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Review Article

Nitrergic Control of Cerebral Vascular Tone and Blood Flow, and a Possible Blood–Brain Barrier Function

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Abstract

Neural control of vascular tone is important for the maintenance of circulatory homeostasis. Neurogenic vascular relaxation is obtained not only by the inhibition of constrictor nerves, but also by the stimulation of dilator nerves. We have reported that the vasodilator nerve innervating the cerebral artery is nitrergic in nature. In anesthetized animals, electrical stimulation (ES) of a pterygopalatine ganglion (PPG) or a greater petrosal nerve (GPN) only dilated cerebral arteries on the stimulated side. Nitric oxide (NO) synthase inhibitors abolished this dilation. Surgical denervation at the PPG instantly produced cerebroarterial constriction. In rats, ES of the nerve bundles from the PPG significantly increased cerebral blood flow, which was inhibited by NO synthase inhibitors. After systemic infusion of FITC (fluorescence)-dextran (10 kD) in anesthetized dogs, ES was applied to one side of the PPG. The fluorescent intensity in certain areas of the brain was higher on the stimulated side. Similar findings were obtained histochemically. T1-weighted MRI enhanced by gadolinium-DTPA during the GPN-stimulation in monkeys showed higher signal intensities in certain brain regions on the stimulated side. These findings suggest that nitrergic nerves tonically dilate the cerebral artery to maintain the cerebral circulation and may play a role in the regulation of blood–brain barrier permeability. [*Tzu Chi Med J* 2009;21(1):1–5]

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1. Cerebroarterial dilation and increase in cerebral blood flow (CBF)

1.1. Discovery of nitrergic nerves

The role of the autonomic nervous system in the circulation of the brain was once thought to be less important than in other organs. The contraction of the cerebral artery caused by sympathetic nerve activation is weaker than that of other arteries. Furthermore, the role of the parasympathetic nerve innervating blood vessels including the cerebral artery was thought to be negligible.

Toda first found that isolated cerebral arteries responded to transmural electrical stimulation (TES) with relaxation, which is resistant to both beta-blockers and atropine [1]. The relaxation caused by TES was independent of the absence of endothelium, resistant

to several receptor antagonists and desensitized to several peptides, but was abolished by treatment with nitric oxide (NO) synthase inhibitor(s), restored by the addition of L-arginine, and abolished by treatment with tetrodotoxin. Furthermore, a histochemical study demonstrated that the neuronal type of NO synthase (nNOS)-containing nerve fibers were mainly in the adventitial layer of the cerebral artery. The results also showed that nerve stimulation by electrical pulses increased the release of NO_X (nitrate and nitrite) from the endothelium-denuded cerebral artery and the content of cyclic GMP in this artery. These findings led us to consider that the vasodilating neurotransmitter of the cerebral artery was unstable NO itself or stable NO analog(s).

We performed experiments to examine whether the neurotransmitter was NO itself or instead stable analogs of NO such as S-nitrosocysteine, dinitrosyl-iron-cysteine complex, nitroxyl and hydroxylamine [2]. Isolated cerebral arteries without the endothelium after treatment with diethyldithiocarbamic acid, an inhibitor of copper/zinc superoxide dismutase, responded to TES with relaxation that was markedly inhibited by treatment with duroquinone, a superoxide anion-generating agent. The inhibition was partially reversed by exogenously applied superoxide dismutase. These findings suggest that the neurotransmitter liberated from vasodilator nerves innervating cerebral arteries was NO itself rather than stable analogs of NO [3]. Therefore, we concluded that the vasodilator transmitter released from nerves innervating the cerebral artery was actually NO in several animal models [4], including dogs [5] and monkeys [6].

1.2. Anatomy of nitrergic nerves innervating the cerebral artery

Ethanol was injected into the vicinity of one side of the pterygopalatine ganglion (PPG) in dogs to chemically degenerate the PPG. After 1 week of injections, isolated middle cerebral artery (MCA) and posterior cerebral artery (PCA) strips from both sides (impaired and intact sides) were transmurally stimulated. The changes in tension were examined. The MCA and PCA on the intact side were relaxed by the stimulation, which was abolished by treatment with NO synthase inhibitors, whereas stimulation failed to relax the arteries on the impaired side. Furthermore, positive NADPH diaphorase staining of perivascular nerves was found in the MCA and PCA on the intact side, but not on the impaired side [7]. This enzyme in the neuronal tissues is thought to be identical to nNOS [8]. Thus we thought that the nitrergic nerves innervating the cerebral arteries originated from the PPG. Another histochemical study also demonstrated that the PPG was the major source of positive fibers in the cerebral arteries [9].

1.3. Studies with cerebral angiography

In anesthetized dogs [10] and monkeys [11], the application of electrical stimulation (ES) to one side of the PPG or to the greater petrosal nerve (GPN), which is the preganglionic nerve of PPG, dilated cerebral arteries only on the stimulated side, as observed using cerebral angiography. NO synthase inhibitors abolished this dilation. Systemic treatment with hexamethonium, a ganglionic blocking agent, abolished the vasodilation caused by GPN stimulation, but failed to affect the dilation caused by PPG stimulation. Surgical denervation of the postganglionic nerve from the PPG in dogs instantly produced cerebroarterial constriction [10]. These findings suggest that impulses possibly from the superior salivary nucleus located at the midbrain that run through the GPN and nerve terminals of the GPN release acetylcholine (ACh). The released ACh binds to and activates nicotinic receptors on the dendrites or the cell bodies of nitrergic nerves in the PPG. We also suggest that nitrergic nerves tonically dilate cerebral arteries.

1.4. Measurement of CBF

In rats chronically treated with capsaicin to denervate the sensory nerves, application of ES to one side of the nerve bundles derived from the PPG significantly increased the CBF on the stimulated side, as assessed using a laser-Doppler flowmeter. NO synthase inhibitors dose-dependently inhibited the increase in CBF and the addition of L-arginine restored the CBF [12].

1.5. Summary

GPN, one of the parasympathetic efferent nerves, is derived from the brain stem and forms synapses at the PPG. Nitrergic nerves, postganglionic nerves from the PPG, innervate cerebral arteries and participate in the physiological regulation of the vascular tone and blood flow in the brain [13].

2. Possible opening of the blood–brain barrier (BBB)

2.1. The BBB

The BBB is the major defense mechanism of the brain and regulates the entry of substances from the blood into the nerve cells of the brain. Generally, molecules larger than 0.5 kDa or hydrophilic substances cannot freely cross the BBB. However, it has been shown that the defense mechanisms of the brain impair the access of therapeutic drugs to focal brain

lesions (inflammation, neoplasm and degenerative diseases).

2.2. Studies with fluorescence measurements

Recently, Yarnitsky et al reported that stimulation of the PPG produces a transient increase in the vascular permeability of the BBB in the rat brain [14]. To confirm this finding in medium-sized animals, we collaborated with their group in experiments on dogs. In anesthetized dogs, FITC (fluorescent marker)-dextran (10 kDa) was systemically infused and ES was applied to one side of the PPG. The fluorescent intensity in certain brain areas (frontal lobe, olfactory bulbs, striatum, hypothalamus and hippocampus), which were irrigated by arteries innervated by nitrergic nerves derived from the PPG, was higher on the stimulated side of the brain than that on the non-stimulated side [15]. In contrast, the fluorescent intensity in the cerebellum and pons, which were irrigated by arteries innervated by nitrergic nerves that originated from a place other than the PPG, was not affected by the stimulation. Results of histochemical studies support these findings.

2.3. MRI study

To further investigate the effects of PPG activation on the BBB function, we performed an MRI study in monkeys. T_1-weighted MRI enhanced by gadolinium-DTPA, a non-ionic contrast agent, usually enhances proton signaling in regions with very slow blood flow (cavernous sinus, choroid plexus, cerebral arteriovenous malformation, etc.) [16] or with impaired BBB function (tumor, inflammation, etc.). The MRI study performed during unilateral GPN-stimulation in monkeys demonstrated higher signal intensities in the temporal lobe, striatum and hippocampus on the stimulated side. A midline shift, suggestive of unilateral brain edema, was not observed. Thus we found that stimulation of monkey GPN elicits excitation of nitrergic nerves derived from the PPG innervating the cerebral artery, dilates the arteries, and may increase BBB permeability.

3. Clinical aspects

3.1. Cerebral infarction

As described above, CBF may, at least in part, be controlled by NO released from nitrergic nerves innervating the cerebral artery, indicating that dysfunction of the nitrergic nerves may induce cerebral ischemia and then lead to infarction. Koketsu et al [17] measured the infarct sizes following MCA occlusion in spontaneous hypertensive rats after parasympathetic denervation. The volume of the infarction was larger after selective sectioning of parasympathetic nerves containing nitrergic nerves derived from the PPG, suggesting that dysfunction of nitrergic nerves may exacerbate cerebral ischemia. A significant increase in the infarct size developed by the treatment with a NO synthase inhibitor in rats after occlusion of MCA, and additional intravenous injection of L-arginine decreased the infarct volume [18]. Therefore, it seems that NO released from nitrergic nerves innervating cerebral arteries protects the brain from cerebral ischemia and infarction. Thus stimulation of the PPG may minimize the size of lesions in cerebral ischemia and infarction.

3.2. Cerebral vasospasm after subarachnoid hemorrhage

Delayed cerebral vasospasm after subarachnoid hemorrhage (SAH) produces severe neurological deficits or death of patients if successful treatment is not performed for ruptured intracranial aneurysm. In experimental animals, including dogs and monkeys, two injections of autologous blood into the cisterna magna induced delayed vasospasm, as seen in humans [19]. The injections of oxyhemoglobin into the cisterna magna induces vasospasm in a few hours [20], whereas injection of a NO synthase inhibitor produces vasoconstriction within 1 hour and additional injection of L-arginine restores the constriction [21,22]. NO synthase inhibitors act more potently on the nerve than on the endothelium. Oxyhemoglobin is well known as a strong scavenger of NO. Thus deletion of the nerve-derived NO by oxyhemoglobin metabolized from hemoglobin after SAH may initiate the delayed vasospasm. Recently, subacute sodium nitrite infusions were reported to prevent delayed cerebral vasospasm in a primate model of SAH [23]. Therefore, stimulation of nitrergic nerves may prevent delayed cerebral vasospasm after SAH.

3.3. Migraine and cluster headaches

The clinical effectiveness in patients with headaches of ergotamine, flunarizine, a Ca^{2+} channel blocker, and serotonin 5-HT_1 agonists, including sumatriptan, which constrict intra- and extra-cranial arteries, are well recognized. However, the etiology of migraines and cluster headaches has yet to be clarified. The hypothesis has long been proposed that dilation of cerebral arteries is related to vascular headaches. It has been reported that NO donors, such as nitroglycerin, produce vascular headaches [24], and NO synthase inhibitors prevent the headaches [25]. These findings support the hypothesis that endogenous NO may

participate in the etiology of migraines and cluster headaches.

Sumatriptan antagonized the NO-mediated vasodilator response to nerve stimulation in cerebral arteries in dogs (26). Flunarizine seems to inhibit the synthesis and release of NO by reducing Ca^{2+} influx (27). Sanders and Zuurmond reported that radiofrequency lesioning in the PPG was effective in 61% of patients suffering from cluster headaches, which did not respond to therapy with medication (28). Therefore, NO derived from nitrergic nerves may also be involved in the vascular mechanism of headaches (29).

3.4. *New possibilities of selective drug delivery to the brain*

Unwanted effects usually emerge during chemotherapy because the BBB impairs the access of drugs to focal brain legions (inflammation, neoplasm, etc.), which results in need for relatively high dose of drugs compared with that for the therapy of other organs. Both Yarnitsky et al and our group reported that large molecule substances may cross the BBB during PPG stimulation, possibly via transient disruption of the BBB (14,15).

These findings lead us to investigate a novel drug-delivery procedure that could save patients with the fewer doses of drugs than usual, when stimulation of the PPG is safely and appropriately applied to the patients with focal brain inflammation and neoplasm. This procedure may also be applied during immune therapy for patients with neurodegenerative diseases.

4. Conclusion

We conclude that nitrergic nerves, postganglionic nerves of the PPG, tonically dilate the cerebral arteries and maintain the cerebral circulation. Furthermore, the nitrergic nerves appear to regulate permeability of the BBB. Studies on nitrergic nerve function are expected to help alleviate patient suffering from several diseases in the brain.

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