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Review Article

Proton as a Possible Neurotransmitter for Perivascular Axo-axonal Transmission in the Rat Mesenteric Resistance Artery

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Abstract

The rat mesenteric resistance arteries are densely innervated by adrenergic vasoconstrictor nerves, CGRPergic vasodilator nerves and nitric oxide-containing nerves. Those nerves have axo-axonal interactions to modulate vascular nerve function and to regulate vascular tone. The present study focused on a possible transmitter, which is involved in axo-axonal transmission of adrenergic nerves and CGRP nerves. When nicotine is applied in the rat perfused mesenteric artery, nicotine stimulates nicotinic $\alpha_3\beta_4$ nicotinic acetylcholine receptors on adrenergic nerves. This stimulation leads to the release of proton from adrenergic nerves. Released proton activates transient receptor potential vanilloid-1 receptors on neighboring CGRP nerves and CGRP is released to cause vasodilation. The present findings would explain why CGRPergic nerves, which are capsaicin-sensitive sensory nerves, have efferent function in spite of being primary afferent nerves. Namely, efferent adrenergic nerves, which momentarily and constantly regulate the vascular tone, may send their information to the neighboring CGRPergic nerves. Proton may be used as the transmitter for axo-axonal transmission to counteract excess vasoconstriction as the efferent function of afferent sensory nerves. Recent study demonstrated that periarterial nerve stimulation of perfused mesenteric arteries resulted in a decrease in the pH level of the perfusate concomitant with CGRPergic nerve-mediated vasodilation, which was abolished by the adrenergic neuron blocker, guanethidine. These findings suggest that proton acts as a transmitter for axo-axonal transmission between adrenergic and CGRPergic nerves in mesenteric resistance arteries. In conclusion, the present study presents evidence that perivascular nerves have strong axo-axonal interaction to modulate nerve function and to regulate vascular tone. [*Tzu Chi Med J* 2009;21(2):95–98]

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1. Introduction

It is widely accepted that the vascular tone is mainly maintained by sympathetic adrenergic nerves via the release of the neurotransmitter norepinephrine (NE). However, accumulating evidence reveals that non-adrenergic, noncholinergic (NANC) vasodilator nerves play a role in the regulation of vascular tone. We have demonstrated that the rat mesenteric artery has dense innervation of calcitonin gene-related peptide (CGRP)-containing (CGRPergic) nerves, which release a transmitter CGRP to cause vasodilation [1]. Recently, we reported that nitric oxide (NO)-containing nerves innervating rat mesenteric arteries are involved in modulation of adrenergic neurotransmission [2]. The rat mesenteric artery is also densely innervated by adrenergic nerves, which contain neuropeptide Y (NPY) with NE. Observations that double immunostainings in immunohistochemical study showed both NPY- and CGRP-immunoreactivities in the same neuron of the rat mesenteric artery [3] led to a hypothesis for the existence of some interactions between adrenergic nerves and CGRPergic nerves.

Our previous reports presented evidence that functional deficiency of CGRPergic nerves augments adrenergic nerve-mediated vasoconstriction. Conversely, adrenergic nerves via NE release presynaptically inhibit the neurogenic release of CGRP from the nerve to decrease CGRPergic nerve function [4,5]. Thus, we have proposed that CGRPergic vasodilator nerves and sympathetic vasoconstrictor nerves reciprocally regulate the tone of the mesenteric resistance artery. Recently, we discovered a new axo-axonal interaction of perivascular adrenergic and CGRPergic nerves in the rat mesenteric artery [3,6,7]. Therefore, the present study focused on a possible transmitter, which is involved in axo-axonal transmission of adrenergic nerves and CGRP nerves.

2. Reciprocal interaction between adrenergic nerves and NANC nerves

We reported that the rat mesenteric resistance artery has innervation of NANC vasodilator nerves in which CGRP, a potent vasodilator peptide, acts as a vasodilator neurotransmitter [1]. In rat perfused mesenteric vascular beds with active tone, the field stimulation of CGRPergic nerves, which contain CGRP, releases CGRP from the nerves to cause a potent vasodilation. Inhibition of CGRPergic function by capsaicin (CGRP depleting agent) and CGRP(8-37) (CGRP receptor antagonist) augmented adrenergic nerve-mediated vasoconstriction in response to field stimulation, suggesting that CGRPergic nerves suppress sympathetic nerve-mediated vasoconstriction [4]. Conversely, CGRPergic nerve-mediated vasodilation was blunted by α_2-adrenoceptor agonists (clonidine and NE) and NPY without affecting exogenous CGRP-induced vasodilation. This finding suggests that sympathetic nerves presynaptically inhibit the release of CGRP from the nerve to decrease CGRPergic nerve function [5,8]. Thus, we have proposed that CGRPergic vasodilator nerves along with sympathetic vasoconstrictor nerves reciprocally regulate the tone of the mesenteric resistance artery.

Immunohistochemical studies showed that the rat mesenteric arteries are densely innervated by NPY-like immunoreactive (LI) nerve fibers, which are eliminated by cold-storage denervation and 6-hydroxydopamine but not capsaicin. The CGRP-LI nerve fibers also densely innervate the mesenteric arteries and are abolished by cold-storage denervation and capsaicin but not 6-hydroxydopamine [9], indicating that CGRPergic nerves are capsaicin-sensitive nerves. In additional studies, double immunostainings showed appearance of both NPY- and CGRP-LI nerve fibers in the same neuron of the rat mesenteric artery [3]. These findings indicate that there are close contact areas between adrenergic and CGRPergic nerves and both nerves interact at these areas.

Our recent study demonstrated that L-NAME (NO synthase inhibitor) augments adrenergic nerve-mediated vasoconstriction in rat mesenteric arteries without endothelium, and that augmented effect of L-NAME is reversed by L-arginine [2]. Additionally, L-NAME facilitates neurogenic release of NE from perivascular adrenergic nerves. Capsaicin abolishes L-NAME-induced augmentation of adrenergic nerve-mediated vasoconstriction. However, L-NAME has no effect on CGRPergic nerve-mediated vasodilation. From these findings, we suggest that NO-containing nerves presynaptically regulate adrenergic neurotransmission by inhibiting transmitter NE release, but it is unlikely that the NO nerves directly cause vasodilation [2].

3. Axo-axonal interaction in vasodilation induced by nicotine and nicotinic acetylcholine receptor agonists

We have reported that, in rat perfused mesenteric vascular beds with active tone and without endothelium, perfusion of nicotine and nicotinic acetylcholine (ACh) receptor agonists (cytisine and epibatidine) for 1 minute caused concentration-dependent vasodilation without inducing vasoconstriction [6,7]. The nicotine-induced vasodilation was abolished by cold-storage denervation, suggesting that perivascular nerves are responsible for the effect of nicotine. Additionally, vasodilation induced by nicotine and nicotinic ACh receptor agonists was blunted by mecamylamine ($\alpha_3\beta_4$ nicotinic ACh receptor antagonist), guanethidine (adrenergic neuron

blocker) and capsaicin, but not by α-bungarotoxin (α_7 nicotinic ACh receptor antagonist) and dihydro-β-erythroidine ($\alpha_4\beta_2$ nicotinic ACh receptor antagonist). Therefore, it is very likely that intact adrenergic nerves and CGRPergic nerves mediate the nicotine- and nicotinic ACh receptor agonist-induced vasodilation [6,7]. Based on these results, it can be hypothesized that nicotine initially stimulates $\alpha_3\beta_4$ nicotinic ACh receptors, which are located on adrenergic nerves, to release adrenergic neurotransmitter(s) and related substances. These substances activate receptors located on adjacent CGRP nerves and then release CGRP to cause vasodilation.

4. Transmitter(s) mediating nicotine-induced vasodilation

To estimate possible substances transmitting nicotine-induced vasodilation, several antagonists, which antagonize the effect of adrenergic neurotransmitters, were used in additional studies. The nicotine-induced vasodilation was not inhibited by L-NAME (NO synthase inhibitor), propranolol (β-adrenoceptor antagonist), L-DOPA cyclohexyl ester (3,4-dihydroxyphenylalanine [DOPA] receptor antagonist), SCH23390 (selective dopamine D_1 receptor antagonist), haloperidol (dopamine D_2 receptor antagonist), α,β-methylene ATP (ATP P_{2x} receptor desensitizing agonist), 8(p-sulfophenyl) theophylline (adenosine A_2 receptor antagonist) or BIBP3226 (NPY-Y1 receptor antagonist) [3]. Additionally, perfusion of 3-methoxytyramine (COMT-metabolite of dopamine) and normetanephrine (COMT-metabolite of noradrenaline), but not other MAO- and COMT-metabolites of dopamine and noradrenaline, induced concentration-dependent vasodilation in rat denuded mesenteric vascular beds. However, capsaicin treatment, which abolished nicotine-induced vasodilation, did not affect vasodilation induced by both metabolites. Therefore, it is unlikely that catecholamines, catecholamine metabolites and other transmitters, which are released from perivascular adrenergic nerves, are involved in the nicotine-induced vasodilation.

5. Involvement of vanilloid-1 (transient receptor potential vanilloid-1; $TRPV_1$) receptors in nicotine-induced vasodilation

Additional studies were focused on presynaptic receptors in CGRPergic nerves, since CGRPergic nerves are capsaicin-sensitive sensory nerves. It is well known that the primary sensory nerves are activated by acid and heat via vanilloid-1 ($TRPV_1$) receptors. To estimate possible transmitter(s) for mediating the nicotine-induced vasodilation, we studied the effects of $TRPV_1$ receptor antagonists on the nicotine-induced vasodilation. We have demonstrated that capsazepine ($TRPV_1$ receptor antagonist) and ruthenium red (inhibitor of $TRPV_1$ receptor response) concentration-dependently inhibited the nicotine-induced vasodilation without affecting vasodilation in response to exogenously applied CGRP [3]. Furthermore, immunohistochemical staining of the mesenteric artery showed dense innervation of CGRP- and $TRPV_1$-receptor-immunopositive nerves, with both immunostainings appearing in the same neuron [3]. Therefore, these results strongly suggest that CGRPergic nerves innervating mesenteric arteries have $TRPV_1$ receptors and that $TRPV_1$ receptors are involved in the nicotine-induced vasodilation.

6. Proton as a possible transmitter for axo-axonal transmission

Based on our findings, it is possible to hypothesize that nicotine releases endogenous agonist for $TRPV_1$ receptors from adrenergic nerves. Since $TRPV_1$ receptors have been shown to be stimulated by proton, anandamide and lipid metabolites, we therefore focused on proton as an endogenous agonist for $TRPV_1$ receptors.

The additional studies were conducted to investigate whether proton have a vascular effect in the perfused mesenteric vascular bed. Direct injection of HCl into the perfusate proximal to the denuded preparation induced an initial sharp vasodilation followed by long-lasting vasodilation, while NaCl injection had no effect, suggesting that proton, but not chloride anion (Cl^-), is responsible for the vasodilation. The HCl-induced vasodilation was inhibited by capsazepine, ruthenium red, capsaicin and CGRP(8-37). Taken together, it is likely that proton (H^+) induces vasodilation, which is associated with activation of $TRPV_1$ receptors in the CGRP nerves.

To confirm further involvement of proton in the nicotine-induced vasodilation, we measured changes in the pH value of the perfusate, which had flowed out from the preparation and was continuously monitored with a pH meter. The perfusion of nicotine caused a concentration-dependent decrease in pH values, which appeared during the vasodilation. The nicotine-induced pH reduction was abolished by mecamylamine and guanethidine, suggesting involvement of proton (H^+) in the nicotine-induced vasodilation. These results clearly show that nicotine acts on presynaptic nicotinic $\alpha_3\beta_4$ receptors in adrenergic nerves to release proton (H^+) together with adrenergic neurotransmitter(s) or related substances and the released proton then stimulates $TRPV_1$ receptors on CGRPergic nerves, resulting in CGRP release and vasodilation.

7. Conclusions

The rat mesenteric resistance arteries are densely innervated by adrenergic vasoconstrictor nerves, NANC CGRPergic vasodilator nerves and NO nerves. Those nerves have axo-axonal interactions to modulate the vascular nerve function and to regulate vascular tone. When nicotine is applied, nicotine stimulates nicotinic $\alpha_3\beta_4$ nicotinic ACh receptors on adrenergic nerves. This stimulation leads to the release of proton from adrenergic nerves. Released proton activates $TRPV_1$ receptors on neighboring CGRP nerves, and CGRP is released and causes vasodilation. However, another process may be considered to explain this mechanism. For example, it could be expected that released adrenergic transmitters, such as NE and ATP, may act on vascular smooth muscles to release proton or endogenous $TRPV_1$ agonists. These substances may stimulate $TRPV_1$ receptors in CGRPergic nerves. Another mechanism may explain that there are unknown nerves in the artery and released adrenergic transmitters activate the unknown nerves, which can release proton to stimulate $TRPV_1$. However, further study needs to be done to clarify these mechanisms.

The present findings would explain why CGRPergic nerves, which are capsaicin-sensitive sensory nerves, have efferent function in spite of being primary afferent nerves. Namely, efferent adrenergic nerves, which momentarily and constantly regulate the vascular tone, may send their information via proton to the neighboring CGRPergic nerves. This proton may be used as the transmitter for axo-axonal transmission to counteract excess vasoconstriction as the efferent function of afferent sensory nerves. We have reported interesting results that capsazepine and ruthenium red inhibited the CGRPergic nerve-mediated vasodilation in response to perivascular nerve stimulation of the mesenteric artery with intact adrenergic and CGRPergic nerves (3). Furthermore, primary studies showed that periarterial nerve stimulation of perfused mesenteric arteries resulted in decrease in the pH level of the perfusate concomitant with CGRPergic nerve-mediated vasodilation. These findings also suggest that proton acts as a transmitter for axo-axonal transmission between adrenergic and CGRPergic nerves in mesenteric resistance arteries.

In conclusion, the present study demonstrates evidence that perivascular nerves have strong axo-axonal interaction to modulate nerve function and to regulate vascular tone.

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