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Invited Review

Neuromedins and their involvement in the regulation of growth, structure and function of the adrenal cortex

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Summary. Current data on the synthesis and the mechanism of action of neuromedins on adrenal cortex are presented. The localization of these biologically-active peptides in all components of the hypothalamo-pituitary-adrenal axis as well as their action on the adrenal cortex both *in vivo* and *in vitro* suggest their involvement in the regulation of growth, structure and function of the adrenal cortex. Neuromedins may exert both direct and indirect effect on the adrenal cortex. Direct effect is proven by the stimulation of glucocorticoid synthesis by adrenocortical cells in culture (NMK, NML) while indirect effects may be mediated by ACTH, vasopressin (aldosterone secretagogue effect) and angiotensin (prompt proliferative response) or by substances of medullary origin. The last mechanism of action is well documented for NMU.

Key words: Hypothalamus, Pituitary, Adrenal, Neuropeptides, Neuromedin K, Neuromedin L, Neuromedin B, Neuromedin C, Neuromedin N, Neuromedin U

Glossary: The following abbreviations have been used: NMK, neuromedin K; NML, neuromedin L; NMC, neuromedin C; NMU, neuromedin U; NMU-R, neuromedin U receptor; BM, bombesin; NMB, neuromedin B; NMB-R, neuromedin B receptor; SP, substance P; NT, neurotensin, NT-R, neurotensin receptor; GRP, gastrin-releasing peptide; GRP-R, gastrin-releasing peptide receptor; CRH, corticotropin-releasing hormone; DX, dexamethasone; ZG, zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis

Introduction

Neuromedins are smooth muscle-stimulating peptides originally isolated from the porcine spinal cord. Their sequences and biological activity are similar to some of those of known neuropeptides and therefore they are commonly divided into four groups:

- kassinin-like tachykinins: neuromedin K (NMK) and neuromedin L (NML).
- bombesin-like neuromedins: neuromedin B (NMB) and neuromedin C (NMC).
- neurotensin-like: neuromedin N (NMN).
- neuromedins U: neuromedin U-8 (NMU-8) and neuromedin U-25 (NMU-25) for which no substantial homology with other known neuropeptides was found.

Like other biologically active neuropeptides the neuromedins are widely distributed within the central nervous system and on the periphery. Usually they are synthesized in the form of large polyprotein precursors, the splitting of which yields the neuromedin.

Numerous neuropeptides have been localized to cells comprising the hypothalamo-pituitary-adrenal axis and their role in regulation of the growth, structure and function of the adrenal cortex has recently been reviewed (Hinson, 1990; Malendowicz, 1993).

In the present review the current data on the influence of neuromedins on the above mentioned axis are presented and discussed.

Kassinin-like tachykinins: neuromedin K (NMK) and neuromedin L (NML)

Tachykinins are neuropeptides that contain the sequence Phe-X-Gly-Leu-Met-NH_2 at their carboxyl terminus, where X denotes variable amino acid (Maggio, 1988). Recently, two mammalian genes coding precursor proteins for tachykinins have been described. Preprotachykinin A gene encoded prohormone proteins contain substance P (SP) and NML sequences while the sequence of NMK is contained in prohormone encoded by preprotachykinin B gene (Nawa et al., 1983; Kawaguchi et al., 1986; Kotani et al., 1986; Nakanishi, 1986; Bonner et al., 1987; Krause et al., 1987).

NMK is also called neurokinin B or neurokinin ß, while NML is also known as neurokinin A, neurokinin α or substance K. Both, NMK and NML are decapeptides structurally related to SP (Fig. 1) while neuropeptide K, the fourth mammalian tachykinin, is the N-terminally-

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extended form of NML (Tatemoto et al., 1985; Deacon et al., 1987).

The effects elicited by the tachykinins are mediated by membrane receptors which belong to the family of the guanosine-nucleotide-binding-regulatory-protein (G)-coupled receptors, with seven transmembrane domains. Three types of tachykinin receptors - NK1, NK2 and NK3 show high affinity for SP, NML and NMK respectively. Although all types of receptors can be activated by all three ligands, each of them requires different concentration. Activation of receptors induces transient inositol 1,4,5-triphosphate formation and Ca^{2+} mobilization, increases output of arachidonic acid and prostaglandin E_2, stimulates cAMP generation and *de novo* DNA synthesis, and induces «immediately early» genes *c-fos* and *c-jun* (Masu et al., 1987; Quirion and Dam, 1988; Helke et al., 1990; Shigemoto et al., 1990; Eistetter et al., 1991; Fong et al., 1992; Yokota et al., 1992).

The mammalian tachykinin peptides and their receptors are widely distributed in the central and the peripheral nervous system. They regulate the variety of physiological processes, among others smooth muscle contraction, hypotension, inflammation, perception of pain, endocrine and exocrine secretion, neurotransmission and proliferation (for review see Holrer-Petsche et al., 1985; Maggio et al., 1988).

The distribution of SP within the hypothalamo-pituitary-adrenal axis and the role of neuropeptide in regulation of adrenocortical growth and function has recently been reviewed (Hinson, 1990; Jessop et al., 1992; Malendowicz, 1993).

The distribution of NMK in the rat brain is similar to the distribution of SP, however there are regions where the two distributions are different (Jessop et al., 1990; Merchenthaler et al., 1992). As demonstrated by RIA, the hypothalamus has the highest concentration of this neuromedin in the entire brain (Minamino et al., 1984a; Tateishi et al., 1989). Moreover, in situ hybridization histochemistry revealed the distribution of preprotachykinin B mRNA in the rat hypothalamus, among others in nucleus paraventricularis (Warden and Young, 1988). The presence of a high NMK concentration in the rat hypothalamus was also confirmed by means of the HPLC-RIA method (Brown et al., 1990) as well as by RIA based on the highly specific anti-NMK sera (Merchenthaler et al., 1992).

The distribution of NMK in the rat brain, as determined by immunocytochemistry with the antisera directed toward its amino terminus, revealed only a few immunoreactive cells in the magnocellular division of the paraventricular nucleus (Merchenthaler et al., 1992).

SP:	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
NML:	H-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
NMK:	H-Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2

Fig. 1. Amino acid sequences of substance P (SP), neuromedin L (NML) and neuromedin K (NMK).

This immunoreactivity, however, could not be eliminated or even decreased by the preabsorption of the primary antiserum with NMK. Scattered immunoreactive nerve fibres were present in the internal zone of the median eminence where nerve terminals were also seen in close association with the pituitary portal capillaries. The intense immunocytochemical reaction for NMK was also seen in hypothalamus of the cat (Hunter et al., 1985).

RIA determinations also revealed a high concentration of NML in the hypothalamus of the rat (Minamino et al., 1984a,b). These observations have been confirmed by *in situ* hybridization of combined preprotachykinin A mRNA levels as well as by HPLC-RIA assays (Brown et al., 1990; Debeljuk et al., 1990; Larsen et al., 1993). The results of these studies show a high concentration of NML in the nucleus para-ventricularis (medial parvocellular part). However, the origin of this neuropeptide in the paraventricular nucleus is not fully explained. On a ground of experiments with monosodium glutamate Jessop et al. (1991) suggest that the arcuate nucleus is a significant source of the NML found in the nucleus paraventricularis.

On the other hand, in the anterior pituitary, only NML is present (Jonassen et al., 1987; Brown et al., 1990; Debeljuk et al., 1991, 1992; Merchenthaler et al., 1992). The absence of NMK in the anterior pituitary of rat is consistent with the Northern blot analysis showing the lack of pituitary NMK-encoding mRNA (Brown et al., 1990). So far, the cellular localization of NML in the anterior pituitary has not been described.

To our knowledge, there is no report on the presence and localization of NMK and NML in the adrenal gland. However, the presence of SP in the gland implies the presence of NMK and NML (for review see Malendowicz, 1993).

Few data are available on the effects of NMK or NML on the hypothalamo-pituitary-adrenal axis. Faria et al. (1991) demonstrated that contrary to SP, NML had no effect on CRH-41 secretion from the rat hypo-thalamus *in vitro*.

In the cultured bovine adrenocortical cells both NMK and NML stimulated cortisol secretion. However, the response was notably lower than that evoked by SP, the potency order was SP>NML>NMK (Yoshida et al., 1992). In the rat, Kalra and Kalra (1993) revealed that within 20-60 min the intracerebroventricular administration of 10 nmol NML markedly stimulated plasma corticosterone level, while the lower dose of neuromedin was ineffective.

The above cited data suggest the direct stimulatory effect of NMK and NML on glucocorticoid secretion. However, *in vivo* effects of these neuromedins may be mediated by vasopressin (Massi et al., 1991) or via modulation of adrenaline and noradrenaline secretion (Khalil et al., 1988), which in turn are able to modify secretory activity of adrenocortical cells (Bornstein et al., 1990, 1991).

Bombesin-like neuromedins: Neuromedin B (NMB) and Neuromedin C (NMC)

Bombesin (BM) is a tetradecapeptide originally isolated from the skin of frog *Bombina bombina* by Anastasi et al. (1971). Gastrin-releasing peptide (GRP) (neuropeptide containing 27 amino acids) is the mammalian counterpart of BM. Despite this, recently a true amphibian GRP, distinct from amphibian BM, has been identified and cloned (Nagalla et al., 1992). According to their C-terminal tripeptides, BM-related peptides have been divided into three subfamilies - bombesin, ranatensin and litorin (Erspamer et al., 1988).

Two known BM-like neuromedins are NMB, and NMC - C-terminal decapeptide of GRP. Both neuromedins share structural homologies and exert similar biological effects.

NMB was isolated from porcine spinal cord by Minamino et al. (1983). This neuromedin is derived from the 116 amino acid precursor protein (preproNMB) that contains a single copy of NMB (Krane et al., 1988). The 10 amino acid NMB has 7 of the same C-terminal amino acids as GRP.

GRP, the 27 amino acid peptide homologous to the carboxyterminus of BM, was isolated from porcine stomach by McDonald et al. (1979). Subsequently Minamino et al. (1983) and Reeve et al. (1983) isolated a smaller form of GRP - the carboxyl terminal decapeptide of GRP. This peptide - GRP-10, (also called neuromedin C) is present in all species which also express GRP (Minamino et al., 1983, 1984c,d; Reeve et al., 1983; Orloff et al., 1984; Lemaire et al., 1989). Human, porcine, canine, guinea pig and avian GRP are all 27 amino acids, while rat GRP has 29 amino acids (McDonald et al., 1979, 1980; Reeve et al., 1983; Orloff et al., 1984; Shaw et al., 1987; Lebacq-Verheyden et al., 1988). The sequence of GRP-10 is identical in all species except for position 2 which is Ser in rats, chicken and frogs, and Asn in humans, pigs and dogs (Nagalla et al., 1992). GRP is also synthesized in a form of 148 amino acid precursor protein (preproGRP) that contains a single copy of GRP (Spindel et al., 1984; Sausville et al., 1986).

In mammalian tissues, two distinct receptors with binding preferences for either NMB (NMB-R) or GRP (GRP-R) have been distinguished, and complementary DNA (cDNA) clones encoding both receptors have been isolated. Both are members of the BM receptor family within the G-protein-coupled receptor superfamily. The fifth transmembrane segment of NMB-R is critical for high affinity NMB binding (Fathi et al., 1993). NMB-R contains 390 amino acid residues and has 54% sequence homology to GRP-R which contains 384 amino acid residues. Activation of NMB-R and GRP-R stimulates inositol polyphosphate release (Heslop et al., 1986; Fisher et al., 1988; von Schrenck et al., 1989; Spindel et al., 1990; Battey et al., 1991, 1993; Corjay et al., 1991; Wada et al., 1991; Benya et al., 1992; Dobrzanski et al., 1993). BM binds to both GRP-R and NMB-R, but NMB only to NMB-R.

Stimulation of NMB-R and GRP-R regulates numerous biological processes including gastrointestinal hormone release, smooth muscle contraction, and neuronal activity modulation (for review see Battey et al., 1991). Both neuromedins are potent mitogens. NMB and its mRNA have been identified in small cell lung carcinoma(SCLC). Like BM, NMB may be an autocrine growth factor for SCLC (Cardona et al., 1991; Giaccone et al., 1992).

NMB-like immunoreactivity was found to be present in both the peripheral and the central tissues and a high concentration of neuropeptide is localized in the hypothalamus, the pituitary and the adrenal gland (Minamino et al., 1984c; Namba et al., 1985; Moody et al., 1986; Steel et al., 1988; Domin et al., 1989a,b,c; Ladenheim et al., 1991; Jones et al., 1992). High concentration of GRP-immunoreactivity was also found in the hypothalamus (Panula et al., 1984; Kita et al., 1986).

NMB mRNA and GRP mRNA are differentially distributed in the rat central nervous system, both being present in hypothalamic nuclei (Wada et al., 1990). Moreover, Wada et al. (1991) demonstrated the distinct regional distribution of NMB-R mRNA and GRP-R mRNA in the rat. GRP-R mRNA was most prominent in the hypothalamus, among others in the suprachiasmatic and the paraventricular nuclei. NMB-R mRNA was also expressed in the hypothalamic paraventricular nucleus.

Within the adenohypophysis, NMB-immunoreactivity has been shown to be localized in the thyrotropes (Steel et al., 1988). This neuropeptide is locally synthesized within this gland and, as experimental data suggest, NMB may be involved in the local - paracrine or autocrine - regulation of TSH and the growth hormone's synthesis and secretion (Rettori et al., 1989; Tajima et al., 1989; Houben and Denef, 1990a,b; Jones et al., 1992). GRP- and proGRP-immunoreactivity, on the other hand, is detectable in the prolactin and ACTH containing cells (Houben and Denef, 1991).

NMC [GRP-(18-27)] is present in the bovine adrenal medulla and NMC is almost exclusively localized in secretory granules. Its release from the perfused bovine adrenal gland by 500 μM carbamylcholine is stimulated 4.5 fold over basal value (Lemaire et al., 1989).

Using dispersed anterior pituitary cells Hale et al. (1984) and Familari et al. (1987) observed the stimulatory effect of GRP on basal ACTH release. Moreover, low doses of GRP or BM potentiated the ACTH-releasing effect of CRH. On the other hand, in the studies of Pirkut and Joseph (1986), and Watanabe and Orth (1988) these neuropeptides were ineffective in the ACTH release regulation.

BM: pGlu-Gln-Arg-Leu-Gly-Asn-Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2
NMB Gly-Asn-Leu-Trp-Ala-Thr-Gly-His-Phe-Met-NH_2
NMC: Gly-Asn-His-Trp-Ala-Val-Gly-His-Leu-Met-NH_2

Fig. 2. Amino acid sequence of bombesin (BM), neuromedin B (NMB) and neuromedin C (NMC).

Microinfusion of BM directly into the paraventricular nucleus of rat hypothalamus stimulated the pituitary ACTH secretion (probably via stimulation of CRH release (Gunion et al., 1989)), while intraventricular injection was without effect (Rivier et al., 1978). Recently, Olsen et al. (1992) have shown that *in vivo* GRP affects ACTH secretion directly at the supra-pituitary level by stimulating the vasopressin and CRH release to the pituitary portal blood and directly at the pituitary level to augment the effect of vasopressin and CRH on corticotropes. The above mentioned GRP effects could be observed only after central infusion of neuropeptide. Still, in normal men, intravenous GRP infusion caused a dose-dependent increase in the plasma level of ACTH (Knigge et al., 1988).

As far as the adrenocortical effects of NMB and NMC are concerned, Thomas and Sander (1985), and Sander and Thomas (1991) demonstrated that in conscious dogs the i.v. GRP or BM infusion caused significant dose-dependent increase in plasma ACTH and cortisol secretion. In our experiments (Malendowicz et al., 1994a), adult male and female rats were administered for 2 (s.c.) or 7 days (intraperitoneal infusion) with NMB. The neuropeptide markedly stimulated blood corticosterone concentration and its output by adrenal slices and statistically insignificantly elevated ACTH blood level. In 2-day experiments a notable increase in the average volume of zona fasciculata (ZF) cells was found while after 7 days the cells were smaller than in control groups. NMB has no effect on total number of the parenchymal cells in the gland nor on the basal or ACTH stimulated corticosterone secretion by isolated inner zone adrenocortical cells. These results suggest the stimulatory effect of NMB on corticosterone secretion in the rat, an effect probably mediated by increased ACTH secretion.

In studies on the role of BM and NMB in the regulation of the proliferative activity of the rat adrenal cortex, adult female rats were treated with a single subcutaneous dose of 3 μg of the neuropeptide. The adrenocortical proliferative activity was assessed by the metaphase-arrest technique (Markowska et al., 1993). BM administration resulted in a marked increase in the number of metaphases in the zona glomerulosa (ZG) and the ZF, and in the entire cortex. This increase appeared in the ZG 24 h after injection and in the ZF 48 h after injection. NMB administration, on the contrary, caused a prompt increase in the number of metaphases in the ZG and the entire cortex at 12th h, followed by a subsequent drop below the control level at the 24th and 48th h of the experiment. These findings indicate that BM and NMB, acting via different receptors or different mediators, enhance the rat adrenocortical cells' proliferative activity.

Neurotensin-like neuromedin: Neuromedin N (NMN)

NMN is a hexapeptide originally isolated from the porcine spinal cord by Minamino et al. (1984e). The carboxy terminal tetrapeptide sequence of NMN is identical to that of the neurotensin (NT) (Fig. 3).

NT and NMN are encoded in the same gene (Kislauskis et al., 1988). The rat NT/NMN gene is divided into four exons by three introns. NT and NMN coding domains are located in tandem on exon 4. The rat gene is transcribed to yield two distinct mRNAs - 1.0 and 1.5 kb in size. The smaller form predominates in intestine and both forms are nearly equally abundant in the hypothalamus, the brain stem and the brain cortex. These two mRNAs differ in the extent of their 3' untranslated regions.

In rat, bovine and dog the NT/NMN precursor consists of 169-170 highly conserved amino acids, the C-terminal region of which contains one copy of both NT and NMN (Dobner et al., 1987, 1992; Kislauskis et al., 1988). Thus, the cell may produce and secrete both NT and NMN. However, marked variations of the relative distribution of NT and NMN in discrete brain areas or in the other organs suggest the differential processing of their common precursor (Shaw et al., 1990; Kitabgi et al., 1991).

The rat receptor for NT contains 424 amino acid residues and has the typical structure of the GTP-binding protein-coupled receptor superfamily (Tanaka et al., 1990). This receptor is coupled to phospholipase C through a pertussis toxin-insensitive binding protein and stimulates phosphoinositides hydrolysis (Goedert et al., 1984; Amar et al., 1986; Kanba and Richelson, 1987; Hermans et al., 1992).

Recent data indicate that NMN, although less efficiently than NT, also specifically activates NT-R, while the presence of specific NMN-R has never been proven (Hermans et al., 1992).

NMN evokes NT-like effects such as analgesia, hypothermia, spontaneous motor activity, or excites neurons. In general, NMN is less potent than NT. This lower potency is due to NMN's higher sensitivity to enzymatic degradation (Minamino et al., 1984e; Checkler et al., 1986; Dubuc et al., 1988; Coquerel et al., 1988; Hermans et al., 1992; Rompre and Gratton, 1992).

The localization of NT in the hypothalamo-pituitary-adrenal axis and the role of this neuropeptide in the regulation of growth, structure and function of the adrenal cortex has recently been reviewed (Malendowicz, 1993). Since NT and NMN are synthesized in common precursor protein, the presence of NT also implies the presence of NMN in the same cells. However, as underlined previously, their relative distribution may vary.

Using highly specific antibodies, Carraway and Mitra (1987), in cat, and Nadai et al. (1989), in rat, demonstrated similar distribution of NT and NMN,

NT: pGlu-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu-OH
NMN: H-Lys-Ile-Pro-Tyr-Ile-Leu-OH

Fig. 3. Amino acid sequence of neurotensin (NT) and neuromedin N (NMN).

among others in hypothalamus. However, there are marked regional variations in the ratio of NT over NMN. In the posterior hypothalamus of the rat, the ratio value is 4.5, while in the anterior and the medial hypothalamus it is 2.6 and 2.8, respectively (Kitabgi et al., 1991). Moreover, both NT and NMN are released from mouse hypothalamus slices upon potassium-induced depolarization (Kitabgi et al., 1990). The ratio of released NMN/NT was 0.3 - identical to the ratio of endogenous neuropeptides.

The high concentration of NMN is present in the pituitary and chromaffin cells of the cat adrenal gland (Carraway and Mitra, 1987).

There are only a few reports on the effect of NMN on the rat adrenal cortex. Within 2 days NMN administration (3 or 9 μg/rat/day) resulted in an increase in adrenal gland weight, caused by both the hypertrophy and the hyperplasia of the adrenocortical cells (Malendowicz et al., 1992a). The adrenal enlargement was due to the increases in volume of ZF and ZR and in the average volume of the parenchymal cells in all the cortical zones and in the number of cells in ZG and ZR. Higher doses of NMN notably raised the serum ACTH and the aldosterone concentrations, while blood corticosterone levels remained unchanged. These changes are very similar to those induced by NT (Malendowicz et al., 1991a,b,c, 1992b; Markowska et al., 1992). Moreover, like NT, NMN (6 μg/100 g for 2 days) partially reversed the dexamethasone (DX)-induced inhibition of ACTH release and the consequent adrenal atrophy and the decrease in the corticosterone plasma concentration in rats (Malendowicz et al., 1992b, 1993a). Furthermore, NMN provoked a significant rise in the plasma aldosterone level. Thus, the NMN adrenocorticotropic action mechanism involves the hypophyseal ACTH secretion stimulation, and NMN, like NT, may overcome the inhibitory effect of small DX doses of the rat hypothalamo-hypophyseal axis.

It seems that the NMN aldosterone secretagogue action depends on the stimulation of the release of some other regulatory peptides exerting mineralocorticoid secretagogue action. As recently demonstrated, vasopressin (AVP) is a factor mediating the NT aldosterone secretagogue effect (Leśniewska et al., 1992; Malendowicz et al., 1992b; Mazzocchi et al., 1993). In view of these facts, it seems reasonable to suggest that the glomerulotropic effect of NMN may also be mediated by AVP.

However, not all the adrenocortical effects of NMN are similar to those evoked by NT. As recently demonstrated, both NMN and NT markedly stimulate the proliferative activity of rat adrenal cortex, though a time-course of changes evoked by the two neuropeptides is different. NMN injection results in a prompt increase in the number of metaphases at 12 h, while after NT administration the peak of metaphases is seen after 48 h. Furthermore, NMN-evoked increase in proliferation is prevented by the concomitant saralasin administration. This last finding suggests the prompt proliferative effect of NMN on the rat adrenocortical cells mediated by angiotensin II (Markowska et al., 1994a,b).

Neuromedin U (NMU)

Neuromedin U (NMU) is the smooth muscle-stimulating peptide, isolated from porcine spinal cord by Minamino et al. (1985, 1988). Originally, this neuropeptide was isolated in two molecular forms, one containing 25 (NMU-25) and the other 8 (NMU-8) amino acid residues. Subsequently NMU was isolated from the tissues of human (NMU-25), rat (NMU-23), dog (NMU-25 and NMU-8), guinea pig (NMU-8) and frog (NMU-25) (Conlon et al., 1988; Domin et al., 1989a,b,c; Murphy et al., 1990; O'Harte et al., 1991). In the mammalian NMUs a common C-terminal sequence - Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2 - contains the active site of the neuropeptide, which is formed by the amino acid residues between positions 2 and 8 (Hashimoto et al., 1991; Sakura et al., 1991).

Little is known about the synthesis of NMUs, but recently Nandha et al. (1993) described receptor of NMU from the rat uterine. The binding of [^{125}I] rat NMU by uterine membrane preparation is saturable, specific and reversible, and time- temperature-, and pH-dependent, and is reduced by the nonhydrolysable analogue of GTP. This latter finding suggests the coupling of the binding site to a G-protein. Scatchard plot analysis reveals a single class of binding site with a maximal binding capacity of 580±140 fmol/mg membrane protein and dissociation constant of 0.35±0.09 nM. NMU binding sites on the rat uterus have an Mr of 48,500.

The NMU's physiological role is not entirely recognized. These neuropeptides potently contract smooth muscles, increase arterial blood pressure and modify intestinal ion transport (Minamino et al., 1985; Sumi et al., 1987; Brown and Quito, 1990; Gardiner et al., 1990; Maggi et al., 1990; Hashimoto et al., 1991; Sakura et al., 1991).

The NMU-like immunoreactivity has been found in various parts of the central nervous system, and the urogenital and the gastrointestinal tract (Domin et al., 1986, 1987; Honzawa et al., 1987; Augood et al., 1988; Ballesta et al., 1988; Timmermans et al., 1989). Numerous nerve fibres containing the NMU-immunoreactive substances are present in the rat hypothalamus, among others in paraventricular and supraoptic nucleus (Steel et al., 1988).

RIA and immunohistochemistry demonstrated the NMU presence in the intermediate and the anterior pituitary gland lobes of various species, among them:

NMU-25: H-Phe-Lys-Val-Asp-Glu-Glu-Phe-Gln-Gly-Pro-Ile-Val-Ser-Gln-Asn-Arg-Arg-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2

NMU-8: H-Tyr-Phe-Leu-Phe-Arg-Pro-Arg-Asn-NH_2

Fig. 4. Amino acid sequence of neuromedin U-25 (NMU-25) and neuromedin U-8 (NMU-8).

mouse, rat and human (Domin et al., 1986, 1987, 1989; Ballesta et al., 1988; Steel et al., 1988). In the mouse, rat and human adenohypophysis the NMU immunoreactivity was found in corticotropes (Steel et al., 1988). In a subsequent study, by means of immunohistochemistry and electron microscopy, Cimini et al. (1993) found NMU-like immunoreactivity in some thyrotropes and most corticotropes of the rat pituitary gland. NMU was colocalized with galanin and ACTH in the same secretory granules.

To our knowledge, up to now there has been no report on the presence of NMU in the adrenal gland.

The interrelationship between NMU and the hypothalamo-pituitary-adrenal axis was studied by the group of Bloom and Polak. They demonstrated that despite the adrenalectomy-caused increase in the number and size of corticotropes, the extractable anterior pituitary NMU-like immunoreactivity remained unchanged (Steel et al., 1988; Cimini et al., 1993). Nevertheless, TRH treatment increased the anterior lobe content of NMU, an effect dependent on the circulating thyroid hormone concentration (Domin et al., 1989).

The administration of NMU results in profound changes in the rat pituitary-adrenal axis. As recently demonstrated, a single injection of 6 µg/100 g of NMU-8 into adult female rats results in the transient increase in ACTH blood concentration between 3-12 h, and sustained elevation of blood corticosterone level (Malendowicz et al., 1993b). The NMU-8 48-hour treatment (2 or 6 µg/100 g/day) lowered adrenal gland weight, an effect caused by the decrease in the volume of all adrenocortical zones and loss of the parenchymal cells. Under this condition the blood ACTH level remained unchanged, but the serum corticosterone level was significantly raised. These data demonstrate a potent stimulating effect of NMU-8 on the rat adrenal cortex, possibly partially due to the direct neuropeptide effect on the gland. NMU-8 has no effect on basal and ACTH-stimulated corticosterone secretion by isolated inner zone adrenocortical cells. However, this neuropeptide stimulates corticosterone ouptut by adrenal slices, an effect demonstrated only at NMU concentration of 10^{-8}M (Malendowicz et al., 1994b).

Detailed analysis of the NMU-8 action on the rat adrenal slices revealed that at all concentrations tested (10^{-8}-10^{-6} M) neuropeptide increased basal pregnenolone and total post-pregnenolone steroid ouput by gland slices containing both cortex and medulla (Malendowicz et al., 1994c). However, NMU-8 is able to stimulate corticosterone secretion only at the lowest concentration. At higher concentration the NMU-8 stimulating effect is masked by the strong enhancement of the steroid 18-hydroxylation. This effect of NMU-8 is not observed in the adrenocortical autotransplants, which are deprived of chromaffin cells and which display a normal secretory response to ACTH. These results indicate that NMU-8 affects the steroid secretion indirectly by acting on the medullary chromaffin cells, which in turn may paracrinally stimulate the cortical part of the gland. It has been suggested that the medullary mediator of NMU-8 action on adrenal cortex may specifically activate 18-hydroxylation and the aldosterone synthase activity.

General discussion

The data presented above suggest the neuromedin involvement in the control of growth, structure and function of the adrenal cortex. Like their parent neuropeptides, almost all neuromedins are localized and probably synthesized in all components of the hypothalamo-pituitary-adrenal axis. NMK seems to be the only exception. Neither hybridocytochemistry nor RIA estimations revealed this neuromedin presence in the rat anterior pituitary gland.

The mechanism of the neuromedin action on adrenal cortex may involve both direct and indirect effects. As evidenced by means of experiments with isolated adrenocortical cells, NMK and NML directly stimulate glucocorticoid secretion, while NMB and NMN are ineffective. Of special importance is the demonstration that NMU exerts a stimulating effect on rat adrenal cortex only in adrenal slices containing the medullaris zone. This observation suggests the zona medullaris-mediated effect of NMU on the rat adrenal cortex. As indicated in numerous reports, adrenal medulla (via intraadrenal paracrine or endocrine routes) may regulate the growth and the function of the cortical part of the suprarenal gland (for review see Hinson, 1990; Malendowicz, 1993).

Modification of release of the hypothalamic and the pituitary hormones, controlling growth and function of adrenal cortex, seems to play a prevailing role in the neuromedin-evoked adrenocortical changes *in vivo*. Of these factors the modification of CRH, vasopressin and angiotensin synthesis and release, sensitivity modulation of pituitary corticotropes and secretion modification of these hormones seem to be involved. As presented above, numerous neuromedins (NMN, NMB and NMU) enhance plasma ACTH level. Aldosterone secretagogue effect of NMN, on the other hand, seems to be mediated by vasopressin, while prompt and profound stimulation of adrenocortical proliferative activity by NMB and NMN seems to be partially mediated by angiotensin II.

Thus, neuromedin involvement in regulation of the adrenocortical growth and function is well documented. However their mechanism of action on hypothalamo-pituitary-adrenal axis is far from being completely understood.

References

Amar S., Kitabgi P. and Vincent J.P. (1986). Activation of phosphatidyl inositol turnover by neurotensin receptors in the human colonic adenocarcinoma cell line HT 29. FEBS Lett. 201, 31-36.

Anastasi A., Erspamer V. and Bucci M. (1971). Isolation and structure of bombesin and alytesin, two analogous active peptides from the skin of the European amphibians Bombina and Alytes. Experientia 27,

166-167.

Augood S.J., Keast J.R. and Emson P.C. (1988). Distribution and characterization of neuromedin U-like immunoreactivity in rat brain and in guinea pig intestine. Regul. Pept. 20, 281-292.

Ballesta J., Carlei F., Bishop A.E., Steel J.H., Gibson S.J., Fahey M., Hennessey R., Domin J., Bloom S.R. and Polak J.M. (1988). Occurrence and developmental pattern of neuromedin U-immunoreactive nerves in the gastrointestinal tract and brain of the rat. Neuroscience 25, 797-816.

Battey J.F., Way J., Corjay M.H., Shapira H., Kusano K., Harkins R., Wu J.M., Slattery T., Mann E. and Feldman R. (1991). Molecular cloning of the bombesin/GRP receptor from Swiss 3T3 cells. Proc. Natl. Acad. Sci. USA 88, 395-399.

Battey J.F., Harkins R. and Feldman R.I. (1993). Purification and molecular cloning of bombesin/gastrin-releasing peptide receptor. Methods Neurosci. 12, 85-109.

Benya R.V., Wada E., Battey J.F., Fathi Z., Wang L.-H., Mantey S., Coy D.H. and Jensen R.T. (1992). Neuromedin B receptors retain functional expression when transfected into BALB 3T3 fibroblast: analysis of binding, kinetics, stoichiometry, modulation by guanine nucleotide-binding proteins, and signal transduction and comparison with natively expressed receptors. Mol. Pharmacol. 42, 1058-1068.

Bonner T.I., Affolter H.-U., Young A.C. and Young W.S. (1987). A cDNA encoding the precursor of the rat neuropeptide, neurokinin B. Mol. Brain Res. 2, 243-249.

Bornstein S.R., Ehrhart-Bornstein M., Scherbraum W.A., Pfeiffer E.F. and Holst J.J. (1990). Effects of splanchnic nerve stimulation on the adrenal cortex may be mediated by chromaffin cells in a paracrine manner. Endocrinology 127, 900-906.

Bornstein S.R., Ehrhart-Bornstein M., Usadel H., Böckmann M. and Scherbaum W.A. (1991). Morphological evidence for a close interaction of chromaffin cells with cortical cells within the adrenal gland. Cell Tissue Res. 265, 1-9.

Brown D.R. and Quito F.L. (1990). Neuromedin U octapeptide alters ion transport in porcine jejunum. Eur. J. Pharmacol. 155, 159-162.

Brown E.R., Harlan R.E. and Krause J.E. (1990). Gonadal steroid regulation of substance P (SP) and SP encoding messenger ribonucleic acids in the rat anterior pituitary and hypothalamus. Endocrinology 126, 330-340.

Cardona C., Rabbitts P.H., Spindel R., Hatei M.A., Bleehen N.M., Bloom S.R. and Reeve J.G. (1991). Production of neuromedin B and neuromedin B gene expression in human lung tumor cell lines. Cancer Res. 51, 5205-5211.

Carraway R.E. and Mitra S.P. (1987). The use of radioimmunoassay to compare the tissue and subcellular distributions of neurotensin and neuromedin N in the cat. Endocrinology 120, 2092-2100.

Checler F., Vincent J.P. and Kitabgi P. (1986). Neuromedin N: High affinity interaction with brain neurotensin receptors and rapid inactivation by brain synaptic peptidases. Eur. J. Pharmacol. 126, 239-244.

Cimini V., Van Noorden S., Timson C.M. and Polak J.M. (1993). Modulation of galanin and neuromedin U-like immunoreactivity in rat corticotropes after alteration of endocrine status. Cell Tissue Res. 272, 137-146.

Coquerel A., Dubuc J., Kitabgi P. and Costentin J. (1988). Potentiation by thiorphan and betastatin of the naloxone-insensitive analgesic effects of neurotensin and neuromedin N. Neurochem. Int. 12, 361-366.

Conlon J.M., Domin J., Thim L., Dimarzo V., Morris H.R. and Bloom S.R. (1988). Primary structure of neuromedin U from the rat. J. Neurochem. 51, 988-991.

Corjay M.H., Dobrzanski D.J., Way J.M., Viallet J., Shapira H., Worland P., Sausville E.A. and Battey J.F. (1991). Two distinct bombesin receptor subtypes are expressed and functional in human lung carcinoma cells. J. Biol. Chem. 266, 18771-18779.

Deacon C.F., Agoston D.V., Nau R. and Conton J.M. (1987). Conversion of neuropeptide K to neurokinin A and vesicular colocalization of neurokinin A and substance P in neurons of the guinea pig small intestine. J. Neurochem. 48, 141-146.

Debeljuk L., Ghosh P. and Bartke A. (1990). Neurokinin A levels in the hypothalamus of rats and mice: Effects of castration, gonadal steroids and expression of heterologous growth hormone genes. Brain Res. Bull. 25, 717-721.

Debeljuk L., Lam E.W. and Bartke A. (1991). Effects of castration and sex steroids on neurokinin A concentrations in the anterior pituitary of male rats. Neuroendocrinol. Lett. 13, 5-14.

Debeljuk L., Villanua M.A. and Bartke A. (1992). Neurokinin A in the anterior pituitary of female rats: effects of ovariectomy and estradiol. Peptides 13, 1001-1005.

Dobner P.R., Barber D.L., Villa-Komaroff L. and McKiernan C. (1987). Cloning and sequencing of cDNA for the canine neurotensin/neuromedin N precursor. Proc. Natl. Acad. Sci. USA 84, 3516-3520.

Dobner R., Kislauskis E. and Bullock P.B. (1992). Cooperative regulation of neurotensin/neuromedin N gene expression in PC12 cells involves AP-1 transcription factors. Ann. NY Acad. Sci. 668, 17-29.

Dobrzanski D., Sharoni Y., Wada E., Battey J. and Sausville E. (1993). Neuromedin-B receptor transfected BALB/3T3 cells: Signal transduction and effects of ectopic receptor expression on cell growth. Regul. Pept. 45, 341-352.

Domin J., Ghatei M.A., Chochan P. and Bloom S.R. (1986). Characterization of neuromedin U-like immunoreactivity in rat, porcine, guinea pig and human tissue extracts using a specific radioimunoassay. Biochem. Biophys. Res. Commun. 140, 1127-1134.

Domin J., Ghatei M.A., Chochan P. and Bloom S.R. (1987). Neuromedin U - a study of its distribution in the rat. Peptides 8, 779-784.

Domin J., Yiangon Y., Spokes R.A., Atkin A., Parmar K.B., Chrysanthou B.J. and Bloom S.R. (1989a). The distribution, purification and pharmacological action of an amphibian neuromedin U. J. Biol. Chem. 264, 20881-20885.

Domin J., Polak J.M. and Bloom S.R. (1989b). The distribution and biological effects of neuromedins B and U. Ann. NY Acad. Sci. 547, 391-403.

Domin J., Steel J.H., Adolphus N., Burrin J.M., Leonhardt U., Polak J.M. and Bloom S.R. (1989c). The anterior pituitary content of neuromedin U-like immunoreactivity is altered by thyreotropin-releasing hormone and thyroid status in the rat. J. Endocrinol. 122, 471-476.

Dubuc I., Nouel D.,Coquerel A., Menard J.F., Kitabgi P. and Costentin J. (1988). Hypothermic effect of neuromedin N in mice and its potentiation by peptidase inhibitors. Eur. J. Pharmacol. 151, 177-191.

Eistetter H.R., Church D.J., Mills A., Godfrey P.P., Capponi A.M., Brewster R., Schulz M.-F., Kawashima E. and Arkinstall S.J. (1991). Recombinant bovine neurokinin-2 receptor stably expressed in Chinese hamster ovary cells couples to multiple signal transduction pathways. Cell Regul. 2, 767-769.

Erspamer G.F., Severini C., Erspamer V., Melchiorri P., delle Fave G. and Nakajima T. (1988). Parallel bioassay of 27 bombesin-like

peptides on 9 smooth muscle preparations. Structure-activity relationships and bombesin receptor subtypes. Regul. Pept. 21, 1-11.

Familari M., Funder J.W. and Giraud A.S. (1987). Bombesin potentiation of CRF stimulated ACTH release is dependent on presence of glucocorticoids. Regul. Pept. 19, 107-112.

Faria M., Navarra P., Tsagarakis S., Besser G.M. and Grossman A.B. (1991). Inhibition of CRH-41 release by substance P, but not substance K, from the rat hypothalamus *in vitro*. Brain Res. 538, 76-78.

Fathi Z., Benya R.V., Shapira H., Jensen R.T. and Battey J.F. (1993). The fifth transmembrane segment of the neuromedin B receptor is critical for high affinity neuromedin B binding. J. Biol. Chem. 268, 14622-14626.

Fisher J.B. and Schonbrunn A. (1988). The bombesin receptor is coupled to a guanine nucleotide-binding protein which is insensitive to pertussis and cholera toxins. J. Biol. Chem. 263, 2808-2816.

Fong T.M., Huang R.-R. and Strader C.D. (1992). Localization of agonist and antagonist binding domains of the human neurokinin-1 receptor. J. Biol. Chem. 267, 25664-25667.

Gardiner S.M., Compton A.M., Bennett T., Domin J. and Bloom S.R. (1990). Regional hemodynamic effects of neuromedin U in conscious rats. Am. J. Physiol. 258, R32-R38.

Giaccone G., Battey J., Gazdar A.F., Oie H., Draoui M. and Moody T.W. (1992). Neuromedin B is present in lung cancer cell lines. Cancer Res. 52, 2732-2736.

Goedert M., Pinnock R.D., Downes C.P., Mantyh P.W. and Emson P.C. (1984). Neurotensin stimulates inositol phospholipids hydrolysis in rat brain slices. Brain Res. 323, 193-197.

Gunion M.W., Tache Y., Rosenthal M.J., Miller S., Butler B. and Zib B. (1989). Bombesin microinfusion into the rat hypothalamic paraventricular nucleus increases blood glucose, free fatty acids and corticosterone. Brain Res. 478, 47-58.

Hale A.C., Price J., Ackland J.F., Doniach I., Ratter S., Besser G.M. and Rees L.H. (1984). Corticotropin-releasing factor mediated adrenocorticotrophin release from rat anterior pituitary cells is potentiated by C-terminal gastrin-releasing peptide. J. Endocrinol. 102, 121-123.

Hashimoto T., Masui H., Uchida Y., Sakura O. and Kimura K. (1991). Agonistic and antagonistic activities of neuromedin U-8 analogs substituted with glycine or D-amino acid on contractile activity of chicken crop smooth muscle preparation. Chem. Pharm. Bull. 39, 2319-2322.

Helke C.J., Krause J.E., Mantyh P.W., Couture R. and Bannon M.J. (1990). Diversity in mammalian tachykinin peptidergic neurones: Multiple peptides, receptors, and regulatory mechanisms. FASEB J. 4, 1606-1615.

Hermans E., Maloteaux J.-M. and Octave J.-N. (1992). Phospholipase C activation by neurotensin and neuromedin N in Chinese hamster ovary cells expressing the rat neurotensin receptor. Mol. Brain Res. 15, 323-338.

Heslop J.P., Blakely D.M., Brown K.D., Irvine R.T. and Berridge M.J. (1986). Effects of bombesin and insulin on inositol (1,4,5)triphosphate and inositol (1,3,4)triphosphate formation in Swiss 3T3 cells. Cell 47, 703-709.

Hinson J.P. (1990). Paracrine control of adrenocortical function: A new role for the medulla? J. Endocrinol. 124, 7-9.

Holrer-Petsche U., Schimek E., Amann R. and Lemberk F. (1985). In vivo and in vitro actions of mammalian tachykinins. Naunyn-Schmiedeberge. Arch. Pharmacol. 330, 130-135.

Honzawa M., Sudok T., Minamino N., Tokyama M. and Matsuo H. (1987). Topographic localization of neuromedin U-like structures in the rat brain: an immunohistochemical study. Neuroscience 23, 1103-1122.

Houben H. and Denef C. (1990a). Stimulation of growth hormone and prolactin release from rat pituitary cell aggregates by bombesin- and ranatensin-like peptides is potentiated by estradiol, 5α-dihydrotestosterone, and dexamethasone. Endocrinology 126, 2257-2266.

Houben H. and Denef C. (1990b). Regulatory peptides produced in the anterior pituitary. TEM 1, 398-403.

Houben H. and Denef C. (1991). Evidence for the presence of gastrin-releasing peptide immunoreactivity in rat anterior pituitary corticotrophs and lactotrophs, AtT_{20} cells, and GH_3 cells: Failure to demonstrate participation in local control of hormone release. Endocrinology 128, 3208-3218.

Hunter J.C., Hannah P.A. and Maggio J.E. (1985). The regional distribution of kassinin-like immunoreactivity (KLI) in central and peripheral tissues of the cat. Brain Res. 341, 228-232.

Jessop D.S., Chowdrey H.S. and Lightman S.L. (1990). Substance P and substance K in the median eminence and paraventricular nucleus of the rat. Neuropeptides 17, 135-140.

Jessop D.S., Chowdrey H.S., Biswas S. and Lightman S.L. (1991). Substance P and substance K in the rat hypothalamus following monosodium glutamate lesions of the arcuate nucleus. Neuropeptides 18, 165-170.

Jessop D.S., Chowdrey H.S., Larsen P.J. and Lightman S.L. (1992). Substance P: Multifunctional peptide in the hypothalamo-pituitary system? J. Endocrinol. 132, 331-337.

Jonassen J.A., Mullikin-Kilpatrick D., McAdam A. and Leeman S.E. (1987). Thyroid hormone status regulates preprotachykinin-A gene expression in male rat anterior pituitaries. Endocrinology 121, 1555-1561.

Jones P.M., Withers D.J., Gathei M.A. and Bloom S.R. (1992). Evidence for neuromedin-B synthesis in the rat anterior pituitary gland. Endocrinology 130, 1829-1836.

Kalra P.S. and Kalra S.P. (1993). Neuropeptide K stimulates corticosterone release in the rat. Brain Res. 610, 330-333.

Kanba K.S. and Richelson E. (1987). Comparison of the stimulation of inostiol phospholipids hydrolysis and of cyclic GMP formation by neurotensin, some of its analogs and neuromedin N in neuroblastoma clone N13115. Biochem. Pharmacol. 36, 869-874.

Kawaguchi Y., Hosimaru M., Nawa H. and Nakanishi S. (1986). Sequence analysis of cloned cDNA for rat substance P precursor. Biochem. Biophys. Res. Commun. 139, 1040-1046.

Khalil Z., Marley P.D. and Livett B.G. (1988). Mammalian tachykinins modulate the nicotinic secretory response of cultured bovine adrenal chromaffin cells. Brain Res. 459, 289-297.

Kislauskis E., Bullock B., McNeil S. and Dobner P.R. (1988). The rat gene encoding neurotensin and neuromedin N: Structure, tissue-specific expression and evolution of exon sequences. J. Biol. Chem. 263, 4963-4968.

Kita T., Chihara K., Abe H., Minamitani N., Kaji H., Kodama H., Chiba T., Fujita T. and Yanaihara N. (1986). Regional distribution of gastrin-releasing peptide- and somatostatin-like immunoreactivity in the rabbit hypothalamus. Brain Res. 398, 18-22.

Kitabgi P., Nadai F., Cuber J.C., Dubuc I., Nouel D. and Costentin J. (1990). Calcium-dependent release of neuromedin N and neurotensin from mouse hypothalamus. Neuropeptides 15, 111-114.

Kitabgi P., Masuo Y., Nicot A., Berod A., Cuber J.-C. and Rostene W.

(1991). Marked variations of the relative distributions of neurotensin and neuromedin N in micropunched rat brain areas suggest differential processing of their common precursor. Neurosci. Lett. 124, 9-12.

Knigge U., Holst J.T., Knuhtsen S., Bach F.W. and Bang P. (1988). Corticotropin-releasing activity of gastrin-releasing peptide in normal man. J. Clin. Endocrinol. Metabol. 65, 1291-1295.

Kotani H., Hoshimaru M., Nawa H. and Nakanishi S. (1986). Structure and gene organization of bovine neuromedin K precursor. Proc. Natl. Acad. Sci. USA 83, 7074-7078.

Krane I.M., Naylor S.L., Helin-Davis D., Chin W.W. and Spindel E.R. (1988). Molecular cloning of cDNAs encoding human bombesin-like peptide neuromedin B. J. Biol. Chem. 263, 13317-13323.

Krause J.E., Chirgwin J.M., Carter M.S., Xu Z.S. and Hershey A.D. (1987). Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. Proc. Natl. Acad. Sci. USA 84, 881-885.

Ladenheim E.E., Moran T.H. and Jensen R.T. (1991). Identification and characterization of neuromedin B receptors in rat central nervous system. Methods Neurosci. 5, 426-441.

Larsen P.J., Jessop D.S., Lightman S.L. and Chowdrey H.S. (1993). Preprotachykinin A gene expression in distinct hypothalamic and brain stem regions of the rat is affected by a chronic osmotic stimulus: A combined immunohistochemical and in situ hybridization histochemistry study. Brain Res. Bull. 30, 535-545.

Lebacq-Verheyden A., Krystal G., Sartor O., Way J. and Battey J.F. (1988). The rat prepro gastrin releasing peptide gene is transcribed from two initiation sites in the brain. Mol. Endocrinol. 2, 556-563.

Lemaire S., Trifaro J.M., Chouinard L., Cecyre D., Dessureault J., Mercier P. and Dumont M. (1989). Structural identification, subcellular localization and secretion of bovine adrenomedullary neuromedin C [GRP-(18-27)]. Peptides 10, 355-360.

Leśniewska B., Nussdorfer G.G., Nowak M. and Malendowicz L.K. (1992). Comparison of the effects of neurotensin and vasopressin on the adrenal cortex of dexamethasone-suppressed rats. Neuropeptides 23, 9-13.

Maggi C.A., Patacchini R., Guiliani S., Turini D., Barbanti G., Rovereo P. and Meli A. (1990). Motor response of the human isolated small intestine and urinary bladder to porcine neuromedin U-8. Br. J. Pharmacol. 99, 186.

Maggio J.E. (1988). Tachykinins. Annu. Rev. Neurosci. 11, 13-28.

Malendowicz L.K. (1993). Involvement of neuropeptides in the regulation of growth, structure and function of the adrenal cortex. Histol. Histopath. 8, 173-186.

Malendowicz L.K., Nussdorfer G.G., Leśniewska B., Markowska A. and Nowak M. (1991a). Comparison of the effects of neurotensin and ACTH on the pituitary-adrenocortical axis of dexamethasone-suppressed rats. J. Steroid Biochem. Molec. Biol. 39, 115-117.

Malendowicz L.K., Nussdorfer G.G., Markowska A. and Leśniewska B. (1991b). Neurotensin enhances the level of circulating ACTH in rats, without altering the blood concentration of glucocorticoids. Neuroendocrinol. Lett. 13, 269-273.

Malendowicz L.K., Leśniewska B., Miśkowiak B., Nussdorfer G.G. and Nowak M. (1991c). Effects of neurotensin on the pituitary-adrenocortical axis of intact and dexamethasone-suppressed rats. Exp. Pathol. 43, 205-211.

Malendowicz L.K., Nussdorfer G.G., Nowak K.W., Torliński L. and Leśniewska B. (1992a). Neuromedin-N increases the levels of circulating ACTH and aldosterone without changing the blood concentration of corticosterone in rats. Neuroendocrinol. Lett. 14, 343-348.

Malendowicz L.K., Nussdorfer G.G., Majchrzak M., Nowak M. and Leśniewska B. (1992b). Neurotensin stimulates the growth and secretion of rat adrenal zona glomerulosa. In Vivo 6, 523-526.

Malendowicz L.K., Nussdorfer G.G., Markowska A., Nowak K.W. and Torliński L. (1993a). Effects of neuromedin-N on the pituitary-adrenocortical axis of dexamethasone-suppressed rats. Neuropeptides 24, 1-4.

Malendowicz L.K., Nussdorfer G.G., Nowak K.W. and Mazzochi G. (1993b). Effects of neuromedin U-8 on the rat pituitary-adrenocortical axis. In Vivo 7, 419-422.

Malendowicz L.K., Nowak K.W., Nussdorfer G.G., Markowska A. and Nowak M. (1994a). Effects of neuromedin-B on the rat pituitary-adrenocortical axis. Neuroendocrinol. Lett. 16, 17-24.

Malendowicz L.K., Nussdorfer G.G., Markowska A., Tortorella C., Nowak M. and Warchol J.B. (1994b). Effects of neuromedin U (NMU)-8 on the rat hypothalamo-pituitary-adrenal axis. Evidence of a direct effect of NMU-8 on the rat adrenal gland. Neuropeptides 26, 47-53.

Malendowicz L.K., Andreis P.G., Markowska A., Nowak M., Warchol J.B., Neri G. and Nussdorfer G.G. (1994c). Effects of neuromedin U-8 on the secretory activity of the rat adrenal cortex: Evidence for an indirect action requiring the presence of the zona medullaris. Res. Exp. Med. (in press).

Markowska A., Nussdorfer G.G. and Malendowicz L.K. (1992). Studies on the preventive action of neurotensin on dexamethasone-induced adrenocortical atrophy. In Vivo 6, 279-282.

Markowksa A., Nussdorfer G.G. and Malendowicz L.K. (1993). Effects of bombesin and neuromedin-B on the proliferative activity of the rat adrenal cortex. Histol. Histopath. 8, 359-362.

Markowska A., Nussdorfer G.G. and Malendowicz L.K. (1994a). Different effects of neurotensin and neuromedin-N on the proliferative activity of rat adrenal cortex. Histol. Histopath. 9, 449-452.

Markowska A., Nussdorfer G.G. and Malendowicz L.K. (1994b). Proliferogenic effect of neurotensin (NT) and neuromedin-N (NMN) on the rat adrenal cortex: Evidence that angiotensin-II mediates the effect of NMN, but not of NT. Neuropeptides 26, (in press).

Massi M., Saija A., Polidori C., Perfumi M., Gentili L., Costa G. and de Caro G. (1991). The hypothalamic paraventricular nucleus is a site of action for the central effect of tachykinins on plasma vasopressin. Brain Res. Bull. 26, 149-154.

Masu Y., Nakayama K., Tamaki H., Harada Y., Kuno M. and Nakanishi S. (1987). cDNA cloning of bovine substance K receptor through oocyte expression system. Nature 329, 836-838.

Mazzocchi G., Malendowicz L.K., Rocco S., Musajo F. and Nussdorfer G.G. (1993). Arginine-vasopressin release mediates the aldosterone secretagogue effect of neurotensin in rats. Neuropeptides 24, 105-108.

McDonald T.J., Jornvall H., Nilsson G., Vagne M., Ghatei M., Bloom S.R. and Mutt V. (1979). Characterization of a gastrin releasing peptide from porcine non-antral gastric tissue. Biochem. Biophys. Res. Commun. 90, 227-233.

McDonald T.J., Jornvall H., Ghatei M., Bloom R. and Mutt V. (1980). Characterization of avian gastric (proventricular) peptide having sequence homology with the porcine gastrin-releasing peptide and the amphibian peptides bombesin and alytesin. FEBS Lett. 122, 45-48.

Merchenthaler I., Maderdrut J.L., O'Hare F. and Conlon J.M. (1992). Localization of neurokinin B in the central nervous system of the rat. Peptides 13, 815-829.

Minamino N., Kangawa K. and Matsuo H. (1983). Neuromedin B: A novel bombesin-like peptide identified in porcine spinal cord. Biochem. Biophys. Res. Commun. 114, 541-548.

Minamino N., Matsuda H., Kangawa K. and Matsuo H. (1984a). Regional distribution of neuromedin K and neuromedin L in rat brain and spinal cord. Biochem. Biophys. Res. Commun. 124, 731-738.

Minamino N., Kangawa K., Fukuda A. and Matsuo H. (1984b). Neuromedin L: A novel mammalian tachykinin identified in porcine spinal cord. Neuropeptides 4, 157-166.

Minamino N., Kangawa K. and Matsuo H. (1984c). Neuromedin B is a major bombesin-like peptide in rat brain: Regional distribution of neuromedin B and neuromedin C in rat brain, pituitary and spinal cord. Biochem. Biophys. Res. Commun. 124, 925-932.

Minamino N., Kangawa K. and Matsuo H. (1984d). Neuromedin C: A bombesin-like peptide identified in porcine spinal cord. Biochem. Biophys. Res. Commun. 119, 14-20.

Minamino N., Kangawa K. and Matsuo H. (1984e). Neuromedin N: A novel neurotensin-like peptide identified in porcine spinal cord. Biochem. Biophys. Res. Commun. 156, 355-360.

Minamino N., Kangawa K. and Matsuo H. (1985). Neuromedin U-8 and U-25: novel uterus stimulating and hypertensive peptides identified in porcine spinal cord. Biochem. Biophys. Res. Commun. 130, 1078-1085.

Minamino N., Kangawa K., Honzawa M. and Matsuo H. (1988). Isolation and structural determination of rat neuromedin U. Biochem. Biophys. Res. Commun. 156, 355-360.

Moody T.W., Korman L.Y and O'Donohue T.L. (1986). Neuromedin B-like peptides in rat brain: Biochemical characterization, mechanism of release and localization in synaptosomes. Peptides 7, 815-820.

Murphy R., Turner C.A., Furness J.B., Parker L. and Giraud A. (1990). Isolation and microsequence analysis of a novel form of neuromedin U from guinea pig small intestine. Peptides 11, 613-617.

Nadai F., Cuber J.C. and Kitabgi P. (1989). The characterization and regional distribution of neuromedin N-like immunoreactivity in rat brain using a highly sensitive and specific radioimmunoassay. Comparison wih the distribution of neurotensin. Brain Res. 500, 193-198.

Nagalla S.R., Gibson B.W., Tang D., Reeve J.R.Jr. and Spindel E.R. (1992). Gastrin-releasing peptide (GRP) is not mammalian bombesin. Identification and molecular cloning of a true amphibian GRP distinct from amphibian bombesin in *Bombina orientalis*. J. Biol. Chem. 267, 16916-16922.

Nakanishi S. (1986). Structure and regulation of the preprotachykinin gene. TINS 9, 41-44.

Namba M., Ghatei M.A., Bishop A.E., Gibson S.J., Mann D.J., Polak J.M. and Bloom S.R. (1985). Presence of neuromedin B-like immunoreactivity in the brain and gut of rat and guinea pig. Peptides 6, 257-263.

Nandha K.A., Benitoorfila M.A., Smith D.M. and Bloom S.R. (1993). Characterization of the rat uterine neuromedin-U receptor. Endocrinology 133, 482-486.

Nawa H., Hirose T., Takashima H., Inayama S. and Nakanishi S. (1983). Nucleotide sequences of cloned cDNAs for two types of bovine substance P precursor. Nature 306, 32-36.

O'Harte F., Bockman Ch.S., Abel P.W. and Conlon J.M (1991). Isolation, structural characterization and pharmacological activity of dog neuromedin U. Peptides 12, 11-15.

Olsen L., Knigge U. and Warberg J. (1992). Gastrin releasing peptide stimulation of corticotropin secretion in male rats. Endocrinology 130, 2710-2716.

Orloff M.S., Reeve J.R.Jr., Ben-Avram C.M. Shively J.E. and Walsh J.H. (1984). Isolation and sequence analysis of human bombesin-like peptides. Peptides 5, 865-870.

Panula P., Yang H.-Y. and Costa E. (1984). Comparative distribution of bombesin/GRP- and substance-P-like immunoreactivities in rat hypothalamus. J. Comp. Neurol. 224, 606-617.

Pirkut D.T. and Joseph S.A. (1986). Co-existence of CRF and vasopressin immunoreactivity in parvocellular paraventricular neurons of rat hypothalamus. Peptides 7, 891-898.

Quirion R. and Dam T.-V. (1988). Multiple neurokinin-receptors. Recent developments. Regul. Pept. 22, 18-25.

Reeve J.R., Walsh J.H., Chew P., Clark B., Hawke D. and Shively J.E. (1983). Amino acid sequences of three bombesin like peptides from canine intestine extracts. J. Biol. Chem. 258, 5582-5588.

Rettori V., Milenkovic L., Fahim A.M., Polak J.M., Bloom S.R. and McCann S.M. (1989). Role of neuromedin B in the control of the release of thyrotropin in the rat. Proc. Natl. Acad. Sci. USA 86, 4789-4792.

Rivier C., Rivier J. and Vale W. (1978). The effect of bombesin and related peptides on prolactin and growth hormone secretion in the rat. Endocrinology 102, 519-522.

Rompre P.-P. and Gratton A. (1992). A comparison of the effects of mesencephalic injections of neurotensin (1-13) and neuromedin N on brain electrical self-stimulation. Peptides 13, 713-719.

Sakura N., Ohta S., Uchida Y., Kurosawa K., Okimura K. and Hashimoto T. (1991). Structure-activity relationships of rat neuromedin U for smooth muscle contraction. Chem. Pharm. Bull. 39, 2016-2020.

Sander L.D. and Thomas R.M. (1991). Gastrin releasing peptide, but not pentagastrin, stimulates ACTH and cortisol secretion in conscious dogs. Regul. Pept. 35, 127-133.

Sausville E., Lebacq-Verheyden A.M., Spindel E.R., Cuttitta F., Gazdar A.F. and Battey J. (1986). Expression of the gastrin releasing peptide gene in human small cell lung cancer. J. Biol. Chem. 261, 2451-2459.

Shaw C., Thim L. and Conlon J.M. (1987). Primary structure and tissue distribution of guinea pig gastrin-releasing peptide. J. Neurochem. 49, 1348-1354.

Shaw C., McKay D., Johnston C.F., Halton D.W., Fairweather I., Kitabgi P. and Buchanan K.D. (1990). Differential processing of the neurotensin/neuromedin N precursor in the mouse. Peptides 11, 227-235.

Shigemoto R., Yokota Y., Tsuchida K. and Nakanishi S. (1990). Cloning and expression of rat neuromedin K receptor cDNA. J. Biol. Chem. 265, 623-628.

Spindel E.R., Chin W.W., Price J., Besser L.H. and Habener J.F. (1984). Cloning and characterization of cDNAs encoding human gastrin releasing peptide. Proc. Natl. Acad. Sci. USA 81, 5699-5703.

Spindel E.R., Giladi E., Brehm T.P., Goodman R.H. and Segerson T.P. (1990). Cloning and functional characterization of a cDNA encoding the murine fibroblast bombesin/GRP receptor. Mol. Endocrinol. 4, 1956-1963.

Steel J.H., Van Norden S., Ballesta J., Gibson S.J., Ghatei M.A., Burrin J., Leonhard U., Domin J., Bloom S.R. and Polak J.M. (1988). Localization of 7B2, neuromedin B, and neuromedin U in specific cell types of rat, mouse, and human pituitary, in rat hypothalamus

and in 30 human pituitary and extrapituitary tumors. Endocrinology 122, 270-282.

Sumi S., Inoue K., Kogire M., Doi R., Takaori K., Suzuki T., Yajima H. and Tobe T. (1987). Effect of synthetic neuromedin U-8 and U-25, novel peptides identified in porcine spinal cord, on splanchnic circulation in dogs. Life Sci. 41, 1585-1590.

Tajima K., Namba M., Oda Y., Matsui I., Mori M., Kitajima K., Mashita K. and Tarui S. (1989). Inhibitory effect of neuromedin B on the release of thyrotropin from perifused rat pituitaries. Biomed. Res. 10, 443-446.

Tanaka K., Masu M. and Nakanishi S. (1990). Structure and functional expression of the cloned rat neurotensin receptor. Neuron 4, 847-854.

Tateishi K., Matsuoka Y. and Hamaoka T. (1989). Establishment of highly specific radioimmunoassays for neurokinin A and neurokinin B and determination of tissue distribution of these peptides in rat central nervous system. Regul. Pept. 24, 245-257.

Tatemoto K., Lundberg J.M., Jornavall H. and Mutt V. (1985). Neuropeptide K: Isolation, structure and biological activities of a novel brain tachykinin. Biochem. Biophys. Res. Commun. 128, 947-953.

Thomas R.M. and Sander L.D. (1985). Influence of CCK and bombesin on ACTH and cortisol secretion in the conscious dog. Peptides 6, 703-707.

Timmermans J.P., Scheuermann D.W., Stach W., Adriaensen D., De Groodt-Lassel M. and Polak J.M. (1989). Neuromedin U-immunoreactivity in the nervous system of the small intestine of the pig and coexistence with substance P and CGRP. Cell Tissue Res. 258, 331-337.

von Schrenck T., Heinz-Erian P., Moran T., Mantex S.A., Gardner J.D. and Jensen R.T. (1989). Neuromedin B receptor in esophagus: evidence for subrypes of bombesin receptors. Am. J. Physiol. 256, G747-G758.

Wada E., Way J., Lebacq-Verheyden A.M. and Battey J.F. (1990). Neuromedin B and gastrin-releasing peptide mRNAs are differentially distributed in the rat central nervous system. J. Neurosci. 10, 2917-2930.

Wada E., Way J., Shapira H., Kusano K., Lebacq-Verheyden A.M., Coy D., Jensen R. and Battey J. (1991). cDNa cloning, characterization, and brain-region-specific expression of a neuromedin B-preffering bombesin receptor. Neuron 6, 421-430.

Warden M.K. and Young W.S. (1988). Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system. J. Comp. Neurol. 272, 90-113.

Watanabe T. and Orth D.N. (1988). Effect of several in vitro systems on the potencies of putative adrenocorticotrophin secretagogues on rat anterior pituitary cells. Endocrinology 122, 2299-2308.

Yokota Y., Akazawa C., Ohkubo K. and Nakanishi S. (1992). Delineation of structural domains involved in the subtype specificity of tachykinin receptors through chimeric formation of substance P/substance K receptors. EMBO J. 11, 3585-3591.

Yoshida T., Mio M. and Tasaka K. (1992). Cortisol secretion induced by substance P from bovine adrenocortical cells and its inhibition by calmodulin inhibitors. Biochem. Pharmacol. 43, 513-517.