Effect of Neuropeptide Y on Vasodilation Mediated by Calcitonin Gene-Related Peptide (CGRP)-Containing Nerves in the Mesenteric Resistance Vessel of the Rat

Chikako NUKI, Hiromu KAWASAKI* and Koichiro TAKASAKI
Department of Pharmacology, Miyazaki Medical College, 5200 Kiyotake, Miyazaki 889-16, Japan
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Abstract—The role of neuropeptide Y (NPY) in the neurotransmission of calcitonin gene-related peptide (CGRP)-containing vasodilator nerves was investigated in perfused mesenteric vascular beds of the rat with active tone. NPY (5 and 10 nM) significantly inhibited the neurogenic vasodilation induced by perivascular nerve stimulation (1–8 Hz). However, NPY had no effect on vasodilation in response to bolus infusion of rat CGRP (10 and 100 pmol). These results suggest that NPY modulates the release of CGRP from CGRP-containing nerves in mesenteric resistance vessels.

Stimulation of the perivascular nerves (PNS) of rat mesenteric resistance blood vessels produces neurogenic vasodilation (1). Calcitonin gene-related peptide (CGRP), a novel 37-amino-acid peptide translated from the calcitonin gene (2) and a potent vasodilator (3), has been proposed as the potential transmitter for the neurogenic vasodilation of the mesenteric resistance blood vessels (1).

Neuropeptide Y (NPY), a vasoconstrictor peptide with 36 amino acid residues, is abundantly present in sympathetic perivascular nerves and is considered to be co-stored with noradrenaline (NA) in sympathetic nerve terminals (4). NPY is released along with NA on stimulation of sympathetic nerves. Recently, we have demonstrated that exogenous or neuronally released NA inhibits neurogenic vasodilation mediated by CGRP-containing nerves (1, 5). Therefore, the present study was designed to investigate the role of NPY in the neurotransmission of CGRP-containing nerves in the mesenteric resistance blood vessels of the rat.

Male Wistar rats weighing 300 and 350 g were used in this study. Under sodium pentobarbital anesthesia (50 mg/kg, i.p.) the mesenteric vascular beds were isolated from rats and prepared for perfusion as described previously (1). The isolated mesenteric vascular bed was placed on a water-jacketed organ bath maintained at 37°C and was both perfused (5 ml/min) and superfused (0.5 ml/min) with a modified Krebs bicarbonate solution (Krebs solution) using a peristaltic pump (SJ-1215, ATTO). Krebs solution of the following composition was used: 120.0 mM NaCl, 5.0 mM KCl, 2.4 mM CaCl₂, 1.2 mM MgSO₄, 25.0 mM NaHCO₃, 0.027 mM EDTA 2 Na and 11.0 mM dextrose (pH=7.4). Changes in the perfusion pressure were measured with a pressure transducer (MPU-0.5 A, Nihon Kohden) and recorded on a polygraph (RM-25, Nihon Kohden).

To maintain the active tone of the mesenteric artery, the preparation was contracted with continuous perfusion of methoxamine at a submaximal concentration of 7 μM in the presence of 5 μM guanethidine, which was added to block adrenergic neurotransmission. After the elevated perfusion pressure was allowed to stabilize, PNS was carried out for 30 sec through bipolar platinum ring electrodes placed around the superior mesenteric artery. Rectangular pulses of 1 msec in duration and
supramaximal voltage (60 V) were applied at 1, 2, 4 and 8 Hz using an electronic stimulator (SEN 3301, Nihon Kohden). In the same preparation, 30–40 min after the experiment of PNS, bolus infusion of rat CGRP was carried out directly into the perfusate proximal to the arterial cannula using an infusion pump (Model 975, Harvard). The volumes of infusion were 100 μl for 10 sec.

The final concentration (5 and 10 nM) of NPY, which was achieved by dilution with Krebs solution containing methoxamine and guanethidine, was perfused throughout the experiment. At the end of each experiment, the preparation was perfused with 100 μM papaverine to relax it completely.

Results, expressed as the mean±S.E., were statistically analyzed using one way ANOVA followed by Dunnett’s test. A P value of less than 0.05 was considered statistically significant.

The following drugs were used: capsaicin (Sigma), rat CGRP (Peptide Institute), guanethidine sulfate (Tokyo Kasei), neuropeptide Y (Peptide Institute), methoxamine HCl (Nihon Shinyaku) and tetrodotoxin (Sigma). Capsaicin was dissolved in 50% ethanol and diluted with Krebs solution. CGRP was dissolved in distilled water and diluted with Krebs solution containing 7 μM methoxamine and 5 μM guanethidine, when injected as a bolus.

In the mesenteric vascular bed contracted by continuous perfusion of 7 μM methoxamine in the presence of 5 μM guanethidine, PNS (1 to 8 Hz) produced a frequency-dependent decrease in perfusion pressure, or vasodilation (Fig.1). The vasodilator response to PNS was slow in both onset (appearing 20–30 sec after the start of stimulation) and decay (returning to the control level within 20–30 min at high frequencies). This vasodilation was abolished in the presence of 300 nM tetrodotoxin (TTX) and by pretreatment with capsaicin for 20 min (data not shown), but it was not affected by 100 nM propranolol and 100 nM atropine (1).

In the perfused mesenteric vascular bed without active tone, perfusion of NPY by itself, at concentrations of 5 and 10 nM, did not alter the resting mean perfusion pressure. There was no significant difference in methoxamine-induced elevated perfusion pressure before PNS between the absence (control: 57.7±11.7 mmHg) and the presence of NPY (5 nM, 59.8±2.5 mmHg; 10 nM, 80.4±16.5 mmHg). As shown in Fig. 1, NPY (5 and 10 nM) caused a significant decrease in perfusion pressure, which was abolished by 300 nM tetrodotoxin (TTX) and by pretreatment with capsaicin for 20 min (data not shown), but it was not affected by 100 nM propranolol and 100 nM atropine (1).
inhibition of the vasodilator response to PNS in a concentration-dependent manner.

In the perfused mesenteric vascular bed with active tone, bolus infusion of 10 and 100 pmol CGRP produced a marked and prolonged vasodilation, which mimicked the vasodilator response induced by PNS. However, NPY at concentrations of 5 and 10 nM did not cause a significant change in vasodilation induced by bolus infusion of CGRP, as shown in Fig. 2.

The present and previous studies (1) demonstrated that in the mesenteric vascular bed with active tone, PNS produced a frequency-dependent vasodilator response. This vasodilation was abolished by TTX, but not by an anticholinergic drug and a beta-adrenoceptor antagonist (1). Therefore, it appears that this vasodilation is mediated by nonadrenergic, noncholinergic (NANC) vasodilator nerves. Capsaicin, which depletes certain neuropeptides including tachykinins (substance P, neurokinin A and neurokinin B) and CGRP from NANC nerves (1, 6), abolished the neurogenic vasodilation of mesenteric vascular beds (1). In the perfused mesenteric vascular bed, bolus infusion of CGRP induced marked vasodilation, which mimicked the PNS-induced vasodilator response. However, no vasorelaxation was produced by tachykinins in the same preparation (1). A preliminary study showed that PNS (2, 4 and 8 Hz) of the perfused rat mesenteric vascular bed evoked a frequency-dependent release of a CGRP-like immunoreactive substance in the perfusate, which was blocked by TTX or calcium-free Krebs solution (7). Inasmuch as CGRP-containing nerves are sensitive to capsaicin, a sensory neurotoxin, CGRP is probably contained in the sensory nerves of the mesenteric artery. Certain sensory nerves in peripheral blood vessels cause not only afferent function but also efferent motor function such as antidromic vasodilation through local axon reflex (8). Therefore, taken together, the neurogenic vasodilation induced by PNS is mediated by CGRP released from NANC vasodilator nerves in the mesenteric resistance vessel.

The present experiment showed that NPY markedly inhibited the neurogenic vasodilation in a concentration-dependent manner. NPY has been shown to amplify vasoconstrictor responses to alpha adrenoceptor agonists such as NA and phenylephrine (9). It is unlikely that decreased neurogenic vasodilation in the presence of NPY results from an enhanced vasoconstrictor response to methoxamine which was used to produce active tone of the mesenteric artery, because there was no significant difference in the methoxamine-induced vasoconstriction between the control (absence) and NPY (presence). Furthermore, the vasodilator response to bolus infusion of CGRP was not altered in the presence of NPY at all doses, suggesting that NPY has little effect on CGRP-induced vasodilation. Thus, these results suggest that decreased neurogenic vasodilation by NPY results from reduced release of CGRP from NANC vasodilator nerves. This notion is supported by recent reports that NPY presynaptically inhibits neurotransmitter release from peripheral nerves including sensory nerves, cholinergic nerves and adrenergic nerves (10–12).
In conclusion, the present results suggest that circulating or neuronally released NPY modulates the release of CGRP from CGRP-containing vasodilator nerves.

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References