Neuropeptides in hypertension: role of neuropeptide Y and calcitonin gene related peptide

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- 1 The effect of neuropeptide Y (NPY) on cardiovascular function at three levels of the noradrenergic axis where the peptide is known to co-exist with noradrenaline (NA) and or adrenaline (A) was studied in normotensive Sprague-Dawley (SD), Wistar-Kyoto (WKY) or spontaneously hypertensive rats (SHR).
- 2 In the perfused mesenteric arterial bed, NPY and the structurally similar peptide intestinal polypeptide (PYY) decreased the periarterial nerve stimulation induced release of NA and potentiated the increase in perfusion pressure to nerve stimulation or exogenously applied agonists (e.g. angiotensin, vasopressin, phenylephrine). In contrast to NPY and PYY, C-terminal NPY fragments inhibited NA release and produced a parallel decrease in perfusion pressure thus supporting the concept of Y₁ (post) and Y₂ (pre) NPY receptors.
- 3 In the mesenteric artery of SHR the prejunctional inhibitory effect of NPY was attenuated while the postjunctional response was enhanced.
- 4 Following intrathecal (Int) injection of NPY, there was a decrease in blood pressure, total peripheral resistance (predominantly by a decrease in mesenteric vascular resistance) and renal nerve activity. The depressor effect of Int NPY was attenuated in the SHR.
- 5 Unilateral injections of NPY into the posterior hypothalamic nucleus increased blood pressure, hindquarter and renal vascular resistance and renal nerve activity. The pressor effect was enhanced in the SHR.
- 6 Periarterial nerve stimulation of the perfused mesenteric artery produced a frequency dependent vasodilation in beds pretreated with guanethidine and precontracted with methoxamine. The vasodilation was unaffected by propranolol or atropine but blocked by capsaicin or calcitonin gene related peptide (CGRP) antisera. CGRP decreased the vasoconstriction produced by nerve stimulation or exogenous NA. NA release was unaltered.
- 7 In SHR, the inhibitory effect of exogenous CGRP on the nerve stimulation induced increase in perfusion pressure was reduced as well as the duration of the vasodilation observed in the presence of guanethidine and methoxamine.
- 8 These results implicate NPY in the development or maintenance of hypertension in the SHR since the effects of NPY which decrease noradrenergic transmission or responsiveness are attenuated in the SHR, while those functions increasing noradrenergic responsive-

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ness are increased in the SHR. Our results are consistent with CGRP serving as a noradrenergic-noncholinergic vasodilator neurotransmitter in the mesenteric arterial bed. A dysfunction of the CGRP system may also contribute to the pathogenesis of hypertension in the SHR.

Keywords neuropeptides hypertension calcitonin gene related peptide

Introduction

It is now known that a variety of vasoactive peptides, stored and released from perivascular nerves or other tissue (smooth muscle, endothelium, myocardium) exert important actions on the cardiovascular system. Such peptides include neuropeptide Y (NPY), calcitonin gene related peptide (CGRP), angiotensin, vasoactive intestinal polypeptide, substance P, atrial natriuretic peptide, vasopressin, endothelin as well as yet unidentified mediators (e.g. endothelial dependent relaxing factor(s) and contracting factors) to name but a few (see Burnstock & Griffith, 1988 for review). The purpose of this paper is to summarize the results of recent studies in our laboratory where we have examined the effect of two of these peptides, NPY and CGRP. NPY has been studied at three levels of the noradrenergic axis where the peptide is known to be co-localized with noradrenaline (NA) and/or adrenaline (A) namely: the vascular noradrenergic neuroeffector junction, where the perfused mesenteric arterial bed has been utilized as a model, the thoracic spinal cord, and the posterior hypothalamic nucleus. Actions of CGRP were examined in the perfused mesenteric arterial bed.

Methods

Various strains of male rats including SHR and Wistar-Kyoto (WKY) (Taconic Farms, Germantown, NY, USA) and Sprague Dawley (SD) (SASCO, St Louis, MO, USA) were used. All animals were maintained under standard vivarium conditions with food and water *ad lib*. Blood pressure was monitored in conscious rats by tail cuff plethysmography 1 or 2 days prior to experimentation or placement of indwelling cannulas, catheters and flow probes. The systolic blood pressure for WKY, SD and SHR of 8–10 week old rats were 106 ± 4 , 105 ± 3 and 160 ± 6 mm Hg, respectively.

The perfused mesenteric arterial bed preparation originally described by McGregor (1965) was set up as described previously (Westfall *et*

al., 1987). Briefly, the mesenteric vascular bed was dissected from the rat, perfused (5 ml min^{-1}) and superfused (0.5 ml min^{-1}) with modified Krebs-bicarbonate buffer bubbled with 95% O_2 and 5% CO_2 at 37°C . A pair of platinum electrodes were placed around the mesenteric artery for periarterial nerve stimulation (various frequencies, 2 ms duration, supramaximal voltage). The preparation equilibrated for 45–60 min prior to experimentation. Perfusion pressure was continuously monitored on pressure transducers coupled to chart recorders. Perfusate effluents were collected in 1 min fractions into tubes containing 0.1 N perchloric acid, concentrated by alumina chromatography separated by h.p.l.c. and quantified by an electrochemical detector (Westfall *et al.*, 1987).

For intrathecal injections (Int), animals were prepared according to the method of Yaksh & Rudy (1976) and described by us in detail previously (Chen *et al.*, 1988). Briefly the atlanto-occipital membrane was exposed by a midline incision through the trapezoid and rhomboid muscles. Keeping the head flexed at 35° , one polyethylene catheter (PE-10) was inserted down the lower thoracic spinal subarachnoid space (T_{10}) through a puncture of the atlanto-occipital membrane. Drugs were dissolved in saline and slowly injected (over 20–30 s) at a volume of $5 \mu\text{l}$ injection of vehicle to wash the catheter. Miniaturized pulse-Doppler flow probes were implanted on the superior mesenteric artery and lower aorta or on the ascending aorta as described by Haywood and co-workers (1981). Arterial pressure, heart rate, hindquarter and mesenteric blood flow or aortic blood flow and in some experiments sympathetic renal nerve activity were monitored continuously throughout the period of study.

For microinjection studies, a cannula was positioned in the posterior hypothalamic nucleus as described by us previously (Martin *et al.*, 1988, 1989). Arterial pressure, heart rate, hindquarter, mesenteric and renal blood flow or aortic blood flow and in some experiments renal sympathetic nerve activity monitored continuously

throughout the study. Drugs were administered in volumes of 0.2–1 μ l over 5–30 s. In both the spinal cord and hypothalamic experiments, arterial pressure and blood flows were used to calculate the percent changes in regional vascular resistance on total peripheral resistance as described previously (Haywood *et al.*, 1981). Heart rate and aortic blood flow were used to calculate the percentage change in stroke volume.

The effect of NPY and NPY fragments on the potassium (K^+)-induced release of NA from slices of the posterior hypothalamus (PH) was carried out as described previously (Ciarleglio *et al.*, 1987; Westfall *et al.*, 1988). K^+ (56 mM) was used during two stimulation periods (S_1 and S_2) to evoke the release of NA measured by h.p.l.c.-EC or by liquid scintillation spectrometry from slices of the PH of SD, WKY and SHR. Drugs were added prior to S_2 . Other experiments examined the effect of drugs on the release of NPY like immunoreactivity (NPY-LI) from brain slices measured by a specific radioimmunoassay.

Results

Effects of NPY in normotensive and hypertensive rats

Pre and postjunctional effects of neuropeptide Y at the vascular noradrenergic neuroeffector junction NPY produced a concentration dependent inhibition of the nerve stimulation induced release of NA from the perfused mesenteric artery which was not altered by either α_1 - or α_2 -adrenoceptor antagonists (Westfall *et al.*, 1987). In contrast, the nerve induced increase in perfusion pressure was potentiated (Westfall *et al.*, 1988). The increase in perfusion pressure produced by various vasoactive substances including angiotensin, arginine vasopressin and phenylephrine was likewise potentiated by NPY (Westfall *et al.*, 1988). The postjunctional effect of NPY was independent of endothelial cells and does not appear to be due to the release of the 26 amino acid peptide endothelin (Han *et al.*, 1989; Knuepfer *et al.*, 1989; Yanagisawa *et al.*, 1988). Removal of the endothelial cells by perfusing the vascular bed with distilled water for 2–3 min resulted in a loss of the vasodilator effect to acetylcholine but did not alter the NPY induced potentiation of exogenously applied NA or vasopressin. Although endothelin was shown to increase basal perfusion pressure and potentiate the contractile effect of periaarterial nerve stimulation, it had no effect on the release of NA.

A series of C-terminal NPY fragments were utilized to examine the hypothesis that there are

distinct pre (Y_2) and post (Y_1) NPY receptors. Various C-terminal fragments including 11-36, 14-36, 16-36, 17-36 and 18-36 synthesized by Dr Jean Rivier of the Salk Institute were all observed to produce a concentration dependent decrease in the nerve stimulation induced release of NA and a parallel decrease in perfusion pressure. In contrast NPY and the closely related peptide intestinal polypeptides Y (PYY) decreased the evoked release of NA while potentiating the increase in perfusion pressure.

In the mesenteric artery obtained from the SHR, the ability of NPY to decrease the evoked release of NA was significantly attenuated (Figure 1) while the increase in perfusion pressure was greatly enhanced (Figure 2). The effect of NPY to potentiate the contractile effect of exogenously administered NA was also enhanced in the mesenteric artery of the SHR.

Intrathecal administration of neuropeptide Y The intrathecal (Int) administration of NPY in conscious or anaesthetized rats at the level of T_4 or T_{10} produced a decrease in blood pressure and heart rate (Chen *et al.*, 1988; Westfall *et al.*, 1988). In addition, Int NPY produced a decrease in total peripheral resistance predominantly due to a decrease in mesenteric vascular resistance with little effect on cardiac output. NPY also significantly decreased renal sympathetic activity but did not change baroreflex sensitivity. The depressor effect of NPY was attenuated by prior Int administration of the α_2 -adrenoceptor antagonist, yohimbine and the β -adrenoceptor antagonist, propranolol or prior treatment of the animal with reserpine. NPY did not alter the basal release of NA measured in the perfusate of a push-pull cannula, while the potassium evoked release of NA was attenuated. The depressor effect of Int NPY was significantly reduced when examined in a 10 week old SHR, in contrast to Wistar-Kyoto, Sprague-Dawley or DOCA-salt hypertensive rats.

Microinjection of NPY into the posterior hypothalamic nucleus In contrast to the effect of NPY in the spinal cord, the microinjection of NPY into the posterior hypothalamic nucleus (PHN) produces an increase in blood pressure in the anaesthetized or conscious rat (Martin *et al.*, 1988, 1989). NPY also elicited an increase in mesenteric vascular resistance and total peripheral vascular resistance while having a minimal effect on hindquarter resistance, and a decrease in stroke volume. The pressor effect was due to an increase in centrally mediated outflow since the effect was antagonized by the ganglion blocker, pentolinium and α -adrenoceptor an-

Figure 1 The effect of NPY on the periaxillary nerve stimulation (8 Hz) induced release of endogenous NA from the perfused mesenteric arterial bed of 8–10 week old WKY (▨) or SHR (■). Data are plotted as percent fractional release vs NPY concentration. Each bar is the mean $\pm$ s.e. mean of 5–7 preparations.

tagonist, phenoxybenzamine but not the vasopressin antagonist, $[d(CH_2)_5(Me)]AVP$. Moreover, we have observed a direct increase in renal sympathetic nerve activity. Microinjection of NPY into the PHN of the SHR resulted in a significantly greater increase in blood pressure

than corresponding normotensive control animals (Figure 3).

Effects of NPY on NA release from brain slices of hypertensive and normotensive rats NPY was shown to decrease the K^+ -evoked release of NA from slices of PH obtained from SD rats. There was a similar inhibition observed in slices obtained from WKY or SHR. Potassium depolarization resulted in a calcium dependent parallel release of NA and NPY-LI from PH. The evoked release of NPY-LI was decreased by clonidine and propranolol and enhanced by yohimbine.

Effects of CGRP at the vascular neuroeffector junction of normotensive and SHR Electrical stimulation of the periaxillary nerves of the per-

Figure 2 The effect of NPY (10^{-7} M) on the periaxillary nerve stimulation (8 Hz) induced increase in perfusion pressure of the perfused mesenteric arterial bed obtained from 8–10 week old WKY or SHR. Data are plotted as the increase in perfusion pressure in WKY or SHR. Each bar is the mean $\pm$ s.e. mean of 5–7 preparations.

Figure 3 The effect of the unilateral microinjection of NPY (2.35 nmol) into the posterior hypothalamic nucleus on mean arterial blood pressure of anesthetized SD (■, $n = 16$) or SHR (□, $n = 8$). Data are plotted as change in mean arterial pressure in mm Hg vs time. Each point is the mean $\pm$ s.e. mean.

Figure 4 The effect of CGRP on the periarterial nerve stimulation induced change in perfusion pressure of the perfused mesenteric arterial bed obtained from 8–10 week old SD (□) or SHR (■). Each preparation was stimulated (16 Hz) twice (S₁ or S₂) at 30 min intervals. CGRP was added during the second stimulation (S₂). Data are plotted as the S₂/S₁ ratio of the change in perfusion pressure vs CGRP concentration. Each point is the mean $\pm$ s.e. mean of at least 5 observations.

fused mesenteric artery produced a frequency dependent vasodilation in beds pretreated with guanethidine to block the release of NA and precontracted with methoxamine. The electrically induced vasodilation was unaffected by pretreatment with propranolol or atropine but was abolished by tetrodotoxin. The nerve stimulation induced vasodilation could be attenuated by prior treatment with capsaicin or by antisera against CGRP. Removal of the endothelial cells did not alter the nerve stimulation induced vasodilation while abolishing acetylcholine induced vasodilation. CGRP had no effect on basal perfusion pressure but reduced the increase in perfusion pressure produced by electrical stimulation when carried out in the absence of guanethidine and methoxamine or following the administration of exogenous NA. In contrast, CGRP had no effect on the nerve stimulation induced release of NA.

In the SHR, the inhibitory effect of exogenous CGRP on the nerve stimulation induced increase in perfusion pressure was reduced (Figure 4). Although the amplitude of the stimulation induced vasodilation (in guanethidine plus methoxamine treated beds) was only slightly decreased in SHR the duration of the vasodilation was significantly shortened compared with SD rats (Figure 5).

Discussion

These results show that NPY produces excitatory or inhibitory effects on the cardiovascular system

Figure 5 The duration of the periarterial nerve stimulation induced vasodilation of the perfused mesenteric artery obtained from 8–10 week old SD (□) or SHR (■). The preparation was treated with guanethidine (5×10^{-6} M) and precontracted with methoxamine (5×10^{-6} M). Data are plotted as time in min vs frequency in Hz. Each point is the mean $\pm$ s.e. mean of at least 5 observations.

depending upon the site of administration. In the perfused mesenteric artery, where NPY is known to be co-localized with NA, NPY exerted both prejunctional and postjunctional effects. Prejunctionally, NPY decreased the evoked release of NA while postjunctionally it potentiated the nerve stimulation induced increase in perfusion pressure. The postjunctional response was independent of endothelial cells and did not appear to be mediated via the release of endothelin. Studies with NPY fragments are consistent with the concept of Y₁ and Y₂ receptors originally proposed by Wahlestedt *et al.* (1986). Since NPY has been shown to be co-localized and co-released with NA in numerous blood vessels, NPY appears to be an important co-transmitter at the vascular neuroeffector junction.

In the spinal cord (at a level of T₁₀), NPY elicited a depressor effect as well as haemodynamic effects and a decrease in renal sympathetic nerve activity. The mammalian spinal cord contains extensive NPY cells and fibre networks. NPY immunoreactivity has been observed in the dorsal horn (Gibson *et al.*, 1984), in cranial parasympathetic ganglia (Chiba & Murata, 1981; LeBlanc *et al.*, 1987), in the sixth lumbar and first sacral segments (Sasek & Elde, 1985) and the intermediolateral cell columns (IML) in thoracic segments (Blessing *et al.*, 1987; Holets *et al.*, 1988). The distribution of these latter fibres of the thoracic and sacral segments closely parallels that of catecholamine fibres and they disappear after spinal cord transection (Holets *et al.*, 1988) in contrast to NPY fibres in the dorsal horn (Gibson *et al.*, 1984). The origin of NPY fibres terminating on pre-ganglionic

sympathetic neurons in the IML include C₁ neurons in the rostral ventrolateral medulla (VLM) (Blessing *et al.*, 1987; LeBlanc & Landis, 1988) as well as neurons in the locus coeruleus (Holets *et al.*, 1988). It has been demonstrated that stimulation of neurons in the rostral VLM of the rabbit result in a release of NPY in the perfused spinal cord (Blessing *et al.*, 1987). Our results suggest that the depressor effect we observed with ITC NYP was primarily due to a decrease in total peripheral resistance predominantly by a decrease in mesenteric vascular resistance. Direct evidence for a decrease in sympathetic nerve activity was obtained by the demonstration of a decrease in renal sympathetic nerve activity.

There is extensive NPY innervation of the hypothalamus (Everitt *et al.*, 1984; Sawchenko *et al.*, 1985). In contrast to the effect in the spinal cord, microinjections of NPY in the PHN produced a pressor response. Our results suggest that NPY elicits an increase in mean arterial blood pressure via centrally mediated sympathetic excitation and not by altering baroreflex activity or via the release of vasopressin. NPY can decrease the release of NA in the hypothalamus presumably by activating Y₂ receptors in a manner similar to that observed in the spinal cord and vascular neuroeffector junction. No differences in the inhibitory effect of NPY on NA release were observed in slices obtained from SHR. The release of NPY-LI from PH slices was also demonstrated. The evoked release of NPY-LI appears to be under inhibitory modulation via α_2 -adrenoceptors and excitatory modulation via β -adrenoceptors.

In addition to NPY exerting important cardiovascular effects at various levels of the neuropeptide-catecholamine axis, our results have demonstrated marked differences in response in the SHR. The effect of NPY on vascular smooth muscle and the posterior hypothalamic nucleus both of which lead to an increase in blood pressure were enhanced. In contrast, the inhibitory effect

on noradrenergic transmission at the vascular neuroeffector junction and the depressor effect in the spinal cord were attenuated. The net effect of these alterations is a greater increase in blood pressure. The mechanism of these changes is not understood. However, since there is evidence for subtypes of NPY receptors (Schwartz *et al.*, 1989; Sheikh *et al.*, 1989) it is possible that there might be differential up and down regulation of Y₁ and Y₂ receptors. It is also possible that there may be differential effects on the intracellular transduction mechanisms. To date NPY has been shown to inhibit the stimulation of cAMP induced by various agonists (Harfstrand *et al.*, 1987; Lundberg *et al.*, 1988), to increase phosphoinositide (Hinson *et al.*, 1988; Perney & Miller, 1989), to produce an increase in intracellular calcium (Motulsky & Michel, 1988; Perney & Miller, 1989) or to inhibit intracellular Ca²⁺ transient and depolarization induced transmembrane Ca²⁺ currents (Colmers *et al.*, 1988; Ewald *et al.*, 1988; Schafeld & Ikeda, 1988; Walker *et al.*, 1988). Alterations in one or more of these transduction mechanisms may occur in blood vessels or neuronal tissue of the SHR.

Our studies are consistent with CGRP serving as a noradrenergic-noncholinergic vasodilator neurotransmitter in the mesenteric arterial bed. CGRP is known to be widely distributed in both the nervous and cardiovascular system (Owman, 1988). The vasodilator effects of CGRP are consistent with the results reported by others (Owman, 1988). The attenuation of the vasodilator effects of CGRP observed in the SHR suggest that dysfunction of the CGRP system may also contribute to the pathogenesis of hypertension in this model.

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