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学位論文の題目	Neuropharmacological Study on Cholinergic Innervation and Function in Rat Mesenteric Arteries （ラット腸間膜動脈におけるコリン作動性神経の分布と機能に関する神経薬理学的研究）
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学位論文内容の要旨

【Aim】

Vascular blood vessels were innervated mainly by sympathetic adrenergic nerves and non-adrenergic calcitonin gene-related peptide (CGRP)-containing (CGRPergic) nerves to regulate vascular tone. The blood vessels have various types of cholinergic acetylcholine (ACh) receptors, but the source of ACh has not been confirmed in the vessels. Additionally, the function of cholinergic innervation in the vessel remains unknown. Therefore, the present study aimed to investigate cholinergic innervation by immunohistochemistry studies and function by pharmacologically analyzing effects of cholinesterase inhibitors (neostigmine and physostigmine), muscarinic ACh receptor antagonist (atropine, pirenzepine, methoctramine, 4-DAMP), and a nicotinic ACh receptor antagonist (hexamethonium) on adrenergic nerve-mediated vasoconstriction and CGRPergic nerve-mediated vasodilation in rat mesenteric vascular beds with or without an endothelium. Also, the present study investigated distribution of muscarinic ACh receptor subtypes (M1, M2 and M3) and their function on CGRPergic nerves in the rat mesenteric arteries.

【Methods and Results】

In Western blot analysis, protein expressions of M1, M2 and M3 receptor subtypes were found in rat mesenteric arteries with endothelium and without endothelium, and in arteries treated with cold storage denervation. The expression of M1 receptor protein was much greater than that of M2 or M3 receptors. Endothelium removal and cold storage denervation tended to decrease M1 receptor expression compared to control. Endothelium removal significantly decreased M2 and M3 receptor expressions compared to control, while expression levels of M1, M2 and M3 receptors were not significantly altered by cold storage denervation. In immunohistochemistry study, choline acetyltransferase (ChAT; ACh synthase enzyme) -immunopositive fibers were found in the mesenteric artery like network. Distribution of CAT-immunopositive fibers was not affected by treatment with capsaicin (CGRP depletor) or 6-hydroxydopamine (adrenergic neurotoxin). In pharmacological analysis, perivascular nerve stimulation (PNS) at 2 to 12 Hz and exogenous norepinephrine (NE) in rat perfused mesenteric vascular beds treated with capsaicin or in the presence of nitric-oxide synthase inhibitor (L-NAME) evoked a frequency- and concentration-dependent vasoconstriction. Atropine, hexamethonium and neostigmine had no effect on vasoconstrictor responses to PNS and NE injections. In preparations without endothelium, these cholinergic agents did not affect the PNS (12 Hz)-evoked release of NE. In denuded preparations with active tone in the presence of guanethidine (adrenergic neuron blocker), PNS (1 to 4 Hz) induced a frequency-dependent vasodilation, which was not affected by atropine, hexamethonium, and neostigmine. In preparations treated with capsaicin and guanethidine, PNS did not induce vascular responses and atropine, neostigmine and physostigmine had no effect on PNS. In perfused mesenteric vascular beds with intact endothelium and with active tone, bolus injection of ACh (1, 10 and 100 nmol) produced a concentration-dependent vasodilation. In denuded preparations, vasodilator response to ACh at 10 and 100 nmol, but not 1 nmol, caused a long-lasting vasodilation. In denuded preparations, pirenzepine (M1 antagonist) and 4-DAMP (M1 and M3 antagonist), but not methoctramine (M2 and M4 antagonist), abolished 10 nmol ACh-induced vasodilation, while 100 nmol ACh induced-vasodilation was inhibited by pirenzepine (68%) and 4-DAMP (53%). In preparations treated with hexamethonium and guanethidine without endothelium, pirenzepine and 4-DAMP abolished vasodilator response to ACh at 1, 10 and 100 nmol.

【Conclusion】

These results suggest that rat mesenteric arteries have cholinergic innervation, which is different from adrenergic and capsaicin-sensitive nerves and not associated with the regulation of vascular tone. It is also suggested that muscarinic ACh receptor subtypes (M1, M2 and M3), mainly M1 subtypes, distribute mesenteric arteries and activation of M1 and M3 subtypes, but not M2 subtype, located on CGRPergic nerves releases CGRP, thereby causing the endothelium-independent vasodilation.

論文審査結果の要旨

学位論文中の引用文献に基づく議論や論理の展開も適切であると判断され、表現や図表も適切であったが、一部表記に関して審査委員によって指摘された点の修正を必要とした。申請者による指摘箇所の訂正の後、メール委員会で最終判定を協議した結果、学位審査委員会は、本研究の学術的意義は十分であり、本研究科で授与する博士（薬学）の学位に値するものと判断し、審査合格と決定した。