Interactions between the neuropeptide Y system and the hypothalamic–pituitary–adrenal axis

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Abstract
The aim of this paper is to review the present knowledge of interactions between the neuropeptide Y (NPY) system and the hypothalamic–pituitary–adrenal (HPA) axis. On the basis of in vitro and in vivo studies of various animal species, we review the effects of NPY on all levels of HPA axis activity. We also describe the effects of glucocorticosteroids on the NPY system in the hypothalamus, including interactions between glucocorticosteroids and insulin. On the basis of available literature, we discuss the role of these interactions in the control of food intake and in the pathogenesis of obesity.

Introduction
Neuropeptide Y (NPY) is a 36-amino-acid peptide that belongs to the family of pancreatic polypeptides. High levels of NPY are detected in the central and peripheral nervous systems both of humans and of many animals (1). NPY functions as a neurotransmitter or neuromodulator (1), and its action is mediated by specific receptors, Y1, Y2, Y3, Y4, Y5, Y6 (2). The major effects of NPY on the central nervous system are as follows: stimulation of food intake, antianxiety effect, regulation of hormone synthesis and release, and control of circadian rhythms (3). In turn, NPY gene expression and NPY levels are regulated by many factors, among which hormones have an important role (3, 4).

The aim of this paper is to summarize the present knowledge of interactions between the NPY system and the hypothalamic–pituitary–adrenal (HPA) axis.

Role of NPY in regulation of the HPA axis in the hypothalamus
The hypothalamic paraventricular nucleus (PVN) is the main site of accumulation of nerve terminals of NPY-containing neurons, the perikaryons of which are located mainly in the hypothalamic arcuate nucleus (ARC) and partially in the brainstem. In the brainstem neurons, NPY is colocalized with epinephrine or norepinephrine (1). The PVN, especially its parvocellular subdivision, is the main site of synthesis of corticotropin-releasing factor (CRF) in the hypothalamus (5).

Electron microscopy has revealed synapses between NPY-containing neurons and the dendrites and cell bodies of CRF-ergic neurons (5). These synapses are asymmetric in shape, indicating a stimulatory character of transmission (6). In vitro (7) and in vivo (8, 9) studies have revealed that NPY stimulates CRF release and increases CRF gene expression (10). Consequently, the level of pro-opiomelanocortin mRNA increases (10), as does the release of ACTH (9–11), cortisol (12), and corticosterone (13, 14). NPY influences the HPA axis in a dose-dependent manner (10). In vivo, the HPA axis is stimulated by NPY given by different routes: into the lateral ventricle (10, 13), the third ventricle (11, 15), the cistern (8), intravenously (10), or directly into the PVN (9, 14). The greatest CRF release was induced by picomolar doses of NPY injected into the PVN, which proves that the PVN has an important role in the activation of the HPA axis by NPY. The weakest stimulatory effect of NPY on CRF secretion was observed after intravenous doses of NPY, because the permeability of the blood–brain barrier for NPY is low.

In dogs subjected to hypoglycemic stress, anti-NPY γ-globulin significantly decreased ACTH and cortisol release, indicating that endogenous NPY plays a part in the physiological regulation of the HPA axis (16). In turn, in NPY knockout mice this peptide has been shown to be unnecessary for the appropriate function of the HPA axis in animals feeding ad libitum or under acute fasting conditions (17).

It is unclear how NPY-containing nerve terminals stimulate CRF-ergic neurons. It seems that NPY directly affects CRF synthesis and release, though an indirect
action (via influencing catecholaminergic neurotransmission) cannot be excluded. The latter is less probable, because the antagonists of \( \alpha_1 \)- and \( \beta \)-adrenergic receptors given at doses that block CRF release in vitro do not alter the NPY-dependent secretion of CRF (7). Also, the in vivo study by Suda et al. (10) has shown that non-selective \( \alpha \)- or \( \beta \)-adrenergic receptor antagonists – phentolamine and propranolol respectively – do not alter the effect of NPY on CRF gene expression and ACTH release. Nevertheless, other authors have suggested that an increase in CRF content after a single central injection of NPY requires normal concentrations of epinephrine and norepinephrine, and that this effect may be mediated via the \( \alpha_2 \)-receptor. They observed that the depletion of these catecholamines with 6-hydroxydopamine allowed NPY to stimulate CRF release profoundly, with no evidence of altered CRF synthesis, the effect common for \( \alpha_2 \)-stimulation (9).

The NPY receptor that mediates an increase in HPA axis activity is as yet unknown. In addition, results from studies of dogs and rats are inconsistent. It has been shown that only intact NPY can stimulate ACTH and cortisol release in dogs, whereas its C-terminal fragment, NPY (19–36), and the NPY analog, NPY (1–36)-OH, are ineffective (12). In contrast, Small et al. (18), using different NPY analogs, have obtained results suggesting that the rat hypothalamic NPY receptor, which increased plasma ACTH concentrations, has a fragment activation profile unlike those of Y1–Y4 and Y6 receptors, and appears distinct from the NPY receptor controlling food intake. They also suggest that the activity of this receptor seems to be similar to that of the Y5 receptor.

The stimulatory effect of NPY injection on HPA axis activity in rats with free access to food after NPY was stronger than the effect observed in rats that were fasted after NPY treatment (19). These data, and the concept (which is not accepted by all authors) that an overnight fast reduces the corticosterone response to NPY treatment (19), support the view that non-selective Y-receptor controlling food intake. They also suggest that the activity of this receptor seems to be similar to that of the Y5 receptor.

Role of NPY in regulation of the HPA axis in adrenal glands

NPY is likely to be involved in the regulation of the HPA axis activity in the adrenal glands, because NPY-containing neurons have been found in the adrenal capsule and zona glomerulosa (ZG) and NPY-binding sites have been detected in the ZG (23). Acute administration of NPY to rats increases the concentrations of circulating aldosterone (24) and enhances the release of aldosterone in adrenals perfused in situ (25) and through isolated specimens of the adrenal capsule and ZG (26). Chronic administration of NPY to rats with damaged HPA axis and renin–angiotensin system (RAS) stimulates ZG to grow and to synthesize steroids (27). NPY also modifies the ACTH-dependent release of aldosterone. At low ACTH concentrations (comparable to those in plasma) NPY stimulates the steroidogenic response in ZG, which is reflected by increased aldosterone secretion. At high ACTH concentrations, NPY inhibits the stimulatory effect of ACTH on the release of aldosterone (28). These changes are reflected by the concentrations of cAMP. Although NPY does not affect cAMP concentrations in the adrenal cortex, it modifies ACTH-induced increases in cAMP; namely it increases ACTH-dependent cAMP concentrations at low concentrations of ACTH, and decreases cAMP concentrations at high concentrations of ACTH (28). However, the significance of the stimulatory effect of NPY on the synthesis and release of aldosterone is not very important. This view is based on studies that have demonstrated that the NPY receptor antagonist, Ac-[3-(2,6-dichlorobenzyl)-Tyr\(^\text{37},d\)-Thr\(^\text{38} \)]NPY-(27–36) (PYX-2), does not alter aldosterone release, despite blocking NPY-induced aldosterone secretion (23), and that an NPY-induced increase in aldosterone release is relatively small (29).

Reports concerning the influence of NPY on corticosterone secretion by the adrenals are contradictory. Some authors believe that NPY does not influence the release of corticosterone, because it did not affect corticosterone secretion in intact and ACTH-stimulated cells of the zona fasciculata/reticularis (28). Moreover, prolonged administration of NPY to rats with damaged HPA axis and RAS did not stimulate the growth of the
zonae fasciculata and did not alter the plasma corticosterone concentrations (27). In contrast, Malендовicz et al. (30) observed that NPY inhibited release of corticosterone from both intact and ACTH-stimulated adrenal cells, and Neri et al. (31) demonstrated that NPY increased corticosterone release in vitro.

It seems that the role of NPY in the physiological regulation of the HPA axis is of less importance in the adrenal glands than in the hypothalamus.

**Regulation of the hypothalamic NPY system by adrenal steroids**

There are two types of glucocorticoid receptors. Type I receptors have high affinity and low capacity, and type II receptors have lower affinity for corticosteroids (32). In physiological conditions, type I receptors are stimulated tonically because they are highly sensitive to glucocorticosteroids, whereas type II receptors are stimulated only if their concentrations are relatively high, for example, at the peak of circadian release of corticosterone or after stress (32). The type I receptor is often called the mineralocorticosteroid receptor because it has high affinity for aldosterone and it is located in aldosterone-dependent organs, such as the kidneys, salivary glands, and intestine. In the central nervous system, type I receptors have been detected mainly in the limbic system, whereas type II receptors are present in the hypothalamus (32).

The sensitivity of NPY-containing hypothalamic neurons to corticosteroids depends on the density of intracellular and membrane-bound type II glucocorticosteroid receptors. In hypothalamic structures of low receptor density, such as the supraoptic nucleus and lateral part of the paraventricular nucleus, glucocorticosteroids produce a slight increase in NPY immunoreactivity (33). In contrast, in hypothalamic structures of very high receptor density, such as the median part of the paraventricular nucleus, NPY activity responds to glucocorticosteroid administration, and it is conditioned by the concentrations of endogenous corticosterone in plasma (34). The important role of the type II receptor in influencing the NPY system through glucocorticosteroids (Fig. 1) has been confirmed by the following findings: (i) dexamethasone (DEX), a selective stimulator of type II receptors, increased the concentrations of NPY mRNA, NPY, or both, in the basomedial hypothalamus (35), the ARC (36), the PVN (36, 37), and in hypothalamic cell culture (this effect was blocked by the type II receptor antagonist, RU 486) (38); (ii) RU 486 (mifepristone), unlike the type I antagonist, RU 318, blocks the effect of NPY on food intake (39); and (iii) circadian NPY activity correlates with circadian corticosterone, but not aldosterone activity (40). An additional piece of evidence for a role of the type II receptor in the regulation of the NPY system in the hypothalamus is that some authors have not detected any changes in the concentrations of NPY mRNA and NPY in the ARC in adrenalectomized rats (41). Other authors, however, question these results, as they have found a decrease in NPY gene expression (42) and in NPY concentrations in the ARC (43). Moreover, adrenalectomy does not alter NPY concentrations in hypothalamic areas, where NPY is contained mainly in the nerve terminals (34, 41), except for hypothalamic structures with particularly abundant terminals of NPY-containing neurons, namely the parvocellular area of the PVN, the dorsomedial nucleus, and the medial preoptic area, where adrenalectomy decreases NPY immunoreactivity (34).
Chronic administration of corticosterone to adrenalectomized rats increases NPY system activity in the ARC and locus coeruleus, the sites of NPY synthesis. This increased NPY activity is confirmed by increases in NPY gene expression, NPY concentrations, Y1 receptor mRNA levels, and in the affinity of this receptor for 125I-labelled peptide YY (34, 44). The results of the studies conducted on adrenalectomized rats indicate that the influence of adrenal steroids is significantly more pronounced in these animals than in intact ones (34). According to Ponsalle et al. (45), glucocorticosteroids are indispensable to an increase in NPY mRNA in fasted animals; however, some authors do not share this opinion (46, 47).

Moreover, glucocorticosteroids are able to control NPY gene expression in hypothalamic structures belonging to the hypothalamic–neurohypophysial system. This is supported by the finding that adrenalectomy increases NPY mRNA concentrations in these structures, probably as a result of the augmentation of plasma osmolarity, and that this increase is abolished by DEX treatment. The increased NPY gene expression after adrenalectomy is not paralleled by increased NPY concentrations, probably because of disturbances in translation, lower intracellular capacity, or enhanced axonal flow resulting in rapid release of newly synthesized peptide (33, 48).

The influence of glucocorticosteroids on the hypothalamic NPY system is not only direct, but also indirect, via the CRF system (Fig. 1). Chronic administration of CRF into the hypothalamus decreases food intake, body weight gain, and insulinemia in rats (32). These effects are opposite to the action of NPY (3). CRF inhibits NPY synthesis and release (3, 49) and decreases NPY-induced food intake (32). Glucocorticosteroids inhibit the release of CRF, thus abolishing its inhibitory effect on NPY-ergic neurons and shifting the balance between NPY and CRF in favor of the former.

An interaction between corticosterone and hypothalamic NPY neurons is important for the circadian rhythmicity of some physiological processes, such as food intake, locomotor activity, corticosterone secretion, and occupation of the type II glucocorticoid receptor, all of which are strongest at the beginning of the active phase of circadian cycle (34).

Involvement of complex interactions between the HPA axis and the NPY system in regulation of food intake and pathogenesis of obesity

The important role of corticosteroids in the control of NPY activity has been confirmed by the facts that adrenalectomy or hypophysectomy inhibits the stimulatory effect of NPY on food intake (50, 51) and that adrenalectomy reverses the increased body weight and plasma concentrations of corticosterone, insulin and triglyceride observed after several days of NPY treatment in intact rats (13).

Strack et al. (52) suggested that glucocorticosteroids and insulin are major antagonistic regulators of energy balance and that their long-term effects on food intake are in part mediated by the regulation of hypothalamic NPY synthesis and release. In contrast to the relationship between NPY and steroids, the relationship between the hypothalamic NPY system and insulin possesses the feature of negative feedback (3). The ARC contains abundant insulin receptors, and insulin decreases the concentrations of NPY and its mRNA in this structure (53). In the hypothalamus of diabetic rats, hypoinsulinemia is accompanied by increased synthesis and concentrations of NPY (38, 53). Insulin also regulates the feedback between glucocorticosteroids and NPY, because it inhibits DEX-induced NPY gene expression in the ARC and increases in NPY concentrations in the ARC and PVN (36).

The opposing effects of insulin and glucocorticosteroids on the hypothalamic NPY system are very important for the regulation of food intake (52). However, it should be borne in mind that NPY activity is also modulated by other factors, such as β-endorphin, serotonin and leptin – a recently discovered fat tissue hormone of potent anorexigenic activity. All these factors influence hypothalamic NPY concentrations and NPY gene expression via both direct and indirect mechanisms, and the net effect results from their mutual interactions (4). This complex regulation may explain the failure of some authors to find and parallel changes in NPY gene expression in the basomedial hypothalamus, in NPY release in the PVN, and in plasma corticosterone concentrations in fasted animals (46, 47).

The positive feedback between corticosterone and NPY, which has an important role in the central regulation of food intake, in pathological conditions may produce a ‘vicious circle’ of events. This has been shown in obese Zucker rats, obese ob/ob mice, patients with anorexia nervosa, animals and humans exposed to stress and to glucocorticosteroids (44, 52, 54).

Obese Zucker rats exhibit increased NPY mRNA and NPY concentrations in the ARC and PVN respectively, which led to increased NPY release (55). The altered activity of the hypothalamic NPY system in these rats results, not only from insensitivity to insulin (53) and mutations of the leptin receptor (4), but also from increased plasma corticosterone concentrations (56) and increased sensitivity to corticosterone (57). In turn, NPY increases, via a positive feedback, plasma corticosterone concentrations (32). Such a vicious circle of events occurring in these conditions is confirmed by the finding that adrenalectomy (58) and hypophysectomy in these rats (59) reduce most symptoms of obesity.

In ob/ob mice, obesity is caused by a genetic disturbance of leptin synthesis. Leptin reverses increases
in the hypothalamic NPY mRNA and NPY concentrations (4) and enhanced HPA axis activity that are observed in these mice (60). Because, in intact rats, leptin blocks corticosterone-induced NPY release (61), it is not surprising that, in adrenalectomized ob/ob mice, glucocorticosteroids accelerate hypothalamic NPY axonal transfer and release in the PVN (62). It is likely that high corticosterone concentrations and increased NPY system activity observed in ob/ob mice may produce a vicious circle of events. This suggestion is supported by the fact that adrenalectomy normalizes many metabolic defects in ob/ob mice (4, 62).

It has been found that patients with anorexia nervosa have increased NPY concentrations in their cerebrospinal fluid (63). The contradiction between decreased food intake in anorexia nervosa and increased concentrations of NPY (which stimulates food intake) may be explained by a masking of hunger by the discomfort associated with eating (63). The increased NPY concentrations in cerebrospinal fluid from patients with anorexia nervosa may result from increased NPY synthesis, transport and release induced by the increased plasma concentrations of ACTH and cortisol in these patients (64). This suggestion may be confirmed by a decrease in cortisol and NPY concentrations after the normalization of body weight (63, 65).

Chronic stress or administration of glucocorticosteroid increase both glucocorticosteroids and insulin in plasma, while their ratio remains normal (52). Consequently, energetic compounds start to accumulate in abdominal adipose tissue instead of muscle tissue. It is likely that some types of abdominal obesity may be due to increased HPA axis activity (54). As corticosterone increases food intake and carbohydrate consumption, a prolonged excess of corticosterone may lead to obesity and insulin resistance, in which the hypothalamic NPY system may play a significant part (52, 54).

**Conclusions**

The relationship between the NPY system and the HPA axis is complex (Table 1) and seems to include positive feedback between NPY and adrenal corticosteroids and negative feedback between CRF and NPY. The stimulation of the HPA axis by NPY takes place mainly in the hypothalamus, primarily as a result of increased CRF synthesis in the PVN and increased CRF release in the median eminence. The role of NPY in the regulation of the HPA axis in the pituitary and adrenal glands is of less significance, but cannot be excluded. In the pituitary, the effects of NPY vary from animal to animal. In the adrenal cortex, the effect of NPY is strongest in the ZG, where NPY affects the synthesis and release of aldosterone. Glucocorticosteroids increase NPY and NPY mRNA concentrations in the rat hypothalamus, and their effects on the hypothalamic NPY system are primarily due to the stimulation of the type II receptor. The feedback loop between glucocorticosteroids and NPY plays a significant part in the regulation of their metabolic functions, and any disturbance may be conducive to metabolic diseases. The activity of the NPY system is also regulated by other factors, among which insulin has an important role. Despite extensive research into the relationship between the NPY system and the HPA axis, not all issues have yet been clarified and therefore they require further study.

**Table 1** Effects of NPY on various components of the HPA axis.

<table>
<thead>
<tr>
<th>Site of action</th>
<th>Type of action</th>
<th>Animal species</th>
<th>Effect</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>Direct</td>
<td>Rat, sheep, dog</td>
<td>Increased CRF synthesis and release</td>
<td>The main site of the HPA axis that is stimulated by NPY</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>Indirect</td>
<td>Rat, sheep, dog</td>
<td>Enhanced secretion of ACTH</td>
<td>Indirect effect on hypothalamic levels is more important than direct effect</td>
</tr>
<tr>
<td></td>
<td>Direct</td>
<td>Sheep Dog</td>
<td>No effect</td>
<td>Direct effect depends on animal species</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>Indirect</td>
<td>Rat, sheep, dog</td>
<td>Increased corticosterone or cortisol release</td>
<td>Indirect effect (secondary to increased secretion of ACTH) is more important than direct effect</td>
</tr>
<tr>
<td></td>
<td>Direct</td>
<td>Rat</td>
<td>Increased aldosterone synthesis and release</td>
<td>Modulation of ACTH-induced aldosterone secretion</td>
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<td></td>
<td></td>
<td></td>
<td>Contradictory results for corticosterone release</td>
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