

In our studies we found the differences in hormonal response to leptin, NPY and galanin administration in non-starved and starved rats. In non-starved rats, leptin injection caused a decrease in LH release and an increase in estradiol and progesterone. Opposite effects were observed in experiments *in vitro*. Leptin increased LH release from pituitary cell culture and it decreased estradiol and progesterone release from cultured granulosa cells. However, in starved rats any effects of leptin action were observed *in vivo* and *in vitro* experiments. Injection *in vivo* of NPY decreased PRL release and decreased progesterone and estradiol release in non-starved rats, *in vitro* experiments NPY did not produce significant changes in gonadal hormones release. In starved rats NPY *in vivo* stimulated estradiol and progesterone. Galanin *in vivo* and *in vitro* experiments stimulated estradiol and progesterone release in non-starved rats. However, in starved rats galanin did not cause significant changes in gonadal hormonal release.

Opposite effects of leptin and NPY action in experiments *in vivo* and *in vitro* suggest that some other factors despite direct effects may be involved in the mechanism of hormone release in response to leptin and NPY. It has been published that NPY, leptin, galanin co-exist with a number of other of neurotransmitters and neuropeptides as a catecholamines, serotonin, acetylcholin, insulin, somatostatin,  $\beta$ -endorphin, enkephalin, GABA (gamma-aminobutyric acid) which may modulate their effects of their action on hormones release and control of appetite [11, 31, 38, 39, 51, 52, 55]. On the other hand it could be speculated that starvation may lead to a decrease of neuropeptide activity and to dysfunction in response and sensitivity of hormone release to neuropeptides action.

## Conclusions

1. The disturbances in neuropeptides activity and in hormones release were observed in starvation.
2. Leptin, NPY and galanin have direct and indirect effects on pituitary and gonadal hormones release.
3. In starvation, the hormonal response to leptin, NPY and galanin is impaired.

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