Pharmacology and Physiology of Perivascular Nerves Regulating Vascular Function

Regulation of Vascular Function by Perivascular Calcitonin Gene-Related Peptide-Containing Nerves

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Received October 2, 2001 Accepted November 12, 2001

ABSTRACT—The rat mesenteric artery is innervated by nonadrenergic noncholinergic (NANC) vasodilator nerves in which calcitonin gene-related peptide (CGRP), a potent vasodilator peptide, acts as a vasodilator transmitter. The inhibition of CGRPergic nerve function potentiates a vasoconstrictor response mediated by the sympathetic adrenergic nerve, suggesting that CGRPergic nerves inhibit adrenergic function and play a role in the regulation of mesenteric vascular tone. In contrast, norepinephrine released from adrenergic nerves presynaptically inhibits neurotransmission of CGRPergic nerves. Thus, both nerves reciprocally control the vascular tone. Pathophysiological studies have shown that an age-related decrease in CGRPergic nerve-mediated vasodilation, neurogenic CGRP release and CGRP mRNA levels in the dorsal root ganglia are found in spontaneously hypertensive rats (SHR), indicating a reduced function of CGRPergic nerves. Long-term treatment with angiotensin converting enzyme inhibitor and angiotensin II-receptor antagonist restores the reduced function of CGRPergic nerves, suggesting the involvement of angiotensin II in the malfunction of CGRPergic nerves in SHR.

Keywords: Calcitonin gene-related peptide-containing nerve, Perivascular vasodilator nerve, Perivascular nerve remodeling, Hypertension

It is widely accepted that resistance blood vessels play an important role in the maintenance of blood pressure and regulation of tissue blood flow (1). The resistance vascular tone is mainly controlled by sympathetic adrenergic nerves through the release of neurotransmitter norepinephrine (NE) (1). Indeed, perivascular nerve stimulation (PNS) of perivascular nerves in blood vessels isolated from various species evokes vasoconstriction, which is blocked by an α-adrenoceptor antagonist (prazosin), adrenergic neuron blocker (guanethidine) and neurotoxin tetrodotoxin. However, PNS of precontracted blood vessels isolated from various species causes vasodilation (2), which can be abolished by tetrodotoxin but not by a β-adrenoceptor antagonist or muscarinic cholinceptor antagonist (atropine). These findings suggest nonadrenergic noncholinergic (NANC) vasodilator innervation in blood vessels.

Neuropeptides and nitric oxide (NO) have been implicated as possible vasodilator transmitters for NANC nerves in peripheral and cerebral arteries isolated from several species (3 – 5). However, the present author has shown that the rat mesenteric resistance artery is innervated by NANC nerves in which calcitonin gene-related peptide (CGRP), a potent vasodilator neuropeptide, acts as a vasodilator transmitter (5).

The present article focused on the innervation of CGRPergic nerves and its physiological role in neural control of peripheral resistance arteries. The pathophysiological role of CGRPergic nerves in hypertension and the effects of the antihypertensive drugs on malfunction of CGRPergic nerves are also presented.

CGRP-containing nerve (CGRPeric nerve) innervation in mesenteric resistance arteries

The present study has used the rat isolated perfused mesenteric vascular bed that includes small resistance
arteries, and its active tone was produced by continuous perfusion of methoxamine (α₂-adrenoceptor agonist) in the presence of guanethidine, which was added to block adrenergic neurotransmission. A previous report showed that PNS of the preparation caused a NANC vasodilation, which was sensitive to tetrodotoxin and insensitive to cholinergic and β-adrenoceptor antagonists (5). Furthermore, this NANC response was abolished by CGRP(8–37) (CGRP receptor antagonist), CGRP receptor desensitization and pretreatment with capsaicin (deleter for CGRP from primary afferent nerves) (5, 6). In addition, PNS in the rat mesenteric vascular bed evokes an increase in CGRP release, which is sensitive to tetrodotoxin and capsaicin and is abolished by calcium removal from the medium (7). Moreover, there are dense innervations of CGRP-like immunoreactive-containing fibers, and exposure of the mesenteric artery to capsaicin abolishes the intensity of these fibers (5). These findings strongly suggest that rat mesenteric resistance arteries have major NANC innervation with CGRPergic nerves.

In studies using the mesenteric arteries of rats and rabbits (5, 8–10), PNS induced tetrodotoxin-sensitive NANC vasorelaxation, which was not inhibited by NO synthase inhibitors and was sensitive to capsaicin and CGRP(8–37). These findings provide evidence that the mesenteric artery is mostly innervated with capsaicin-sensitive sensory nerves in which CGRP acts as a neurotransmitter. Gyoda et al. (11) found that PNS of the guinea pig mesenteric artery induced tetrodotoxin-sensitive NANC relaxation, which was more sensitive to NO synthase inhibitors and partially sensitive to capsaicin or CGRP(8–37). Zheng et al. (12) reported that NO synthase inhibitors partially inhibited capsaicin-sensitive neurogenic NANC relaxation of the guinea pig mesenteric artery, suggesting that NO was released from primary sensory nerves. Therefore, there might be regional and segmental differences in the innervation of mesenteric arteries.

In the rat perfused mesenteric vascular bed precontracted with methoxamine in the presence of guanethidine, removal of endothelial cells by perfusion of sodium deoxycholate abolished acetylcholine-induced vasodilation but did not inhibit the PNS-induced neurogenic vasodilation mediated by CGRPergic nerves and exogenous applied CGRP-induced vasodilation (13). In contrast, vasodilations induced by PNS and exogenous CGRP injection in the endothelium-removed preparation were significantly potentiated (13). These findings suggest that both the CGRPergic nerve-mediated and exogenous CGRP-induced vasodilation are independent of the endothelium and that the endothelium may play an inhibitory role in the CGRPergic nerve function. Furthermore, in preparations without endothelium, perfusion of NO synthase inhibitor had no effect on the neurogenic vasodilation. These findings suggest that nitricergic nerves are probably not present in rat mesenteric resistance arteries.

Physiological role of CGRPergic nerves in the regulation of resistance vessel tone

In rat mesenteric resistance arteries, the adrenergic neurotransmitter, NE, has been reported to exert an inhibitory influence upon CGRP release from CGRPergic nerves by activating presynaptic α₂-adrenoceptors (14). In addition, neuropeptide-Y, a vasoconstrictor neuropeptide colocalized with NE in peripheral adrenergic nerves, inhibited the neurogenic vasodilation and CGRP release in these vessels (15). On the other hand, inhibition of CGRPergic nerve function by capsaicin and CGRP(8–37) potentiated adrenergic nerve-mediated vasoconstriction without changing neuronal release of NE, suggesting that CGRPergic nerves, by releasing CGRP from the nerve, postsynaptically inhibited sympathetic nerve-mediated vasoconstriction in these vessels (16, 17). Therefore, it is proposed that adrenergic and CGRPergic nerves regulate the tone of mesenteric resistance artery by reciprocal interaction (16). Additional evidence was provided by Nuki et al. (18), who showed that exogenously applied CGRP inhibits CGRPergic nerve-mediated vasodilation. This inhibition is antagonized by CGRP(8–37), and low doses of CGRP(8–37) enhance CGRPergic nerve-mediated vasorelaxation (18). This report suggested that CGRPergic nerves innervating the rat mesenteric resistance vessel are endowed with presynaptic CGRP receptors that regulate the release of CGRP from the nerve via a negative feedback mechanism. The findings by Nuki et al. support a physiological role for CGRP as a neurotransmitter in the regulation of peripheral resistance blood vessel tone.

The CGRPergic nerves have been shown to be sensitive to capsaicin and to be the primary sensory afferent nerve. It, therefore, raises the question how the sensory nerve is able to participate in the regulation of resistance vessel tone similar to the adrenergic efferent nerve. This question was resolved by the in vivo study of Taguchi et al. (19) with the pithed rat in which blood pressure was artificially elevated by methoxamine in the presence of hexamethonium, an autonomic ganglion blocker. The findings of that study showed that electrical stimulation of lower thoracic spinal cord (Th9–12) but not upper thoracic cord (Th1–4) caused a frequency-dependent fall in blood pressure without changing heart rate. This depressor response was sensitive to tetrodotoxin and capsaicin, and it is abolished by CGRP(8–37) but not by the muscarinic cholinoreceptor antagonist, β-adrenoceptor antagonist and histamine H₁ and H₂ receptor antagonists. These findings suggest that the depressor response to the spinal cord stimulation is NANC in nature and is mediated by endogenous CGRP, which is released by CGRPergic nerves. It is likely that there may
be an outflow of CGRPeric nerves from the spinal cord to blood vessels and CGRPeric nerves participate in the neuronal control of the tone of resistance blood vessels.

**Pathophysiological role of the CGRPeric nerve in hypertension**

Increased total peripheral vascular resistance maintains the elevated blood pressure in chronic hypertension (20). Therefore, impairment of the control systems regulating peripheral resistance might be a cause of chronic hypertension (21). In studies with spontaneously hypertensive rats (SHR), the enhanced activity of sympathetic vasoconstrictor nerves has been shown to be an important factor in the increased tone of peripheral resistance vessels (22). Both CGRP nerve-mediated vasodilation and PNS-induced CGRP-like immunoreactivities release in SHR have been shown to be smaller than in WKY and both phenomena decreased with age (23, 24). Moreover, the mesenteric arteries of 15- and 30-week-old SHR have been shown to have a lower density of CGRP-like immunoreactive-containing fibers compared with age-matched WKY and 8-week-old SHR (23). Recent studies showed that the depressor response to spinal cord stimulation was significantly smaller in SHR than in WKY (24). The level of CGRP mRNA in dorsal root ganglia, which is a prominent site of CGRP synthesis, was significantly lower in SHR than that in WKY and this phenomenon was also age-related (25). In contrast, the contents of CGRP-like immunoreactivities in the atrium and mesenteric artery of 15 week-old SHR were greater than those in age-matched WKY (26). The latter finding suggests that the increased amount

![Fig. 1. Changes in CGRP-like immunoreactive-containing fibers innervating mesenteric arteries of SHR following 7-week treatment with antihypertensive drugs. C-SHR and C-WKY indicate non-treated 15-week-old SHR and WKY, respectively. Hyd-SHR, Cap-SHR and Tem-SHR show SHR treated with 0.005% hydralazine, 0.05% captopril and 0.005% temocapril for 7 weeks in drinking water, respectively. A white bar at the upper right corner is 50 μm.](image)

![Fig. 2. Changes in expression of CGRP mRNA in dorsal root ganglia following 7-week treatment with antihypertensive drugs in SHR. Each bar indicates the ratio of CGRP mRNA / GAPDH mRNA determined by densitometric assay of Northern hybridization analysis. Values represent means ± S.E.M. of 6 experiments and the fold increase over control SHR. C-SHR and C-WKY indicate non-treated SHR (15 week-old) and non-treated WKY (15 week-old), respectively. Tem and Hyd indicate SHR treated with 0.005% temocapril and 0.005% hydralazine for 7 weeks in drinking water, respectively. **P<0.01, compared with the SHR control.](image)
of CGRP-like immunoreactivities in SHR is due to the reduced release of CGRP from CGRPergic nerve terminals, even though the levels of CGRP mRNA in SHR are decreased. Taken together, these studies strongly suggest that the function of CGRP vasodilator nerves in the mesenteric resistance blood vessel of SHR decreases with age. It is very likely that the outflow of CGRPergic vasodilator nerves from the spinal cord to peripheral blood vessels via the dorsal roots decreases in SHR. Since CGRPergic nerves have been shown to inhibit the function of adrenergic nerves (14), the decreased function of CGRPergic nerves in SHR enhances the sympathetic adrenergic vasoconstriction. A recent study demonstrated that α-CGRP/calcitonin gene knockout mice displayed elevated mean blood pressure compared with wild-type mice, indicating that α-CGRP is involved in the long-term regulation of resting blood pressure (27). Therefore, the reduced CGRPergic nerve function may have caused the increased vascular resistance and high blood pressure in SHR.

Although the malfunction of CGRP nerves involved in peripheral vascular resistance control plays an important role in the development and maintenance of hypertension in SHR, the mechanisms underlying the reduced function of CGRP nerves in SHR remain unresolved. A recent study provided evidence that angiotensin II in the mesenteric artery of SHR prejunctionally inhibits neurotransmission of CGRP nerves through angiotensin II receptors (28), suggesting that the renin-angiotensin system might be involved in the reduced function of CGRP nerves in SHR. Long-term treatment (7 weeks) with captopril and temocapril, angiotensin converting enzyme inhibitors, restored the reduced vasodilator response mediated by CGRP nerves in the mesenteric artery of SHR, whereas calcium antagonists (nicardipine, pranidipine, amlodipine), β-adrenoceptor antagonists (propranolol, pindolol) and a vasodilator drug (hydralazine) had no such effect (29). In non-treated 15-week-old SHR mesenteric artery, directly applied captopril or temocapril did not modify CGRPergic nerve-mediated vasodilation (28). In addition, in the mesenteric artery of SHR treated with captopril, PNS evoked significantly larger release of CGRP than in non-treated SHR (28). Furthermore, long-term treatment of 8 week-old SHR with captopril for 7 weeks restored the reduced depressor response to spinal cord stimulation (25). As shown in Figs. 1 and 2, long-term treatment (7 weeks) with captopril and temocapril but not hydralazine in SHR caused increases in the density of CGRP-like immunoreactive-containing nerves in mesenteric arteries and the level of CGRP mRNA in dorsal root ganglia. These findings strongly suggest that long-term inhibition of the renin-angiotensin system prevents or restores function reduction and remodeling of CGRPergic nerves in SHR.

Conclusions

The available evidence shows that the CGRPergic nerve is the main NANC vasodilator nerve in the rat mesenteric resistance artery and plays a role in the regulation of mesenteric resistance blood vessel tone. Both CGRPergic nerves and sympathetic adrenergic nerves reciprocally control the rat mesenteric vascular tone. It appears that the increased activity of the local renin-angiotensin system induces malfunction and remodeling of CGRPergic nerves. It is hypothesized that remodeling of CGRPergic nerves may increase vascular sympathetic nerve function to elevate vascular tone.

Acknowledgments

This study was supported by Grants-in-Aid (03671100, 08672611, 09672326, 10557244, 13672389) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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