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Non-adrenergic inhibition at prejunctional sites by agmatine of purinergic vasoconstriction in rabbit saphenous artery

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Abstract

We investigated the effects of agmatine, clonidine, xylazine and moxonidine on the purinergic vasoconstriction induced by electrical stimulation in the rabbit isolated saphenous artery without endothelium.

Transmural electrical stimulations induced reproducible responses in the arterial preparations, which were abolished by tetrodotoxin at 0.1 μM or pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid tetrasodium salt (PPADS, 30 μM), but were not affected by 1 μM prazosin.

Clonidine, xylazine and moxonidine induced transient and concentration-independent vasoconstriction, with threshold concentrations of 1, 3 and 30 μM, respectively. Agmatine, in contrast, did not produce any vascular response even at 1 mM. Lower concentrations of clonidine, xylazine and moxonidine (0.01–0.3 μM) concentration-dependently decreased vasoconstrictor responses to electrical stimulation, whereas agmatine (0.1–1 mM) induced an inhibitory followed by a facilitatory effect on electrically evoked responses. Agmatine, clonidine and moxonidine but not xylazine significantly enhanced the vasoconstriction elicited by 1 mM ATP. The concentration–response curve for NA was shifted to the left slightly by 1 mM agmatine, but not affected by 0.3 μM of other three agonists.

Phenoxybenzamine did not affect the vasoconstrictive responses to 1 mM ATP and to electrical stimulations, but abolished those to NA. Agmatine at 1 mM evoked only an inhibitory effect on electrical stimulation-induced vasoconstriction in the preparation pretreated with phenoxybenzamine, and the inhibitory action was enhanced to 38.6% from the control value (without treatment with phenoxybenzamine) of 22.5%. The non-imidazoline compound xylazine at 0.3 μM lost its inhibitory effect on the neurogenic vasoconstriction in the presence of phenoxybenzamine.

In conclusion, agmatine produces a biphasic effect on the purinergic vasoconstriction induced by sympathetic nerve stimulation in the rabbit isolated saphenous artery. The monophasic inhibition of agmatine in the artery treated with phenoxybenzamine is due to an α-adrenoceptor-independent mechanism at prejunctional sites, and the potentiation effect of agmatine is mainly dependent on its enhancement of vasoconstriction at postjunctional sites.

Keywords: Purinergic vasoconstriction; Prejunctional regulation; Postjunctional regulation; Transmural electrical stimulation; Agmatine; Clonidine; Xylazine; Rabbit saphenous artery

1. Introduction

Imidazoline receptors are now being discussed as potential sites of drug action in many tissues (Molderings, 1997). Furthermore, evidence for prejunctional imidazoline receptors has been obtained in the rabbit pulmonary artery, aorta (Göthert and Molderings, 1991; Molderings

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et al., 1991; Molderings and Göthert, 1995) and heart (Fuder and Schwarz, 1993), the rat kidney (Bohmann et al., 1994) and the human atria and pulmonary artery (Likungu et al., 1996; Molderings et al., 1997). Prejunctional imidazoline receptors are hypothesized mainly according to the following facts from in vitro study using isolated tissues preincubated with [^{3}H]noradrenaline. First, imidazoline derivative BDF6143 reduced noradrenaline (NA) release when α_2-autoreceptors were blocked with high concentrations of rauwolscine, phentolamine or phenoxybenzamine, and a rauwolscine-resistant component of inhibitory effects on NA release was also induced by imidazolines such as clonidine, moxonidine, idazoxan and cirazoline (Göthert and Molderings, 1991). Second, BDF6143 concentration-dependently inhibited NA release evoked by transmural electrical stimulation at 0.66 Hz even in the absence of α_2-adrenoceptor antagonists (Göthert and Molderings, 1991). However, Gaiser et al. (1999) investigated 10 agents, which were thought to inhibit NA release via prejunctional imidazoline receptors or α_2-adrenoceptors using the rabbit pulmonary artery under autoinhibition-free conditions (brief high-frequency stimulation), and provided evidence indicating that prejunctional α_2-autoreceptor effects of the 10 agents left nothing unexplained, and there was no need to invoke prejunctional imidazoline receptors.

Agmatine, an endogenous amine found in several mammalian organs including the blood vessel (Raasch et al., 1995) is a ligand for α_2-adrenoceptors and imidazoline receptors, and is recognized as an endogenous clonidine-displacing substance (Li et al., 1994; Piletz et al., 1995) and a putative neurotransmitter or neuromodulator (Li et al., 1994). Intravenous administration of agmatine at higher doses induced a pronounced blood pressure decrease in the anaesthetized spontaneously hypertensive rats (SHR), even under α_2-adrenoceptor blockade conditions (Raasch et al., 2002, 2003), and no reflex tachycardia was evident despite the fact that the baroreflex arch was maintained (Raasch et al., 2002). I_2-binding sites have been reported on vascular smooth muscle cells (Regunathan et al., 1995), and I_3-binding sites were shown to regulate vasoconstriction in the porcine isolated rectal artery (Minyan et al., 2001). In most in vitro studies, however, agmatine does not induce prejunctional or postjunctional effects via imidazoline receptors. Pinthong et al. (1995) reported that agmatine exerted neither agonistic nor antagonistic activity towards the α_2-adrenoceptors; and it failed to alter the contractility of the porcine isolated palmar lateral vein and the rat isolated thoracic aorta, gastric fundus and tail artery (Pinthong et al., 1995; Gonzalez et al., 1996). Agmatine inhibits the acetylcholine release from the guinea-pig ileum by interaction with prejunctional α_2-adrenoceptors of the parasympathetic nerves (Colucci et al., 1998). Recently, we reported that agmatine did not produce any vascular responses in the thoracic aorta and ear vein isolated from the rabbit, and it did not affect action potentials of the rabbit sinoatrial node pacemaker cells at concentrations less than 10 mM (Zhao and Ren, 2003). It was somehow contrary to expectations, agmatine injected intracisternally caused a dose-dependent increase in blood pressure in conscious rabbits and SHR (Szabo et al., 1995; Raasch et al., 2002).

As a neurotransmitter released from sympathetic nerve endings of the arteries, NA acts on both prejunctional and postjunctional sites. In saphenous artery and splenic artery, however, a large component of the vasoconstrictive responses to electrical stimulation is purinergic component (Ren et al., 1996; Ren and Burnstock, 1997; Zhang and Ren, 2001), but the response to nicotine stimulation is adrenergic (Bultmann et al., 1991; Ren et al., 1994). Since the modulation effects of agmatine and imidazoline compounds on vasoconstrictive responses to purinergic transmitters have not been reported, and a stable analogue of adenosine triphosphate (ATP), α,β-methylene ATP, induces similar vasoconstriction like NA in the rabbit isolated saphenous, mesenteric, splenic and ear arteries (Ren and Zhang, 2002), we observed the effects of agmatine, clonidine and moxonidine on the purinergic vasoconstriction induced by electrical stimulation in the rabbit isolated saphenous artery in the present study.

2. Materials and methods

2.1. Arterial preparations

Male New Zealand white rabbits (2.5–3.5 kg) obtained from Experimental Animal Center of Hebei Medical University (Certificate No 0059) were killed with an overdose of pentobarbitone sodium injected via the ear vein and then exsanguinated (Ren and Burnstock, 1997). All animals used in the present study received humane care in compliance with institutional animal care guidelines. The study was approved by the Local Institutional Committee. Then, the saphenous artery was excised and cleaned of excess connective tissue and fat. The vascular endothelium was removed by gently rubbing the lumen with a scored polythene cannula that was slightly smaller in external diameter than the internal diameter of the vessels (O'Connor et al., 1990). Ring segments without endothelium (4 mm in length) were mounted horizontally in a 10-ml organ bath by carefully inserting a tungsten wire through the lumen of the vessel ring and anchoring it to a stationary support. Another wire similarly inserted, was connected to an isometric tension transducer, and responses were recorded on a polygraph (ERT-884, Youlin Electron Co, Kaifeng, China). The preparation was placed under a preload of 2.5 g (Ren and Zhang, 2002) and allowed to equilibrate for 1.5 h in a physiological solution (K-H solution) of the