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Evidence for sympathetic, purinergic transmission in the mesenteric artery of the dog

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Electrical transmural stimulation of isolated mesenteric artery of the dog produced a transient contraction which consisted of adrenergic and non-adrenergic components. In contrast to the adrenergic component, the nonadrenergic component was resistant to prazosin and other adrenoceptor-blocking agents. However, the nonadrenergic component was completely blocked by guanethidine and by desensitization with α,β-methylene-ATP (α,β-MeATP). Desensitization induced by α,β-MeATP also inhibited the contractile response to ATP but not the adrenergic responses induced by electrical transmural stimulation and exogeneous noradrenaline. These results suggest that the nonadrenergic contraction induced by electrical transmural stimulation is a sympathetic, purinergic response.

Introduction In many blood vessels, perivascular nerve stimulation produces sympathetic contraction which is blocked by α-adrenoceptor antagonists. However, there is evidence that the sympathetic responses in some arteries are not blocked by α-antagonists (Hirst & Neild, 1980; Holman & Surprenant, 1980; Muramatsu *et al.*, 1981; Kuriyama & Makita, 1983). Recently, we found that the response of isolated dog mesenteric artery to electrical transmural stimulation was not completely blocked by prazosin and suggested that the prazosin-resistant contraction was not adrenergic in nature (Muramatsu *et al.*, 1984). This paper reports that the prazosin-resistant contraction is completely blocked after treatment with α,β-methylene-ATP (α,β-MeATP), that is, by the desensitization of P_2-purinoceptors (Kasakov & Burnstock, 1983).

Methods Dogs of either sex, weighing 8 to 15 kg, were anaesthetized with thiopentone sodium (20 mg kg^{-1}, i.v.), exanguinated from the common carotid arteries and the mesentery removed. The mesenteric arteries were isolated and cut into helical strips, approximately 2 mm in width and 15 mm in length, under a dissecting microscope. Then, the endothelium layer was rubbed with filter paper in order to avoid the possible involvement of endothelium cell-derived factors in the mechanical respones. The strips were mounted vertically in an organ bath containing 20 ml Krebs-Henseleit solution of the following composition (mM): NaCl 112, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11.5. To block the β-adrenoceptors, propranolol (10^{-6} M) was added to the bath solution throughout the experiment. The bath medium was maintained at 37°C, pH 7.4, and was equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2. Resting tension of 1.0 g was applied and the responses were recorded isometrically through force-displacement transducers. The preparations were allowed to equilibrate for 90 min in the bathing medium before experiments were started. Electrical transmural stimulation was applied through a pair of platinum-wire electrodes. Stimulus parameters were 0.3 ms duration, a frequency of 10 Hz and supramaximum voltage (10 V) for 5 s. Drugs were added directly to the bath.

Results Electrical transmural stimulation of isolated mesenteric artery of the dog caused a transient contraction. This response was abolished by guanethidine (3×10^{-6} M) but not completely inhibited by prazosin (10^{-8} or 10^{-7} M) (Figure 1a). As shown previously (Muramatsu *et al.*, 1984), the prazosin-resistant contraction was not inhibited by other adrenoceptor-blocking agents (yohimbine 10^{-6} M, phentolamine 10^{-6} M and phenoxybenzamine 10^{-7} M). However, the response was abolished after treatment with α,β-methylene ATP 5×10^{-6} M. Figure 1 shows representative results. When the muscle tension returned to the original level after a transient contraction induced by α,β-MeATP, electrical transmural stimulation failed to produce any response in the presence of prazosin (Figure 1a). The contractile response to ATP (10^{-4} M) was also abolished after treatment with α,β-MeATP and sometimes reversed to a relaxation. On the other hand, the response to electrical transmural stimulation in the absence of prazosin was only slightly attenuated by α,β-MeATP (18 ± 2% reduction in amplitude, mean ± s.e. of 5 experiments) and the residual response was abolished by further addition of prazosin 10^{-7} M (Figure 1b). Exogenous noradrenaline (10^{-7} M) produced a contraction that was markedly

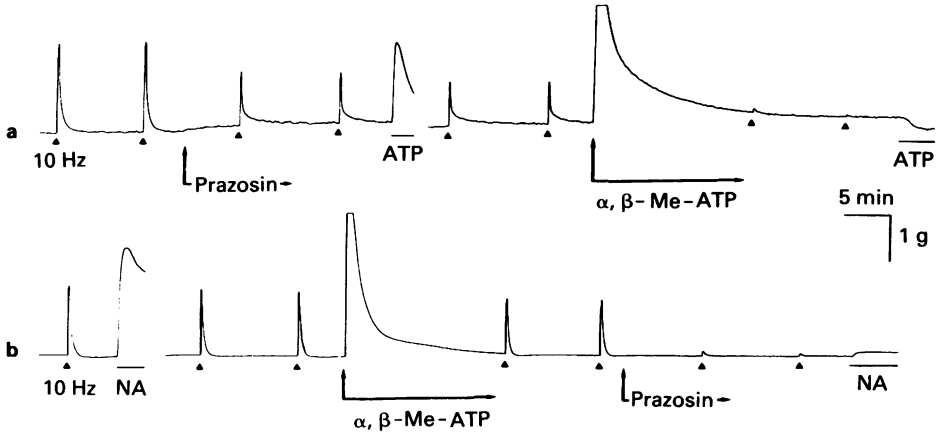

Figure 1 Effects of α,β-methylene ATP (α,β-MeATP, 5×10^{-6} M) on the contractile responses of dog mesenteric arteries to electrical transmural stimulation (10 Hz, for 5 s) in the presence (a) and absence (b) of prazosin 10^{-7} M. α,β-MeATP produced a transient contraction which exceeded the recording range. ATP: 10^{-4} M ATP, NA: 10^{-7} M noradrenaline.

attenuated by prazosin (10^{-7} M) but not by α,β-MeATP (5×10^{-6} M).

Discussion Electrical transmural stimulation produced both adrenergic and nonadrenergic contractions in the dog mesenteric artery. We previously reported that both components were abolished by guanethidine or 6-hydroxydopamine, but that reserpine-treatment selectively inhibited the adrenergic component (Muramatsu *et al.*, 1984). The nonadrenergic component was resistant to prazosin and other α-antagonists. The present study shows that the nonadrenergic component observed in the presence of prazosin is preferentially inhibited by α,β-MeATP. Since the contractile responses to electrical transmural stimulation in the absence of prazosin was not abolished by α,β-MeATP, the preferential inhibition of the nonadrenergic component after α,β-MeATP is not likely to be due to inhibition of transmitter release. α,β-MeATP desensitizes P_2-purinoceptors and inhibits the noncholinergic, nonadrenergic responses which have been put forward as purinergic responses (Kasakov & Burnstock, 1983; Moss & Burnstock, 1985). In the present study, α,β-MeATP inhibited the contractile response to ATP but not to noradrenaline. These results strongly suggest that the nonadrenergic sympathetic response in the dog mesenteric artery is purinergic in nature, as reported for the rat tail artery (Sneddon & Burnstock, 1984).

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