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Sympathetic cotransmission in rabbit saphenous artery *in vitro*: effect of electric stimulation and potentiation by α, β-methylene ATP[1]

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KEY WORDS adrenergic fibers; adenosine triphosphate; norepinephrine; electric stimulation; vasoconstriction; prazosin; purines

ABSTRACT

AIM: To analyze the cotransmission characteristics of contractile responses to electric field stimulation with submaximal voltage and short train in the rabbit saphenous artery. **METHODS**: Isometric vasoconstriction of the rabbit saphenous arterial rings was recorded, and the sympathetic nerves of the arterial rings were activated with electric field stimulation. **RESULTS**: Electric stimulation produced contractile responses in a frequency-dependent manner in the rabbit saphenous artery. Selective α_1-adrenoceptor antagonist, prazosin (1 μmol/L) did not affect the vasoconstriction induced by electric stimulation at 2 Hz significantly, but inhibited 39.9 % - 53.8 % of the vasoconstriction at 8 - 16 Hz. On the other hand, desensitization of the $P2X_1$ receptor with α,β-methylene ATP (3 μmol/L) abolished all the vascular responses induced by stimulation at 2 Hz, and obviously potentiated those induced by stimulation at 16 Hz, but it did not affect the concentration-dependent response curves for exogenous norepinephrine. The vasoconstriction responses induced by electric stimulation were all abolished by the treatment of a combination of prazosin (1 μmol/L) and α,β-methylene ATP (3 μmol/L). **CONCLUSION**: The sympathetic and purinergic contractile responses can be induced by 2 Hz stimulation, and ATP is the sole transmitter causing the vasoconstriction in the rabbit saphenous artery. Contractile responses to higher frequencies are related to both norepinephrine and ATP. Desensitization of the $P2X_1$ receptor with α,β-methylene ATP potentiates the vascular responses to electric stimulation via a presynaptic mechanism.

INTRODUCTION

Adenosine 5′-triphosphate (ATP) is released as a cotransmitter with norepinephrine (NE) from sympathetic nerves endings of several blood vessels in the canines, rabbits and rats[1]. Up to the present, the most important findings in this research field are that there is an obviously different contribution of purinergic component to the neurogenic vasoconstriction among various arteries, and a different sensitivity to electric stimulation frequency for producing vascular contractile responses among various arteries[2-4]. It has been reported that the vasoconstriction induced by electric field stimulation in the rabbit isolated hepatic artery and saphenous artery mainly consists of a purinergic component in comparison with the rabbit ear artery, in the latter artery adrenergic component is dominant. Moreover, electric stimulation at a frequency of less than 8 or 4 Hz could not produce contractile responses in the rabbit hepatic or saphenous artery[2-4]. In our preliminary experiments, however, we found that electric stimulation at 2 Hz and 80 V was able to produce reproducible vasoconstriction in the rabbit saphenous artery, which was similar to our previous results reported in the rabbit splenic arteries[5]. Therefore, we proposed that electric current intensity but not frequency was a more important factor to influence the characteristics of the sympathetic cotransmission in the arteries *in vitro*. In the present study, we investigated the influence of electric stimulation parameters on the characteristics of neurogenic vascular responses to electric field stimulation in the rabbit isolated saphenous artery.

MATERIALS AND METHODS

Rabbits Male New Zealand white rabbits (2.0 -

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3.5 kg) were obtained from Experimental Animal Center of Hebei Medical University (Certificate № 0059).

Chemicals α,β-Methylene adenosine 5′-triphosphate (α, β-meATP), (−)-norepinephrine bitartrate (NE), adenosine 5′-triphosphate (ATP), tetrodotoxin (TTX), prazosin, guanethidine were all obtained from Sigma Chemical Co. All drugs were dissolved in distilled water.

Arterial preparation Rabbits were stunned by a blow and then exsanguinated. The saphenous artery was excised and cleaned of excess connective tissue and fat. Ring segments with 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 Electronic Instrument Co, Kaifeng). The preparation was placed under a resting tension of 1.0 g and allowed to equilibrate for 1h in physiological solution of the following composition (mmol/L): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52, pH 7.2. The solution was maintained at 37℃ and aerated with 95 % O_2 and 5 % CO_2.

Electric stimulation Transmural electric stimulation was delivered to the tissue by means of two platinum wire electrodes placed parallel to, and on each side of the vessel, by a stimulator (YSD-4G, Bengbu Electronic Instrument Co, Bengbu). The artery was stimulated with square-wave pulses of 0.1 ms duration and 80 V, over a frequency range of 2 – 16 Hz for 1 s at 5 min intervals. Electric stimulation parameters were monitored in an oscilloscope (5702, Neimenggu Electronic Instrument Co, Huhehaote, China). Frequency-dependent response curves were repeated 4 or 5 times at 35 min intervals in each preparation. The first and second sets of data were not used in the present experiments.

Drug administration NE was added cumulatively to the organ bath to produce agonist concentration-response curves. The concentration-response curves for NE were repeated 3 times in each preparation at 35 min intervals, and the first set of data was not used in the study. $P2X_1$ receptor agonist α,β-meATP, which rapidly desensitizes its own receptors, was added non-cumulatively at 1 h intervals. Only one set of concentration-response curve for α, β-meATP was generated per preparation.

Antagonists and inhibitors were added to the organ bath 20 min before carrying out the next experimental procedure (electric stimulation, concentration-dependent responses to NE or α,β-meATP), except for TTX which was added 10 min before. Desensitization of the $P2X_1$ receptor was achieved by several exposures (generally 3) of the vessel to α, β-meATP (1 μmol/L) at 5 min intervals until no further contraction was elicited, and vascular tone returned to the baseline. Specific α_1-adrenoceptor antagonist prazosin (1 μmol/L) was used to block the adrenergic component of neurogenic vasoconstriction, and $P2X_1$ receptor desensitizing agent, α,β-meATP, was used to block the purinergic component of the response. The effects of each antagonist, inhibitor, and desensitization of $P2X_1$ receptor with α,β-meATP on the contractile responses to NE or α,β-meATP (as an agonist) were observed.

TTX (0.3 μmol/L) and guanethidine (0.4 μmol/L) were applied to analyze whether the contractile responses to electric stimulation were generated by neurotransmitters from sympathetic nerves.

Statistical analysis Data were expressed as $\bar{x} \pm s$. Two way ANOVA was used to evaluate any differences between frequency-dependent response curves for electric stimulation or concentration-response curves for drugs. If the F statistic was significant, we compared the individual datum with its respective control value by unpaired t-test.

RESULTS

Effects of TTX on the neurogenic vasoconstrictive responses to electric stimulation Electric stimulation (2 – 16 Hz) produced frequency-dependent contractile responses, and the frequency-dependent response curves obtained on the 3rd, 4th, 5th stimulations were not significantly different from each other (data not shown). The contractile responses to electric stimulation were abolished by TTX (0.3 μmol/L, Fig 1). NE (0.01 – 100 μmol/L) induced concentration-dependent response curves, and TTX (0.3 μmol/L) did not affect the vasoconstriction of NE significantly (Tab 1).

Effects of guanethidine on the sympathetic vasoconstrictive responses to electric stimulation The vasoconstrictive responses induced by electric stimulation in the rabbit saphenous artery were abolished

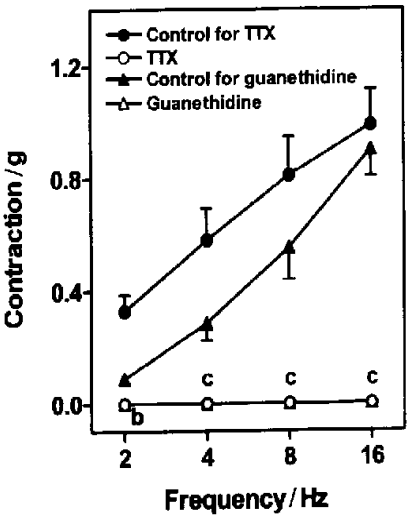

Fig 1. Effect of tetrodotoxin (TTX) 0.3 μmol/L or guanethidine 0.4 μmol/L on vascular responses induced by electric stimulation at 2 - 16 Hz in the rabbit saphenous artery. $n = 5 - 6$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* respective control.

by guanethidine (0.4 μmol/L, Fig 1). On the other hand, guanethidine (0.4 μmol/L) significantly shifted the concentration-dependent response curve for NE to the left (Tab 1), and it did not affect the maximal contractile response (E_{max}) to NE with the E_{max} values of (2.4 ± 0.5) g in control preparations and (2.4 ± 0.4) g in guanethidine-treated preparations ($P > 0.05$, $n = 8$).

Effects of α_1-adrenoceptor antagonist prazosin on the adrenergic component of the neurogenic vasoconstriction Prazosin (1 μmol/L) did not affect the responses to electric stimulation at 2 - 4 Hz, but partially inhibited the responses to 8 and 16 Hz by 39.9 % and 53.8 %, respectively (Fig 2A). Vascular contractile responses to NE at 0.1, 1, 10, and 100 μmol/L were significantly inhibited with prazosin 1 μmol/L by 100 %, 100 %, 99 %, and 44 %, respectively ($P < 0.01$, $n = 8$). A non-cumulative concentration-response curve for α, β-meATP (as an agonist) was constructed from 0.01 to 10 μmol/L, and it was not significantly affected by prazosin (Tab 1).

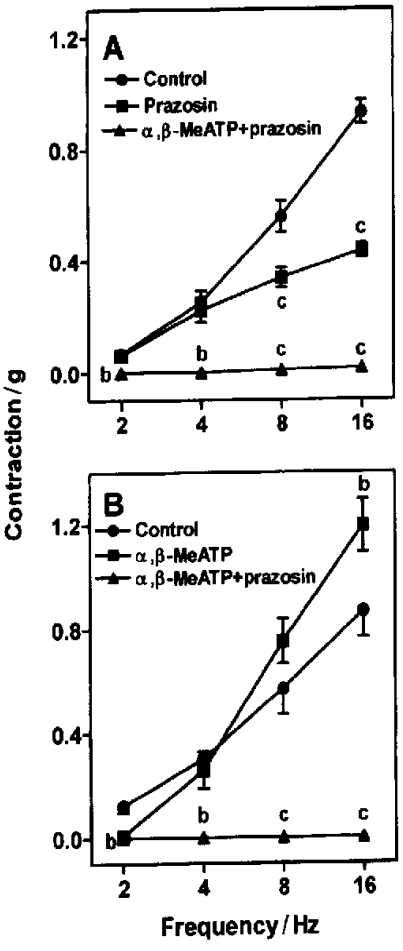

Fig 2. Effects of prazosin 1 μmol/L (A), α,β-meATP 3 μmol/L (B), and a combination of both drugs (A and B) on the contractile responses to electric stimulation at 2 - 16 Hz in the rabbit saphenous artery. $n = 8$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* respective control.

Tab 1. Effects of inhibitors on the vasoconstriction induced by norepinephrine (NE) and α,β-meATP. $n = 5 - 8$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control.

Inhibitor/ μmol·L^{-1}	EC_{50} of NE/μmol·L^{-1}		EC_{50} of α,β-meATP/μmol·L^{-1}	
	Control	Treatment	Control	Treatment
Tetrodotoxin 0.3	3.8 ± 1.3	3.6 ± 1.2	–	–
Guanethidine 0.4	3.4 ± 1.4	0.76 ± 0.26^c	–	–
Yohimbine 1.0	4.8 ± 1.5	6.1 ± 1.5	–	–
Prazosin 1.0	–	–	0.49 ± 0.06	0.62 ± 0.19

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Effects of $P2X_1$ receptor desensitization with α, β-meATP on the purinergic component of neurogenic vasoconstriction Desensitization of $P2X_1$ receptor with α, β-meATP (3 μmol/L) abolished the contractile responses to stimulation at 2 Hz, and did not affect the contractile responses to stimulation at 4 – 8 Hz, however it enhanced the contractile responses to stimulation at 16 Hz significantly (Fig 2B). The vascular contractile responses to NE (0.01 – 100 μmol/L) were not significantly changed by desensitization of $P2X_1$ receptor with α, β-meATP 3 μmol/L (Fig 3B).

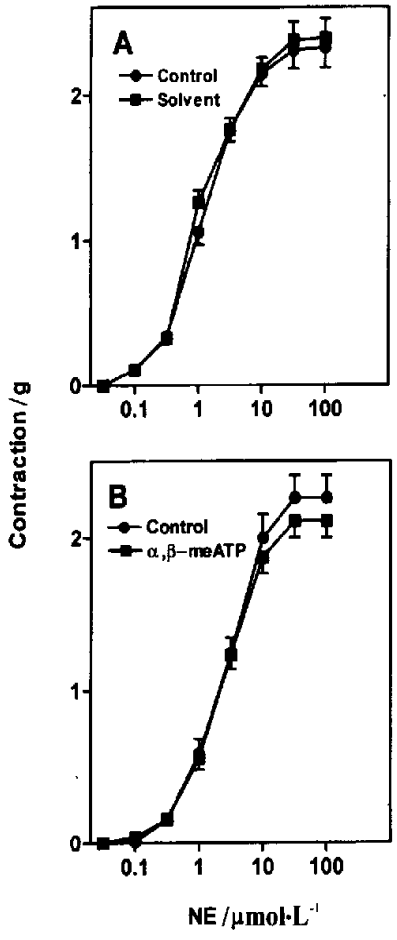

Fig 3. Effects of α, β-meATP 3 μmol/L on the contractile responses to norepinephrine (NE) in the rabbit saphenous artery. (A) Response curves for NE before and after solvent; (B) Response curves for NE before and after treatment with α, β-meATP 3 μmol/L. $n=8$. $\bar{x} \pm s$.

Effects of a combination of both prazosin and $P2X_1$ receptor desensitization on the neurogenic vasoconstriction A combination of both prazosin (1 μmol/L) and desensitization of $P2X_1$ receptor with α, β-meATP (3 μmol/L) abolished all responses to electric stimulation (Fig 2).

Effects of α_2-adrenoceptor antagonist yohimbine on the neurogenic vasoconstriction Vascular contractile responses to NE (0.01 – 100 μmol/L) were not significantly affected by yohimbine (1 μmol/L, Tab 1), but the contractile responses to electric stimulation at 2, 4, 8, and 16 Hz were potentiated significantly by yohimbine with the vascular tension of (0.15 ± 0.04), (0.43 ± 0.13), (0.73 ± 0.16), and (1.11 ± 0.19) g in control preparations and (0.39 ± 0.11), (0.82 ± 0.23), (1.10 ± 0.18), and (1.39 ± 0.25) g in yohimbine-treated preparations ($P < 0.05$, $n = 6$).

DISCUSSION

Results of the present study showed that electric stimulation even at 2 Hz induced an obvious neurogenic vasoconstriction in the rabbit saphenous artery, and ATP or a closely related compound was the sole transmitter to produce this neurogenic vasoconstriction. α, β-MeATP potentiates the vascular responses to electric stimulation by the presynaptic mechanism.

The contractile responses to electric stimulation were abolished by treatment with TTX (0.3 μmol/L)[5,6] or guanethidine (0.4 μmol/L)[7], and both the drugs did not inhibit the vasoconstriction induced by NE, indicating that electric stimulation with the parameters used only activated the sympathetic nerves of the saphenous artery. Contractile responses to electric stimulation at 2 Hz were abolished by desensitization of $P2X_1$ receptor with α, β-meATP, and not affected by prazosin. Prazosin inhibited the responses to 8 – 16 Hz stimulation by 39.9 % – 53.8 %, and the treatment with a combination of prazosin and α, β-meATP abolished all the responses to stimulation at 2 – 16 Hz. Furthermore, yohimb ine at a concentration high enough to block α_2-adrenoceptor did not affect the responses to NE. These results demonstrated that ATP might be the sole transmitter released by sympathetic nerves stimulation at 2 Hz to cause the vasoconstriction, and NE and ATP were implicated in the contractile responses to sympathetic nerve stimulation at other frequencies.

Burnstock *et al*[2-4] reported that electric stimulation at 2 – 100 Hz induced vasoconstriction in the rabbit ear artery, however the stimulation at frequencies less than 8 Hz or 4 Hz did not produce obvious vascular

responses in the rabbit hepatic artery or saphenous artery. MacDonald *et al*[8] observed that contractile responses of the rabbit saphenous artery were only induced by stimulation at 35 V and 4 – 64 Hz, and which were inhibited by 50 % with prazosin. The present results, however, indicated that electric stimulation at 2 Hz could produce the purinergic vasoconstriction in the rabbit saphenous artery. Recently, we obtained a similar result in the rabbit splenic artery[5]. Therefore, it was concluded that electric current intensity but not the frequency was the main factor to cause such neurogenic vascular response. Different sensitivities to electric stimulation frequency for producing vascular contractile responses reported among various arteries might not be a precise concept[2-4].

$P2X_1$ receptor has been reported to exist in cultured rat sympathetic neurons, and ATP evokes NE release by acting at the receptors[9]. α,β-MeATP potentiated the release of NE and ACh induced by electric stimulation via $P2X_1$ receptor of the cholinergic and adrenergic nerves in the guinea-pig ileum[10]. Although MacDonald *et al*[8] observed a potentiation by α,β-meATP of the neurogenic vasoconstriction, they did not clarify the possible mechanism involved in the phenomena. In the present study, desensitization of $P2X_1$ receptor with α,β-meATP potentiated the vascular responses to 16 Hz stimulation significantly, and it did not affect the responses to exogenous NE, indicating that α,β-meATP modulated the release of co-transmitters from the sympathetic nerve terminals via presynaptic mechanism. Because there are no satisfied agonists and antagonists of the P receptor subtypes, definite classification of the presynaptic P receptor has not been known for the present[11,12]. A further study needs a development of new agents for P receptor subtypes.

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兔离体隐动脉交感神经共同传递：电刺激的影响及α,β-亚甲基 ATP 的增强作用[1]

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关键词 肾上腺素能纤维；腺苷三磷酸；去甲肾上腺素；电刺激；血管收缩；哌唑嗪；嘌呤类

目的：分析亚最大电压、超短时程电场刺激诱发兔离体隐动脉收缩反应的神经共同传递特征. **方法**：兔离体隐动脉环等长收缩测定法及电场刺激诱发交感神经源性血管收缩法. **结果**：电场刺激诱发隐动脉交感神经源性血管收缩反应，具有频率依赖性. α_1-受体阻断剂哌唑嗪(1 μmol/L)不影响 2 Hz 电刺激诱发的血管收缩反应，对 8 – 16 Hz 电刺激诱发的血管收缩反应抑制约 39.9 – 53.8 %. 另一方面，以

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α,β-亚甲基 ATP (3 μmol/L)脱敏 $P2X_1$ 受体后，完全抑制 2 Hz 电刺激诱发的血管收缩反应，显著增强 16 Hz 电刺激诱发的血管收缩反应，却不影响外源性 NE 的累积量效曲线．联合应用哌唑嗪(1 μmol/L)和 α,β-亚甲基 ATP (3 μmol/L)完全抑制了各频率电刺激诱发的血管收缩反应．**结论**：2 Hz 电刺激亦可诱发兔离体隐动脉神经源性收缩反应，2 Hz 电刺激诱发的收缩反应仅与嘌呤能神经递质 ATP 或相关核苷酸有关．较高频率电刺激诱发的收缩反应则与 ATP 和 NE 两种递质有关．此外，α,β-亚甲基 ATP 使 $P2X_1$ 受体脱敏，并通过突触前机制增强电刺激诱发的血管收缩反应．

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