Jpn. J. Pharmacol. **88**, 32 – 38 (2002)

FORUM MINIREVIEW

Pharmacology and Physiology of Perivascular Nerves Regulating Vascular Function

Neurogenic Cerebral Vasodilation Mediated by Nitric Oxide

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Received October 2, 2001 Accepted November 12, 2001

ABSTRACT—In cerebral arteries isolated from most of mammals, nerve stimulation produces relaxations in contrast to contractions in peripheral arteries. The relaxant mechanism is found to be non-adrenergic and non-cholinergic, but the neurotransmitter is not clarified until recently. Based on several functional and histological studies with isolated cerebral arteries, nitric oxide (NO) is now considered to be a neurotransmitter of the vasodilator nerve and the nerve has been called a nitroxidergic (nitrergic) nerve. Upon neural excitation, calcium influxed through N-type Ca^{2+} channels activates neuronal NO synthase, and then NO is produced by the enzyme from L-arginine. The released NO activates soluble guanylate cyclase in smooth muscle cells, resulting in relaxation with a cyclic GMP-dependent mechanism. The functional role and neuronal pathway have also been investigated in anesthetized dogs and Japanese monkeys. The nitroxidergic (nitrergic) nerves innervating the circulus arteriosus, including the anterior and middle cerebral and posterior communicating arteries, are found to be postganglionic nerves originated from the ipsilateral pterygopalatine ganglion and tonically dilate cerebral arteries in the resting condition. Our findings suggest that the nitroxidergic (nitrergic) nerve plays a physiologically important role to maintain a steady blood supply to the brain.

Keywords: Nitric oxide, Vasodilator nerve, Cerebral artery, Pterygopalatine ganglion, Neuronal pathway

Neurogenic control of muscle tone plays important roles to maintain the homeostasis of cardiovascular, digestive, respiratory and urinary systems. Most of the organs in these systems are innervated by two or more different types of nerves, and their functions are reciprocally regulated. It has generally known that noradrenergic and cholinergic nerves are responsible for the reciprocal regulation, but this may not be the case in the vascular system.

Histological findings demonstrate the innervation of cholinergic nerve in the vasculatures (1). However, functional evidence supporting the idea that acetylcholine directly controls the vascular tone as a neurotransmitter is only limited to certain regions including canine portal and mesenteric veins (2), rabbit portal vein (3) and monkey ciliary artery (4), and stimulation of the cholinergic nerves elicits vasoconstriction, but not vasodilation, in these blood vessels. Therefore, reciprocal regulation of vascular tone by noradrenergic and cholinergic nerves may not exist.

On the other hand, the presence of vasodilator nerves has been reported in various regions of arteries since 1975 when neurogenic, non-adrenergic, non-cholinergic vasodilation was found in canine cerebral arteries (5). The mechanism of neurogenic vasodilation and the functional role of the vasodilator nerve have only recently been determined.

Mechanism of neurogenic relaxation in cerebral arteries

Since neurons innervating cerebral vascular walls were histochemically found to contain several peptides such as substance P, vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) (6 – 9), which were determined to be potent cerebralvasodilators when applied exogenously (8, 10, 11), these peptides were regarded as candidates for the vasodilator neurotransmitter of the non-adrenergic, non-cholinergic nerve. However, whether or not the peptides in concentrations sufficient to

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produce relaxation were released from the nerve upon stimulation has not been evidenced. Furthermore, functional data against this hypothesis were obtained: a) relaxant responses to nerve stimulation are not influenced in the arterial strips desensitized to VIP and CGRP (10, 12), b) the neurogenic response is not reduced by treatment with capsaicin (13) that depletes these peptides from nerves, and c) substance P-induced relaxation is endothelium-dependent (14), whereas neurogenic relaxation is not dependent on the endothelium (15). As far as canine, bovine and monkey cerebral arteries are concerned (10, 16), these peptides, together with atrial natriuretic peptide, an endogenous cerebral vasodilator (17), were therefore excluded from candidates for the neurotransmitter. However, this conclusion may not be extended to all kinds of mammals because evidence for the involvement of VIP and CGRP has been reported in sheep and cat cerebral artery, respectively (18, 19).

During the analysis of mechanisms underlying the cerebral vasospasm after subarachnoid hemorrhage, oxyhemoglobin or hemolysate was found to abolish the response to nerve stimulation in cerebral arteries (20, 21). This was also true with methylene blue (20), an inhibitor of soluble guanylate cyclase. These findings suggest that cyclic GMP mediates the neurogenic relaxation, and a substance that increases the production of cyclic GMP in smooth muscle is a neurotransmitter (20). Involvement of atrial natriuretic polypeptide that promotes synthesis of cyclic GMP via activation of particulate guanylate cyclase (22) is ruled out (17), as described above.

Endothelium-derived relaxing factor was found to be identical to nitric oxide (NO) synthesized from L-arginine via NO synthase (23), and this enzyme activity is inhibited by L-arginine analogs such as N^G-monomethyl-L-arginine, N^G-nitro-L-arginine and N^G-nitro-L-arginine methylester (24 – 26). We found that N^G-monomethyl-L-arginine and N^G-nitro-L-arginine applied to cerebroarterial preparations abolishes the relaxant response to transmural electrical stimulation (27, 28). The response is restored by the addition of high concentrations of L-arginine. D-Arginine analogs are without effect. L-Arginine does not potentiate the response, but completely prevents the inhibition by NO synthase inhibitors. This may be due to the presence of sufficient amounts of L-arginine in the tissue than the Km values of NO synthase for L-arginine. 7-Nitroindazole, a relatively selective inhibitor of neuronal NO synthase, also inhibits neurogenic relaxation in monkey cerebral arteries (29).

The necessity for extracellular Ca^{2+} in the neurogenic response has been found by using Ca^{2+} depleted media or Ca^{2+} entry blockers. Removal of external Ca^{2+} also abolishes the response to nerve stimulation but not to NO (30). Relaxations and increments in cyclic GMP content induced by nerve stimulation are not influenced by nicardipine, a L-type specific Ca^{2+} channel inhibitor (31), but are dose-dependently inhibited by ω-conotoxin GVIA, a N-type specific Ca^{2+} channel inhibitor (30), suggesting that external Ca^{2+} is introduced into nerve terminals via N-type, but not L-type Ca^{2+} channels upon electrical nerve stimulation. Furthermore, calmodulin inhibitors such as calmidazolium and W-7 depress the neurogenic response (32). These findings suggest the involvement of the constitutive type of NO synthase in the vasodilator nerve function, since Ca^{2+} and calmodulin are required for activation (33).

Electrical nerve stimulation increases the release of nitroxy compounds (NOx) in the superfusate from superfused cerebral arterial strips without the endothelium (15), which is abolished by tetrodotoxin or N^G-nitro-L-arginine. Cyclic GMP content in the endothelium-denuded artery is also increased by nerve stimulation, and an NO synthase inhibitor abolishes the effect (34). Furthermore, in canine cerebral arteries loaded with BNN5M, a caged NO that liberates NO inside muscle cells upon irradiation with ultraviolet light (35), relaxation induced by electrical nerve stimulation is abolished only when the arteries were irradiated. Typical recording is illustrated in Fig. 1.

Moreover, histochemical studies have demonstrated the presence of perivascular nerve fibers and bundles containing NO synthase immunoreactivity or NADPH diaphorase in cerebral arteries. Bredt et al. (36) have clearly demonstrated that perivascular nerves innervating the rat cerebral artery of proximal portion contain NO synthase immunoreactivity. We found networks of the positively stained nerve fibers and bundles in the canine (37) and monkey cerebral arteries and arterioles (38). Neurons are mainly located in the adventitia, and some fine fibers are also seen in the outer layer of media (37). The characteristic localization of the fibers in the media may indicate that NO synthesized and liberated from the nerve is effectively accessible to smooth muscle with a minimal degradation and spacial diffusion. NO synthase-immunoreactive nerve fibers are also observed in human (39) and bovine (40) cerebral arteries.

Taken together, it appears that nerve stimulation elicits Ca^{2+} entry through N-type Ca^{2+} channel, which activates NO synthase located in the nerve terminals; thus NO is synthesized by the enzyme from L-arginine and is released into outside cells, and then the released NO activates soluble guanylate cyclase in smooth muscle and increases the production of cyclic GMP, resulting in vasorelaxation. Since the neurotransmitter is considered to be NO or its stable analog, such as *S*-nitrosocysteine, the nerve has been called ‘nitrergic’ or ‘nitroxidergic’ (41).

Functional role of nitroxidergic (nitrergic) nerve in cerebral artery

Injections of N^G-nitro-L-arginine into the cisterna magna

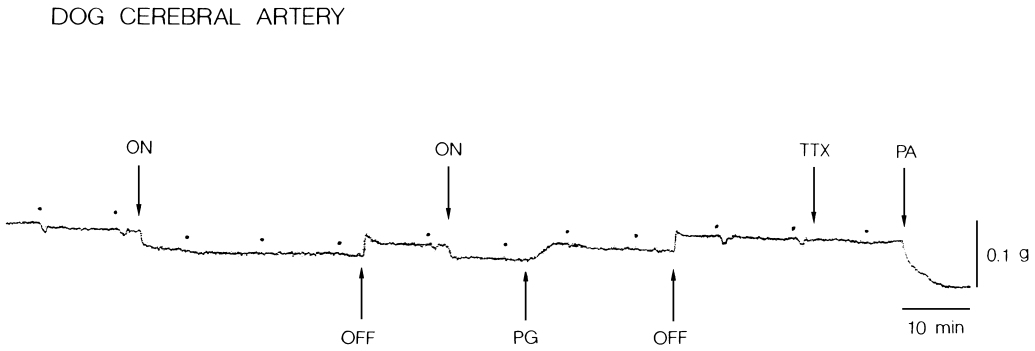

Fig. 1. Typical recording of mechanical responses to transmural electrical stimulation (5 Hz, dots) of a canine middle cerebral artery loaded with bis-*N*-nitroso-caged nitric oxide (BNN5M). The artery was incubated with 1 μM BNN5M for al least 30 min, rinsed with a drug-free Ringer-Locke solution, and then precontracted with prostaglandin $F_{2\alpha}$ under resting tension of 1.5 g. Ultraviolet light at 320 nm was continuously irradiated between ON and OFF. PG indicates the additional application of prostaglandin $F_{2\alpha}$ to raise the arterial tone close to the level before the irradiation. TTX, 3×10^{-7} M tetrodotoxin; PA, 10^{-4} M papaverine.

produce potent basilar arterial constriction that lasted for 4 h or longer in anesthetized dogs (42). The vasoconstrictor effect is reversed by intracisternal injections of L-arginine. The arterial diameter is not affected by N^G-nitro-D-arginine or the vehicle. Treatment with phentolamine does not inhibit the response but rather potentiates the vasoconstriction produced by N^G-nitro-L-arginine. These findings indicate that the magnitude of induced vasoconstriction might reflect vasodilation due to basal release of NO. Treatment with a ganglion blocking agent, hexamethonium, clearly reduces the vasoconstriction induced by N^G-nitro-L-arginine; vasoconstriction relative to the size prior to the injection under phentolamine treatment is 34%, and the value in hexamethonium-treated dogs is 10% (42). These findings suggest that about 2/3 of the vasodilation is associated with NO from the vasodilator nerve that continuously receives efferent impulses from the brain and liberates neurotransmitter, and the remaining 1/3 is due to NO from extraneuronal tissues, possibly the endothelium. Nicotine, a chemical stimulant of nerves, injected into the vertebral artery induces dilatation of the basilar artery that is abolished by treatment with N^G-nitro-L-arginine (42). Therefore, nitrergic (nitroxidergic) nerve is expected to participate in maintaining the dilator tone of large pial arteries in vivo, which is considered to play an important role in the regulation of vascular resistance (43, 44), under resting conditions and when the nerve is stimulated.

Cortical blood flow in anesthetized rats is also increased by electrical microstimulation of the basal forebrain (45) and the nucleus basalis of Meynert (46). Intravenous infusion of N^G-nitro-L-arginine, but not N^G-nitro-D-arginine, results in a significant, dose-dependent attenuation of the stimulation-induced response. Nitroxidergic nerve innervating the cortical vasculature may have some connection with basal forebrain / nucleus basalis Meynert. However, interrelations between the intracerebral neuclei and parasympathetic ganglia outside the skull remains to be determined.

Neuronal pathway of nitroxidergic (nitrergic) nerve in cerebral artery

NO synthase immunoreactivity is observed in nerve cells, bundles and fibers in the pterygopalatine ganglion from Japapnese monkeys (38), dogs (37) and rats (47), and the otic ganglion from dogs and rats. The pterygopalatine ganglion also contains VIP (48) and acetylcholinesterase (49). Unilateral chemical denervation of the pterygopalatine ganglion abolishes the immunoreactivity in the ipsilateral middle cerebral artery of dogs (37, 50), and bilateral denervation is required to abolish the neurons in rat middle cerebral arteries (47). Moreover, the unilateral impairment of the pterygopalatine ganglion also abolishes relaxation in response to nerve stimulation or reverses it to contraction in middle and posterior arteries only from the ipsilateral side (50). The relaxation is sensitive to N^G-nitro-L-arginine, and the induced contraction is suppressed by treatment with phentolamine. Relaxation caused by NO applied exogenously does not differ in the arteries from both sides. Similar histological and functional results are observed in central retinal arteries (50). In contrast, the impairment of the pterygopalatine ganglion does not affect the neurogenic relaxation in temporal arteries, which is also sensitive to the NO synthase inhibitor. Therefore, NO synthase-containing neurons innervating pial cerebral and central retinal arteries in dogs seem to originate mainly from the pterygopalatine ganglion.

Electrical stimulation of the greater petrosal nerve, facial nerve or pterygopalatine ganglion increases cortical or cerebral blood flow in anesthetized rats and dogs; the response is mediated by non-cholinergic (51 – 53) and

cholinergic mechanisms (54). Parasympathetic nuclei in the brain stem send the preganglionic fibers through the geniculate ganglion as the greater petrosal nerve to the pterygopalatine ganglion; the nerve cells of this ganglion send the postganglionic fibers to nasal and lacrimal glands and possibly cerebral vasculatures. When unilateral pterygopalatine ganglion is electrically stimulated, vasodilation of ipsilateral cerebral arteries is observed in anesthetized dogs (55) and Japanese monkeys (56). Typical angiographic recordings are shown in Fig. 2. The response is abolished by intravenous injections of N^G-nitro-L-arginine and the effect is reversed by L-arginine (Fig. 3). Stimulation of the greater petrosal nerve, upstream of the pterygopalatine ganglion, also produces cerebral vasodilation, which is abolished by treatment with the nitric oxide synthase inhibitor and is restored by L-arginine. Treatment with hexamethonium abolishes the response to electrical stimulation of the petrosal nerve, but does not affect the response to pterygopalatine ganglion stimulation. Surgical denervation of the ganglion elicits cerebral vasoconstriction, indicating that vasodilator nerves from the vasomotor center are tonically active in the regulation of vascular tone (56).

Denervation of efferent nerve fibers originated from the pterygopalatine ganglion abolishes the vasodilation, lacrimation and nasal secretion induced on the ipsilateral side by electrical stimulation of the ganglion and petrosal nerve in anesthetized dogs (55). The vasodilator response is suppressed by N^G-nitro-L-arginine but unaffected by atropine, whereas lacrimation and nasal secretion are abolished solely by atropine. These findings indicate that postganglionic nitroxidergic (nitrergic) neurons from the pterygopalatine ganglion innervate the circulus arteriosus, including the middle cerebral and posterior communicating arteries, and the intracranial internal carotid artery in dogs; and preganglionic neurons innervating the pterygopalatine ganglion originate via the greater petrosal nerve, possibly

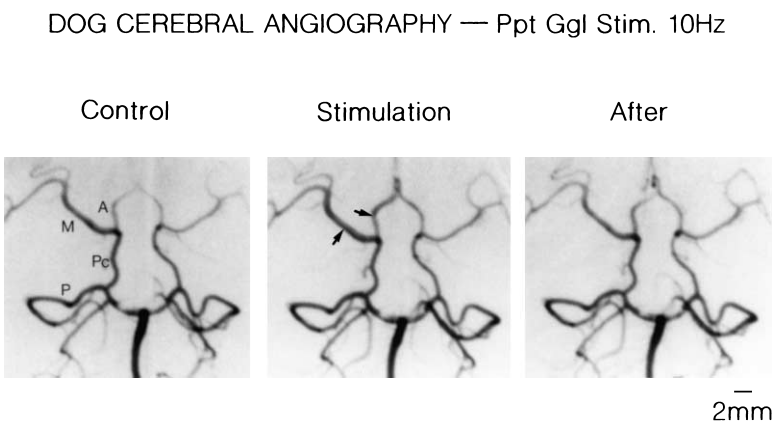

Fig. 2. Typical angiographical recordings of the response to pterygopalatine ganglion stimulation (10 Hz for 15 s) of anterior (A), middle (M) and posterior (P) cerebral and posterior communicating (Pc) arteries before (control), during (stimulation) and 5 min after (after) the stimulation in an anesthetized dog. Arrows indicate vasodilatation of ipsilateral arteries in response to nerve stimulation. (Adopted with a slight modification from Ref. 55 with permission from Lippincott Williams & Wilkins)

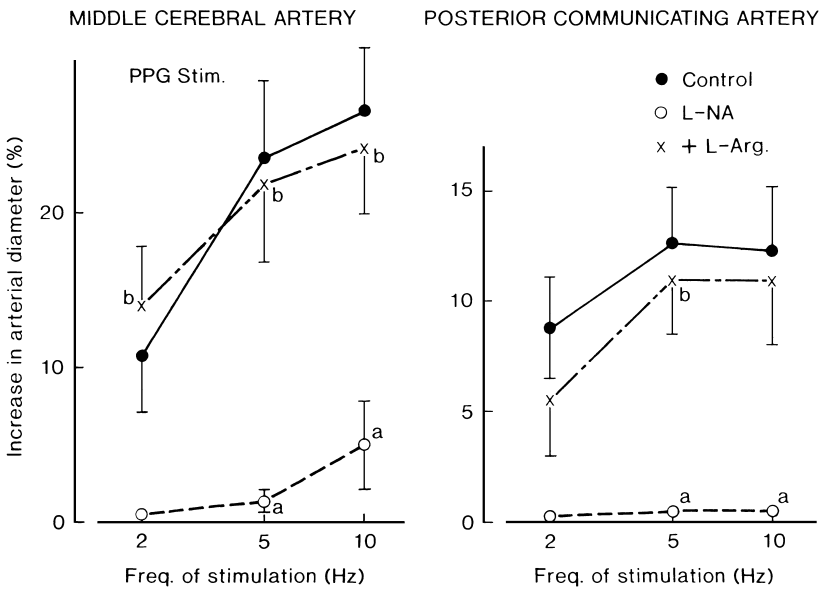

Fig. 3. Modifications by N^G-nitro-L-arginine (L-NA, 5 mg/kg, i.v.) and L-NA + L-arginine (L-Arg, 500 mg /kg, i.v.) of vasodilatation of ipsilateral middle cerebral and posterior communicating arteries by electrical stimulation (2, 5 and 10 Hz) of the pterygopalatine ganglion in anesthetized dogs. The ordinate indicates percent increase in the arterial diameter compared to that prior to the electrical stimulation. Significantly different from the control, $^aP<0.05$; significantly different from the value with L-NA, $^bP<0.05$ (Tukey's test). Experimental number is 7. Vertical bars represent S.E.M. (Adopted with a slight modification from Ref. 55 with permission from Lippincott Williams & Wilkins)

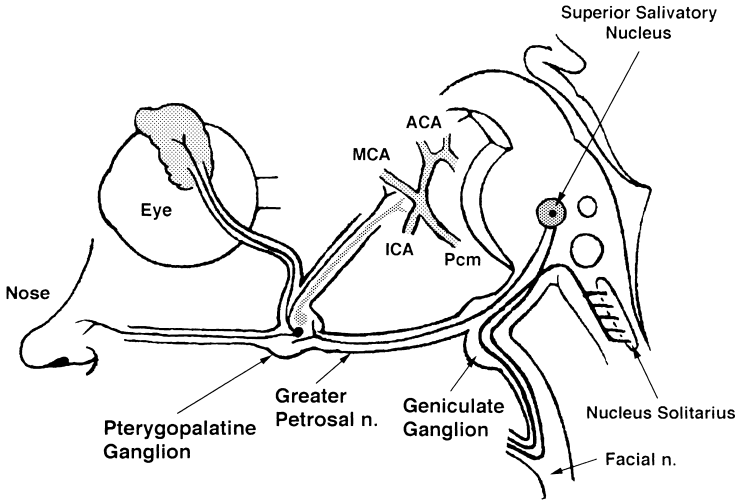

Fig. 4. Schematic presentation of neuronal pathways of nitroxidergic (nitrergic) (a gray and thick line to the cerebral arteries) and cholinergic nerves (black and thin lines) originating from the superior salivatory nucleus via the geniculate ganglion, greater petrosal nerve and pterygopalatine ganglion. The nerves regulate functions of the cerebral arteries (anterior cerebral (ACA), middle cerebral (MCA), intracranial internal carotid (ICA) and posterior communicating (Pcm)) and those of the lacrimal and nasal glands. (Adopted with a slight modification from Ref. 55 with permission from Lippincott Williams & Wilkins)

from the superior salivatory nucleus in the brainstem. Tonic discharges from the nucleus (vasomotor center) significantly participate in the maintenance of cerebral vasodilation. Postganglionic cholinergic neurons are expected to innervate lacrimal and nasal glands and cerebral vasculature (57, 58); cholinergic neurogenic activations stimulate exocrine secretions and possibly inhibit nitroxidergic (nitrergic) vasodilator nerve function as seen in monkey cerebral artery (59). Neuronal pathway of nitroxidergic (nitrergic) and choninergic nerves discussed in this article is schematically summarized in Fig. 4. Endogenous NO released from the vasodilator nerve may contribute to the maintenance of blood flow in main cerebral arteries necessary to supply blood to the different regions of the brain. Without the influence of this nerve, cerebral arteries might be constricted to the extent that blood flow is impaired.

Acknowledgments

The authors wish to thank Dr. K. Fujimori (Department of Chemistry, University of Tsukuba) for the supply of bis-*N*-nitroso-caged nitric oxides. This work was supported in part by the Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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