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Invited Review

Neuropeptide Y-related peptides and hypothalamo-pituitary-adrenal axis function

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Summary. Current data on the localization of neuropeptide Y-related peptides in the hypothalamo-pituitary-adrenal gland (HPA) axis as well as the effects of these peptides on the function of cells comprising HPA axis are presented and discussed. The action of neuropeptide Y, peptide YY, and pancreatic polypeptide on HPA axis are evaluated. Moreover, we report the presence of pancreatic polypeptide immunoreactivity in subset of chromaffin cells in the medulla of rat adrenal gland.

Key words: Hypothalamus, Pituitary, Adrenal, Neuropeptide, Neuropeptide Y, Peptide YY, Pancreatic polypeptide, Peptide Y, ACTH, Aldosterone, Corticosterone

The neuropeptide Y (NPY)-related peptides, their structure and receptors

Neuropeptide Y (NPY) belongs to a family of peptides that also includes peptide YY (PYY), pancreatic polypeptide (PP), and nonmammalian (fish) pancreatic peptide Y (PY). From this family PP was originally discovered by Kimmel et al. (1968, 1975), followed by PYY (Tatemoto and Mutt, 1980), NPY (Tatemoto, 1982; Tatemoto et al., 1982), and finally by PY (Andrews et al., 1985). Neuropeptide Y-related peptides - formerly called pancreatic polypeptide family - share a common secondary structure known as the PP-fold. All of them are composed of 36 amino-acid residues (Wahlestedt and Reis, 1993). Their primary sequence is shown in Table 1. Sequence comparison studies indicate two distinct evolutionary lineages, one containing PP and one containing NPY and PYY (Schwartz et al., 1989). Moreover, available data suggest that NPY is one of the most highly conserved neuropeptides known (Schwartz et al., 1989; Blomqvist et al., 1992).

NPY receptors belong to the superfamily of the G protein-coupled receptors, with typical seven membrane-spanning domains (Herzog et al., 1992; Larhammar et al., 1992; Wahlestedt and Reis, 1993). At least three distinct NPY receptors have been identified by pharmacological criteria. Y1 receptor binds NPY and PYY with equal affinity and is the only receptor able to respond to an analogue of NPY modified at residues 31 and 34 (Leu^{31}, Pro^{34} NPY) (Fuhlendorff et al., 1990; Michel et al., 1990; Wahlestedt et al., 1990, 1992; Michel, 1991). The Y2 receptor also binds NPY and PYY with similar affinity. However, this type of receptor binds NPY peptide derivatives containing carboxyl-terminal fragments (Wahlestedt et al., 1986; Beck-Sickinger et al., 1992). The Y3 receptor binds NPY with at least 100-fold higher affinity than PYY (Balasubramaniam et al., 1990; Grundemar et al., 1991; Wahlestedt et al., 1992).

Brain NPY and PYY receptors in most vertebrate species form fish to man are pharmacologically similar and their expression was found - among others - in hypothalamus and pituitary (Okita et al., 1991).

NPY Y1 and Y2 receptors are widely distributed in the central nervous system and in the periphery, while Y3 subtype has rather limited distribution. The last receptor is present in nucleus of the tractus solitarius, adrenal medulla and heart (Michel, 1991; Grundemar et al., 1991; Wahlestedt et al., 1992; Wahlestedt and Reis, 1993).

Because both Y1 and Y2 receptors bind NPY and PYY equally well, this classification of the receptor subtypes has also been extended to PYY receptor(s) (Inui et al., 1989; Sheik and Williams, 1990; Okita et al., 1991).

Activation of NPY receptors inhibits adenylate cyclase, thus decreasing levels of cAMP and raising intracellular Ca^{++} concentrations. This last effect may be responsible for subsequent weak activation of phosphoinositide turnover (for review see Wahlestedt and Reis, 1993).

Both NPY and PYY exert a wide variety of biological effects; among others they play a role in central regulation of blood pressure (i.e. acting in a vasodepressor way), modulate feeding and appetite

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Table 1. Primary sequence of neuropeptide Y-related peptides. Differences from the human peptide underlined.

NPY	
Human	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
Rat	YPSKPDNPGEDAPAEDMARYYSALRHYINLITRQRY
PYY	
Human	YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY
Rat	YPAKPEAPGEDASPEELSRYYASLRHYLNLVTRQRY
PP	
Human	APLEPVYPGDNATPEQMAQYAADLRRYINMLTRPRY
Rat	APLEPMYPGDYATHEQRAQYETQLRRYINTLTRPRY
PY	
gar	YPPKPENPGEDAPPEELAKYYSALRHYINLITRQRY

behavior, exert influence on learning and memory, and regulate thermoregulation and pituitary hormone secretion. They also contract vascular smooth muscle.

The distribution of NPY and PYY in the components of hypothalamo-pituitary-adrenal (HPA) axis

Hypothalamus and pituitary gland

Paraventricular nucleus of the hypothalamus (NPV) contains a dense system of NPY-immunoreactive terminals which originate from the medulla, locus coeruleus and the arcuate nucleus (Everitt et al., 1984; Bai et al., 1985; Sawchenko et al., 1985; Wahlestedt et al., 1987; Chronwall, 1989; Fuxe et al., 1989). Thus, fibers of the NPV with NPY terminals originate either from catecholamine-containing neurons in the medulla and dorsal pons, or from noncatecholaminergic neurons in the arcuate nucleus. Detailed immunocytochemical studies have revealed that NPY-immunoreactive (NPY-IR) axon terminals have synaptic connections with parvocellular corticotropin-releasing-hormone (CRH) neurons of the NPV (Liposits et al., 1988). Of particular interest is that NPV contains a specific NPY binding site (Martel et al., 1986; Quirion and Martel, 1992).

NPY is secreted into the hypothalamo-pituitary portal system, in which a high concentration of neuropeptide has been demonstrated (McDonald et al., 1987; Sutton et al., 1988).

Only scanty data are available on the localization of NPY to specific cells of anterior pituitary gland. Immunocytochemistry has revealed NPY-immunoreactivity, or NPY mRNA, in cells scattered throughout rat anterior pituitary. These cells were identified as a subset of thyrotropes (Jones et al., 1989), or as gonadotropes, somatotropes, corticotropes, and some lactotropes, but not thyrotropes (Chabot et al., 1988).

Only small quantities of PYY-like material were found in mammalian and lower vertebrate brains (Bottcher et al., 1985; Broome et al., 1985; Ekman et al., 1986). This neuropeptide has a unique distribution and does not overlap with other known peptide distribution, including that of NPY.

Adrenal

In the adrenal glands, NPY-IR as well as its mRNA are present in population of chromaffin cells, some ganglionic cells of the medulla, and in nerves. NPY-IR distribution is very similar in all mammalian adrenal glands studied so far.

NPY-positive fibers are found around the capsular or subcapsular blood vessels and in the zona glomerulosa, where they can form plexuses. This type of fiber is also present in the vicinity of blood vessels of fasciculata and reticularis zones of the cortex (Lundberg et al., 1983; Varndell et al., 1984; Majane et al., 1985; Kondo, 1985; De Quit and Emson, 1986; Kuramoto et al., 1986; Pelto-Huikko, 1989; Maubert et al., 1990; Higuchi et al., 1991; Fernandez-Vivero et al., 1993) (Figs. 1, 2).

By means of hybridocytochemistry NPY mRNA has also been demonstrated in both nerves and chromaffin cells of the rat adrenal gland (Schalling et al., 1988; Higuchi et al., 1991). In the pig adrenal gland NPY was mainly associated with cortical perivascular fibers while chromaffin cells were not stained immunocytochemically (Kong et al., 1989).

Immunocytochemical studies have revealed a colocalization of vasoactive intestinal peptide (VIP) and NPY or VIP and C-PON (NPY C-flanking peptide) in most of the VIP-positive nerve fibers of the adult rat adrenal cortex (Maubert et al., 1990).

The origin of NPY-IR in the rat adrenal gland was studied by RIA by Higuchi and Yang (1986). The level of NPY-IR or its mRNA in the adrenal gland was found to increase considerably with age, mainly due to the increase in the chromaffin cells of the medulla. This phenomenon was abolished by chronic denervation of the splanchnic nerve (Higuchi et al., 1991).

The source of NPY-containing nerve fibers in capsula/glomerulosa zone of the rat adrenal gland was studied by Maubert et al. (1993). Demedullation of the gland notably reduced both NPY content and concentration in the capsule/zona glomerulosa complex in comparison with those in the corresponding part of the contralateral intact gland. Although NPY fibers were regularly distributed in intact capsula/glomerulosa region they were absent in some areas of demedullated gland. The above data suggest a dual origin of the NPY nerves present in the capsula/glomerulosa zone; one part could arise from extra-adrenal site, possibly the suprarenal ganglia, while the other part from intra-adrenal ganglionic cells which also contain NPY.

As reported by Pelto-Huikko (1989), the number of intraadrenal NPY-immunoreactive fibers is not significantly altered after capsaicin or 6-hydroxy-dopamine administration.

Biochemical and immunocytochemical studies have demonstrated colocalization of NPY in chromaffin cells in various mammalian species (rat, mouse, cat, guinea pig, horse, man) (Allen et al., 1983; Varndell et al., 1984;

Majane et al., 1985; Fischer-Colbrie et al., 1986; Kuramoto et al., 1986; Bastiaensen et al., 1988; Pelto-Huikko, 1989). However, still controversial is the exact localization of NPY in particular types of medullary cells. By means of immunohistochemical techniques some authors have claimed that NPY is stored in noradrenaline-containing chromaffin cells (Varndell et al., 1984; Majane et al., 1985). As demonstrated by Lundberg et al. (1986a), NPY-like immunoreactivity was present in chromaffin cells of the adrenaline type in adrenal medulla of mouse, cat, and man, but not in the pig adrenal medulla (with their antibodies). Also, NPY was localized to adrenergic granules in bovine adrenal medulla, in which the molar ratio of adrenaline to NPY is 5000:1 (Bastiaensen et al., 1988). In rat adrenal, NPY was costored (within the chromaffin granules) with chromogranin A and B and secretogranin (Steiner et al., 1989).

Regarding the controversies on the localization of NPY in adrenal chromaffin cells, it seems that the observations of Schalling et al. (1988) partially resolve this issue. They found NPY-IR and NPY mRNA in three components of the rat gland: chromaffin cells, medullary ganglionic cells, and nerve fibers. The chromaffin cells were of both the noradrenaline- and adrenaline-type, while the ganglionic cells were of cholinergic type. Localization of NPY-IR identified by ABC technique in rat adrenal medulla is shown in Figs. 3, 4.

Recently, Wolfensberger et al. (1995) reported localization and coexistence of atrial natriuretic peptide (ANP) and NPY in vertebrate adrenal chromaffin cells immunoreactive to tyrosine-hydroxylase (TH), dopamine-ß-hydroxylase (DBH) and phenylethanol-amine-N-methyl-transferase (PNMT). In all vertebrates

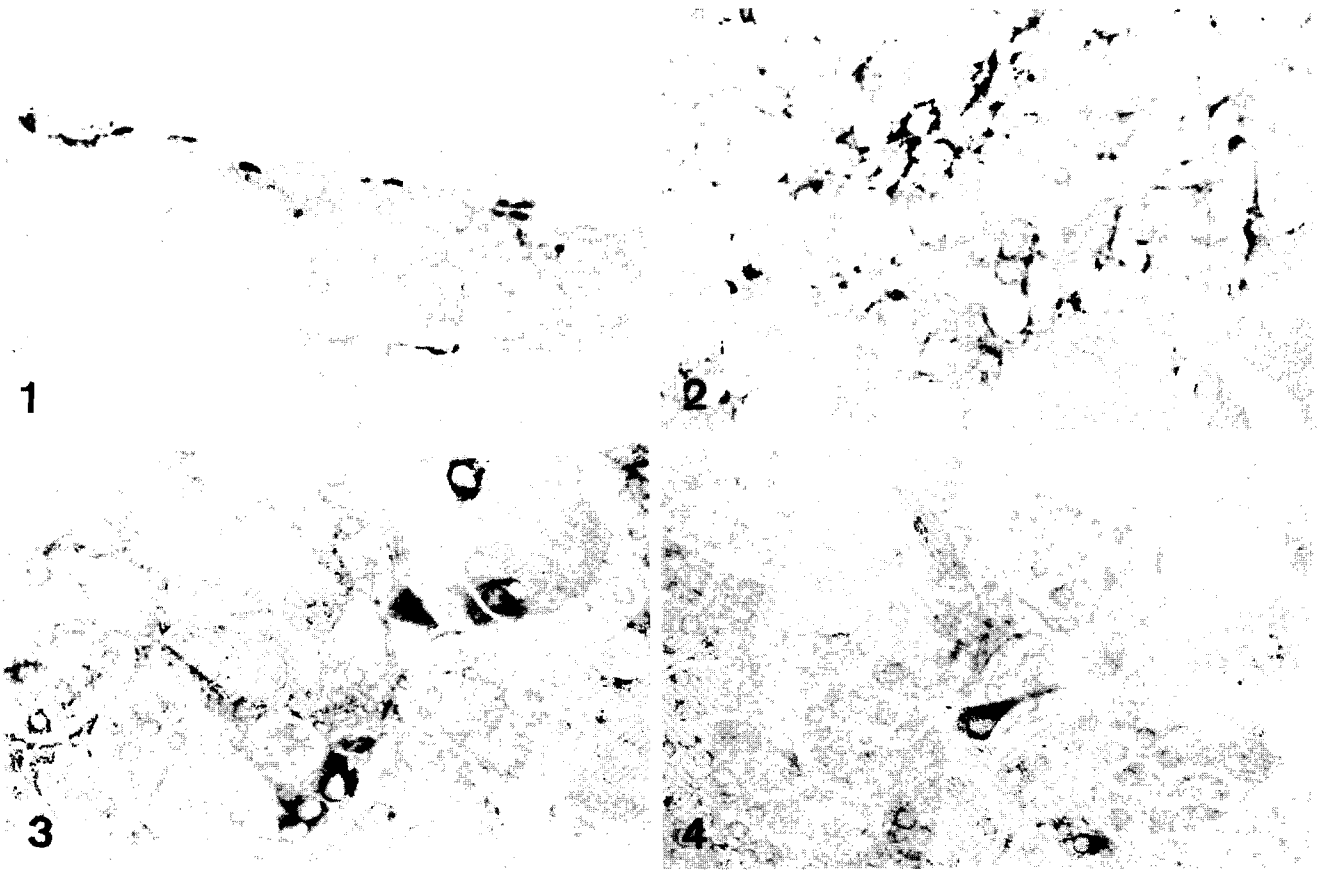

Fig. 1. Adrenal capsula and subcapsular region of the rat adrenal cortex. Immunoreactive NPY is seen in capsular nerve fibers and in a single fiber in the zona glomerulosa. ABC technique, the specific rabbit antisera against NPY (Amersham, England) diluted 1:500. x 250

Fig. 2. Zona reticularis of rat adrenal cortex. A dense network of NPY-containing nerve fibers is visible. ABC technique, the specific rabbit antisera against NPY (Amersham, England) diluted 1:500. x 250

Fig. 3. Rat adrenal medulla. An intense reaction for NPY in ganglionic cells of the medulla. Intense reaction is present around cell nuclei. Only weak reaction in adrenal chromaffin cells is seen. ABC technique, the specific rabbit antisera against NPY (Amersham, England) diluted 1:500. x 250

Fig. 4. NPY-immunoreactive ganglionic cell with a long axon in the rat adrenal medulla. ABC technique, the specific rabbit antisera against NPY (Amersham, England) diluted 1:500. x 250

studied (mammals, birds, reptiles, amphibians, and bony fish) immunoreactivity to NPY occurred in adrenal chromaffin cells but was absent from the cortex or its homolog - the interrenal. The majority of immunoreactivity to NPY was confined to the adrenaline cells. In all species studied immunoreactivities to ANP and NPY partially coexisted and NPY was found in about 30-45% of all chromaffin cells. In rat, coexistence of both neuropeptides amounted to almost 100% and in quail to 95%.

Avian pancreatic polypeptide-immunoreactivity was demonstrated in the adrenal medulla by Lundberg et al. (1980, 1982). However, it is now evident that the peptide responsible for this immunoreactivity was, at least in part, NPY.

Subsequent reports revealed that both NPY and PYY are concurrently secreted with catecholamines following adrenal medullary stimulation (i.e. asphyxia, stimulation of the splanchnic nerve) (Lundberg et al., 1986b). The results regarding PYY stimulation were not confirmed by Gauman et al. (1989). Moreover, numerous experiments on perfused bovine gland suggest that NPY may have the capacity to augment cholinergic receptor - mediated secretion from the gland (Hexum et al., 1987; Hexum and Russet, 1989).

The action of NPY and PYY on CRH, ACTH and adrenal steroid secretion

NPY binding sites are present exclusively in the zona glomerulosa, but not in the inner zones of the bovine adrenal gland (Torda et al., 1988). These binding sites have an apparent dissociation constant (K_d) of 0.45±0.06 nM and a binding capacity (B max) of 134±15 fmol mg^{-1} protein. These findings suggest that NPY may directly affect aldosterone secretion.

Results of studies on the effects of NPY-related peptides on HPA axis differ according to research protocol. Those obtained in vivo depend on dose used, route of drug administration, duration of treatment, and the degree of pharmacological interruption of the HPA axis. On the other hand, results of the in vitro studies depend mainly on preparation of adrenocortical cells, with great differences being observed between responses of isolated adrenocortical cells, and fragments of adrenal glands or glands perfused in situ.

As far as NPY effect on CRH release is concerned, Tsagarakis et al. (1989) demonstrated that relatively high concentrations of neuropeptide were able to stimulate CRH release from hypothalamus in vitro. Moreover, central administration of NPY acutely and selectively increases hypothalamic CRH immunoreactivity in the rat (Haas and George, 1987). On the other hand, Tizabi and Calogero (1992) reported that neither 1 nor 10 nM of NPY had any significant effect on CRH secretion by hypothalamic sections. Of interest is that NPY in cultured placental cells had a stimulating effect on secretion of placental CRH (Petraglia et al., 1989).

The intracerebroventricular administration of 0.01, 0.1, and 1 nmol NPY dose-dependently increased the plasma ACTH levels, as well as the levels of proopiomelanocortin mRNA in the anterior pituitary. The CRH mRNA level in the hypothalamus also increased after administration of 0.1 and 1 nmol NPY in a dose-dependent manner. On the contrary, a peripheral i.v. administration of 1 nmol NPY increased plasma ACTH levels, although the lower doses had no effect (Suda et al., 1993).

At the pituitary level, Tilemans et al. (1992) demonstrated that addition of a physiological dose of NPY (0.1, 1, and 10 nM) into the anterior pituitary reaggregate cell cultures from 14-day-old female rats dose-dependently increased ^{3}H-thymidine incorporation into DNA of cells expressing ACTH-immunoreactivity. This effect was observed only in the absence of the neurointermediate lobe cells and, with great probability, is mediated via growth factors released from gonadotropes.

The earliest studies on the effect of intracerebroventricularly-administered pNPY (1-36) (p-porcine) revealed inhibitory effect of 7.5-25 pmol of neuropeptide on serum levels of aldosterone and corticosterone in the rat. pNPY (1-36) or PYY at a dose of 750-1250 pmol/rat, on the other hand, notably stimulated the secretion of ACTH, aldosterone, corticosterone, and also vasopressin and angiotensin II (Fuxe et al., 1982, 1985, 1989; Harfstrand et al., 1986, 1987). Moreover, direct injection of NPY into the NPV rapidly increased plasma ACTH and corticosterone concentrations in the rat (Wahlestedt et al., 1987; Leibowitz et al., 1988; Albers et al., 1990).

The effects of intracerebroventricular administration of pNPY (13-36) on corticosterone serum level was reinvestigated by Aguirre et al. (1991). NPY at doses 7.5-750 pmol/rat produced a reduction of corticosterone serum levels; however, the highest dose (1250 pmol/rat) had no effect on blood glucocorticoid concentration. Cited authors suggest the inhibitory role of NPY receptors of Y2 type in the central control of corticosterone secretion in the adult male rat while those of Y1 type would be mainly involved in the stimulatory control of corticosterone secretion.

The first report by Inui et al. (1987) indicated that intracerebrally administered PP and PYY stimulated blood ACTH and cortisol concentrations in the dog. In subsequent studies this group demonstrated that pNPY administered into the third or lateral cerebral ventricle of the dog produced a six-fold elevation of plasma ACTH concentrations above baseline at 30 min and a three-fold increase of blood cortisol concentrations at 60 min. Porcine PP and PYY also stimulated corticotropin and cortisol secretion. PYY was the most effective, followed by NPY and PP (Inoue et al., 1989). Stimulatory effect of NPY on ACTH and cortisol secretion was absent in dexamethasone-pretreated dogs and was significantly inhibited in animals administered with α-helical CRH_{9-41}, a specific antagonist of CRH. Of interest is that intravenous administration of NPY failed to increase

plasma ACTH and cortisol concentrations except at the highest dose applied (11.9 nmol). These results indicate that NPY may modulate ACTH secretion at hypothalamic and/or pituitary levels.

Involvement of endogenous NPY in the control of ACTH secretion in the dog was further studied by Inui et al. (1990) by means of immunoneutralization technique with specific antibodies. An intraventricular administration of anti-NPY notably inhibited the ACTH and cortisol release in response to hypoglycemic stress. The integrated adrenocorticotropin and cortisol responses were reduced to 68 and 72% of control values, respectively.

Central injection of NPY (0.15 and 1.5 nmol), delivered by gravity flow into the third cerebral ventricle during both follicular or the luteal phase, caused a notable increase in plasma cortisol levels of the ewes (Porter et al. 1993). A similar effect was observed in ovariectomised-estradiol replaced animals. Moreover, NPY administered intracerebrally evoked a notable stimulation of CRH and arginine-vasopressin secretion into the hypophyseal portal circulation of the sheep (Lin et al., 1994).

A prolonged infusion with NPY (7 days 20 µg/kg/h) in rats whose hypothalamo-hypophyseal axis and renin-angiotensin system were pharmacologically interrupted (treated with dexamethasone and maintenance dose of ACTH and captopril plus angiotensin II) caused a marked hypertrophy of the adrenal zona glomerulosa and its parenchymal cells, while the number of cells in the zone remained unchanged (Rebuffat et al., 1988). Parenchymal cell hypertrophy was due to the increase in the volume of the mitochondrial compartment and in the surface area per cell of mitochondrial cristae and smooth endoplasmic reticulum (SER) membranes. Conversely, the volume of the lipid-droplet compartment was significantly lowered. NPY induced a small but significant increase in the volume of zona fasciculata and the number of its parenchymal cells, but did not affect any other stereological parameters. Neuropeptide applied caused an increase in both basal- and angiotensin II-stimulated aldosterone plasma concentration. NPY had no effect on the blood corticosterone level.

In subsequent studies Lesniewska et al. (1990) investigated the effect of NPY-administration (i.p. injections twice a day for 4 consecutive days, 0.5 µg/injection) on adrenal cortex of intact and dexamethasone-suppressed rats. In intact rats, NPY decreased both pituitary and serum ACTH concentrations (-46% and -31%, respectively), while adrenal and serum corticosterone concentrations and corticosterone output by adrenal homogenates were not significantly affected. Likewise, NPY had no marked effect on the volume of adrenal zones, average volume of parenchymal cells, and the total number of cells in the above zones. NPY administration to dexamethasone-suppressed rats did not provoke significant modifications of adrenal morphometric parameters, with the notable exception of the volume of zona fasciculata cells which displayed a small but significant drop. Pituitary and blood ACTH, already strongly decreased by dexamethasone, did not show evidence of any further change. On the contrary, serum aldosterone level and adrenal corticosterone content were strikingly lowered. No significant changes were observed in the blood concentration and adrenal homogenate output of corticosterone. These findings were interpreted as suggesting an inhibitory effect of NPY on the function of rat zona glomerulosa and perhaps zona fasciculata.

In the intact perfused rat adrenal gland in situ, Hinson et al. (1994a) demonstrated that NPY had only a slight effect on corticosterone secretion. The threshold for stimulation was 100 pmol, but the highest dose used (10 nmol) had no effect at all on glucocorticoid output. In the same experiment NPY significantly increased the rate of aldosterone secretion, with response threshold of 10 pmol, and caused a 30% decrease in the rate of perfusion medium flow through the gland (Hinson et al., 1994b,c).

Bernet et al. (1994a) studied the effects of NPY and PYY on aldosterone and corticosterone secretion by rat capsula/glomerulosa zone preparations in static incubation. Such preparations contained capsular tissue with adhering zona glomerulosa cells and those obtained from female rats had more than 70% of the total aldosterone content of the gland. Aldosterone secretion by the preparation was significantly stimulated by NPY_{1-36}, Leu^{31}-Pro^{34} NPY (the human neuropeptide Y1 receptor agonist - Fuhlendorff et al, 1990), and NPY_{18-36} (the Y2 receptor agonist - Michel et al., 1990) at concentrations ranging from 10^{-8}-10^{-6} M. At lower concentrations (10^{-10}-10^{-9} M) the increase of aldosterone secretion was not significantly different from that of controls. None of the peptides tested were able to significantly modify basal corticosterone secretion by capsule/zona glomerulosa preparation. Since PYY was unable to affect either aldosterone or corticosterone secretion by this adrenal preparation, Bernet et al. (1994a) have suggested the presence of functional NPY receptor of the Y3-like subtype on the capsule/zona glomerulosa cells, with properties similar to those observed on the bovine adrenal chromaffin cells (Wahlestedt et al., 1991). In a subsequent report, Bernet et al. (1994b) confirmed the stimulating effect of agonists of Y1 and Y2 receptors on aldosterone secretion and provided evidence suggesting that this effect may be mediated by stimulation of noradrenaline and adrenaline release.

In isolated inner zones cells of rat adrenal gland, NPY (1.0 µg/ml) decreased basal- and ACTH-stimulated corticosterone secretion. 0.1 µg/ml of NPY had no effect on basal glucocorticoid output and partially prevented the response of suspended adrenocortical cells to ACTH stimulation (Malendowicz et al., 1990). Similar observations were found in suspension of rat capsular (zona glomerulosa) cells. In these cells NPY (10^{-6} M) partially inhibited both basal- and ACTH-stimulated (10^{-8} M) release of aldosterone and 18-OH-cortico-

sterone, without affecting the overall post-pregnenolone yield or basal progesterone output (Neri et al., 1990). Under these conditions exposure to NPY increased 18-deoxy-corticosterone and corticosterone secretion. These data suggest that NPY exerts a direct suppression of 18-hydroxylase activity in rat zona glomerulosa cells without conceivably altering the earlier steps of aldosterone synthesis.

Regarding the effect of NPY on aldosterone secretion, it should be emphasized that this neuropeptide exerts an inhibitory action on renin release by kidney slices and by isolated perfused rat kidney (Hackenthal et al., 1987). Moreover, non-pressor doses of NPY (1-36) and (Pro_{34}) NPY markedly attenuated the renin secretion triggered by isoproterenol, whereas NPY (13-36) had no effect (Aubert et al., 1990, 1992). This suggests that the observed in vivo adrenoglomerulotrophic effects of NPY may be mediated by changes in renin-angiotensin system, one of the most important factors controlling the structure and function of the adrenal zona glomerulosa.

PP-localization to and effects on HPA

PP is released from pancreatic islets after meals or in response to stress. However PP receptors have a rather limited distribution. They have been identified in the area postrema and adjacent nuclei of the nucleus tractus solitarius and dorsal medial nucleus of the vagus (Whitcomb et al., 1990). Moreover, high-affinity PP receptors have been identified on adrenal PC-12 pheochromocytoma cells (Schwartz et al., 1987). Recently, Whitcomb et al. (1992) demonstrated the presence of the PP receptors in the zona fasciculata, zona reticularis, and the medulla of rat adrenal cortex. In the adrenal medulla only a subset of chromaffin cells expressed the PP-binding sites. Binding studies suggest the presence of a single class of PP receptors on both adrenocortical and medullary cells, with K_d of 5.5±0.5 nM and B_{max} of 104±22 fmol/mg membrane protein.

To our knowledge, there is no literature on the localization of PP in the adrenal gland. Our recent immunocytochemical studies have revealed the presence of immunoreactive PP in subset of chromaffin cells of rat adrenal medulla while the cortex was unstained (Figs. 5, 6).

As mentioned earlier, Inui et al. (1987) demonstrated that intracerebrally-administered PP stimulated blood ACTH and cortisol concentration in the dog. In 1993, Andreis et al. revealed that PP dose-dependently enhanced both basal and submaximal ACTH-stimulated corticosterone output by isolated zona fasciculata/zona reticularis cells of the rat adrenal cortex. On the contrary, PP had no effect on aldosterone and corticosterone secretion by zona glomerulosa cells. These studies suggest that PP-receptor coupling activates the same post-receptorial events that transduce the secretory signal of ACTH. In subsequent studies Mazzocchi et al. (1995) found that PP dose-dependently enhanced plasma concentration of corticosterone in hypophysectomised/ACTH replaced rats, but not that of aldosterone.

Regarding the PP action on glucocorticoid secretion it is worth underlying that CRH stimulates and glucocorticoids inhibit PP release (Lantigua et al., 1980; Lytras et al., 1984). Moreover, PP inhibits nicotine- but not KCL-stimulated adrenaline and noradrenaline secretion from culture of bovine chromaffin cells (Higuchi et al., 1988). All these findings suggest that PP may play a physiological role in the rat as a modulator of the adrenal response to various stress stimuli.

Concluding remarks

The above presented data indicate NPY-related peptide involvement in the control of HPA axis function. However, results of studies on the effects of these

Fig. 5. Typical distribution of PP-immunoreactive cells in rat pancreatic islet. ABC technique. the specific antisera against bovine PP (Lilly Laboratories) diluted 1:3000. x 250

Fig. 6. In rat adrenal medulla PP-immunoreactive substance is present in subset of chromaffin cells which form a small island. ABC technique, the specific antisera against bovine PP (Lilly Laboratories) diluted 1:3000. x 250

neuropeptides on HPA axis differ upon research protocol. Of particular interest is the stimulating effect of intracerebrally-administered low NPY doses on ACTH and glucocorticoid secretion while after higher doses concentrations of these hormones remained unchanged. NPY, PYY and PP, apart from their central action, exert a direct effect on secretion of mineralocorticoids and/or glucocorticoids by isolated cells, capsular/zona glomerulosa preparation and by adrenals perfused in situ. Some experimental data suggest that the in vivo observed stimulating effect of NPY and PYY may be mediated by enhanced arginine-vasopressin and/or adrenaline secretion.

A potent vasoconstrictory effect evoked by NPY and the presence of NPY-ergic nerve fibers in the close vicinity of adrenocortical blood vessels as well as the presence of NPY-related peptides (NPY, PYY, PP) in adrenal medulla suggest that these peptides may also exert their effect on adrenocortical secretion by paracrine routes (for details see reviews: Kondo, 1985; Malendowicz, 1993; Vinson et al., 1994).

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