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Distribution of functional $P2X_1$-like receptor in isolated rabbit arteries[1]

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KEY WORDS arteries; purinergic P2 receptors; rabbits; muscle contraction

ABSTRACT

AIM: To clarify the distribution of functional $P2X_1$-like receptors in rabbit arteries. **METHODS:** Isometric contractile responses to noradrenaline (NA) and α,β-methylene ATP (α,β-meATP) were observed in the arteries isolated from rabbit renal (Re), femoral (Fe), saphenous (Sa), mesenteric (Me), splenic (Sp), and ear (Ea). **RESULTS:** The maximal responses to NA ($E_{max \cdot NA}$) varied among the arteries in an order of Re>Fe>Sa>Me>Sp>Ea. After standardization by the maximal response to KCl ($E_{max \cdot KCl}$), however, the values of $E_{max \cdot NA}/E_{max \cdot KCl}$ in the six kinds of arteries were almost the same. EC_{50} values of NA in arteries were different, and the EC_{50} value of NA in Me artery was 54 times that of Fe artery ($P<0.01$). The maximal response to α,β-meATP ($E_{max \cdot \alpha\beta\text{-meATP}}$) varied among the arteries in an order of Re>Sa=Fe>Ea>Sp=Me, and the values of $E_{max \cdot \alpha\beta\text{-meATP}}/E_{max \cdot KCl}$ were still different (Fe<Re<Sa=Ea=Sp=Me). The EC_{50} values of α,β-meATP in regional arteries were almost the same (0.23–0.77 mmol/L). The vasoconstrictive responses induced by α,β-meATP in the Re, Fe, Sa, Me, Sp, and Ea arteries were subject to tachyphylaxis. **CONCLUSION:** There are functional $P2X_1$-like receptors in the six kinds of arteries used, and the vasoconstrictive responses regulated via the receptors are Fe<Re<Sa=Ea=Sp=Me, which is consistent with the sympathetic innervation of the arteries.

INTRODUCTION

The neurogenic vasoconstrictive responses induced by electrical field stimulation consist of purinergic (prazosin-resistant) component and adrenergic (prazosin-sensitive) component in many different regional blood vessels including the rabbit ear[1], saphenous[2], hepatic[3] and splenic artery[4]. Adenosine 5'-triphosphate (ATP) is considered as the purinergic cotransmitter released together with the classical transmitter of noradrenaline (NA) from the sympathetic nerve endings supplying those arteries[1-4]. The proportion of purinergic component to adrenergic component varied considerably among various arteries. In the rabbit ear artery the prazosin-resistant (purinergic) component is less apparent in comparison with the adrenergic component[1], however, a large component of the vasocon-

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striction in the rabbit saphenous artery or splenic artery in response to sympathetic nerve stimulation is purinergic component[2-4]. Regional differences of endogenous ATP release in the rabbit arteries have been reported[5]. But the contribution of functional $P2X_1$-like receptors to the rabbit regional vascular smooth muscle has not been known.

The present study was designed to investigate the contribution of functional α_1-adrenoceptor and $P2X_1$-like receptor to the vascular smooth muscle by observing contractile responses to NA (α-adrenoceptor agonist) and α,β-methylene ATP (α,β-meATP, $P2X_1$ receptor agonist[6]) in the rabbit isolated renal, femoral, saphenous, mesenteric, splenic, and ear arteries. In this study, we first reported the α,β-meATP-induced contractile responses in the rabbit isolated renal, femoral, and mesenteric arteries.

MATERIALS AND METHODS

Rabbits Male New-Zealand white rabbits (2.5–3.5 kg) were obtained from Experimental Animal Center of Hebei Medical University (Certificate № 0059).

Chemicals α,β-Methylene adenosine 5'-triphosphate (α,β-meATP), (–)-noradrenaline (NA), and acetylcholine (ACh) were all obtained from Sigma Chemical Co. All drugs were dissolved in distilled water.

Arterial preparation Rabbits were stunned by a blow, then exsanguinated. The renal (Re), femoral (Fe), saphenous (Sa), mesenteric (Me), splenic (Sp), and ear (Ea) arteries were 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[7]. Ring segments (4-mm in length) with or without endothelium 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). Optimal preloads of 3.0 g, 3.0 g, 2.5 g, 1.5 g, 2.0 g and 2.0 g were applied to the Re, Fe, Sa, Me, Sp, and Ea arterial segments, respectively. The preparations were allowed to equilibrate for 1 h in physiological solution of the following composition (mmol/L)[4]: 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. A successful removal of the arterial endothelium was confirmed by the loss of relaxation response to ACh (1 mmol/L) in precontracted arterial rings[8].

Drug administration NA (0.001–100 mmol/L) was added cumulatively to the organ bath to construct concentration-response curves. The concentration-response curve for NA was repeated twice in each preparation at 35 min interval. The first set of data was not used in analysis.

A selective $P2X_1$ receptor agonist α,β-meATP (0.03–10 mmol/L) was added non-cumulatively at 1 h intervals[4], because it rapidly desensitized its own receptor[6]. Only one concentration-response curve for α,β-meATP was generated per preparation. α,β-MeATP 3 mmol/L was added to the preparation 1 h before constructing the concentration-response curve for α,β-meATP. A single concentration (120 mmol/L) of KCl was added to the preparation in the end of each experiment in order to standardize the vasoconstrictive responses induced by NA or α,β-meATP in the different arteries[9,10]. In the experiment of desensitizing $P2X_1$-like receptor related response, the preparations of the Re, Fe, Sa, Me, Sp, and Ea arteries were exposed to 1 μmol/L α,β-meATP for 20 min, and then the second administration of the same concentration of α,β-meATP was repeated in the preparations without washing.

Statistical analysis Data were expressed as mean±SD. The EC_{50} values were calculated with the equation: $\lg E/(E_{max}-E)=\lg C-\lg K$ (E, response; E_{max}, maximal response; C, agonist concentration; K, equilibrium dissociation constant). Duncan's multiple range test (a computer program of PHARM/PCS-Version 4) was used to evaluate the differences of E_{max} or EC_{50} values of NA and α,β-meATP in the six arteries. If the statistic test was significant, we compared the individual datum by unpaired t-test. P values less than

0.05 were considered statistically significant.

RESULTS

Contractile responses to KCl in the arteries with or without endothelium The maximal contractile responses to KCl 120 mmol/L ($E_{max \cdot KCl}$) in the Sa, Sp, and Ea arteries without endothelium were significantly increased, in comparison with the arteries with endothelium (Fig 1). Endothelium removal did not modify the contraction in Re, Fe, and Me arteries (Fig 1). In the arteries without endothelium, the order of KCl-induced contraction magnitude was Re>Fe>Sa> Me>Sp>Ea (*t* tests).

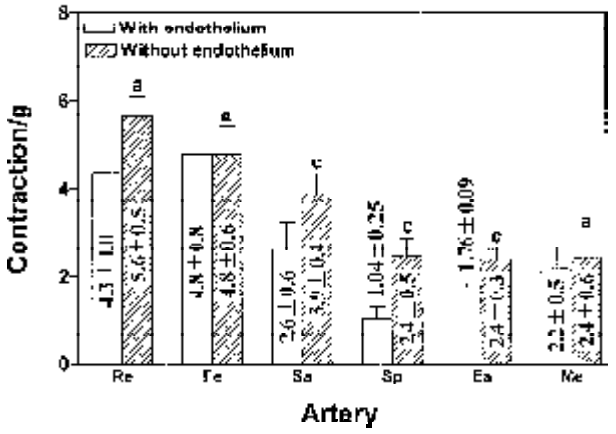

Fig 1. Maximal contractile responses to KCl (120 mmol/L) in the rabbit renal (Re), femoral (Fe), saphenous (Sa), splenic (Sp), ear (Ea), and mesenteric (Me) arteries with or without endothelium. *n*=5–10. Mean±SD. [a] *P*>0.05, [c] *P*<0.01 *vs* the arteries with endothelium.

Contractile responses to NA in the arteries without endothelium NA (0.001–100 μmol/L) produced vasoconstrictive responses in Re, Fe, Sa, Me, Sp, and Ea arteries in a concentration-dependent manner (Fig 2). The maximal contractile responses to NA ($E_{max \cdot NA}$) in the six arteries were significantly different in the contraction magnitude, Re>Fe>Sa>Me>Sp>Ea (*t*-tests). After standardization of $E_{max \cdot NA}$ by $E_{max \cdot KCl}$, however, the values of $E_{max \cdot NA}/E_{max \cdot KCl}$ were almost the same in the six arteries (P>0.05, Duncan's new multiple range test, Tab 1). On the other hand, the EC_{50} value of NA in Me artery was 54 times that of Fe artery (P<0.01, Tab 1).

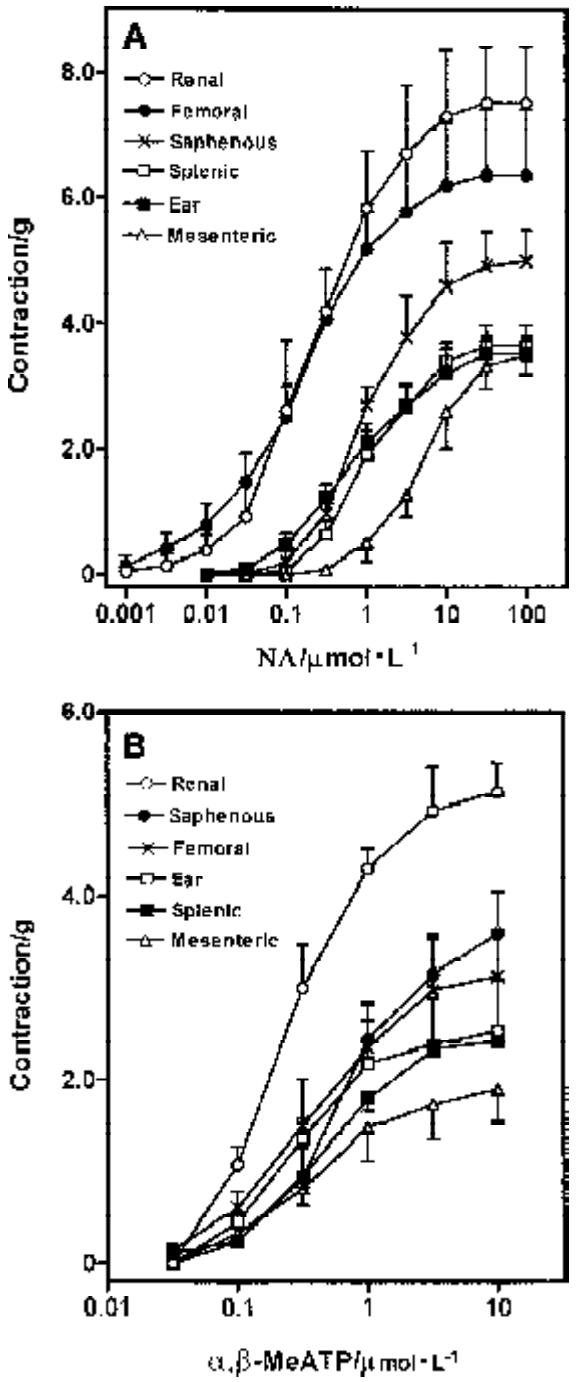

Fig 2. Concentration dependent response curves for NA (A, *n*=6) and a , b -meATP (B, *n*=4) in the rabbit renal, femoral, saphenous, splenic, ear, and mesenteric arteries without endothelium. Mean±SD.

Contractile responses to a , b -meATP in the arteries without endothelium α,β-MeATP (0.03–10 μmol/L) produced vasoconstrictive responses in Re, Fe, Sa, Me, Sp, and Ea arteries in a concentration-dependent manner (Fig 2). The maximal contractile responses

Tab 1. Values of EC_{50} and E_{max} for NA or a , b -meATP in the rabbit arteries without endothelium. *n*=6 for NA. *n*=4 for a , b -meATP. Mean±SD.

Artery	NA			α,β-MeATP		
	E_{max} (g)	$E_{max\cdot NA}/E_{max\cdot KCl}$ (%)	EC_{50} (μmol·L^{-1})	E_{max} (g)	$E_{max\cdot \alpha,\beta\text{-meATP}}/E_{max\cdot KCl}$ (%)	EC_{50} (μmol·L^{-1})
Renal	7.6±0.9	140±17	0.33±0.12	5.2±0.3	87.6±1.0	0.23±0.04
Femoral	6.4±1.1	137±17	0.17±0.05	3.3±0.5	67±8	0.45±0.10
Saphenous	5.1±0.6	130±19	1.4±0.4	3.8±0.5	99±3	0.773±0.019
Mesenteric	4.0±0.4	155±23	9.2±1.8	2.0±0.4	98±5	0.44±0.09
Splenic	3.78±0.21	162±27	1.4±0.3	2.6±0.6	105±11	0.279±0.014
Ear	3.59±0.5	155±13	0.93±0.19	2.67±0.10	110±7	0.47±0.07

to α,β-meATP ($E_{max\cdot \alpha\beta\text{-meATP}}$) in the six arteries were different in the contraction magnitude, Re>Sa=Fe>Ea>Sp=Me (*t*-tests). After standardization of $E_{max\cdot \alpha\beta\text{-meATP}}$ with $E_{max\cdot KCl}$, however, the values of $E_{max\cdot \alpha\beta\text{-meATP}}/E_{max\cdot KCl}$ were still different, Fe<Re<Sa=Ea=Sp=Me (*t*-tests). The EC_{50} values of α,β-meATP in the six arteries were 0.23–0.77 mmol/L (Tab 1).

Vasoconstrictive responses (g) induced by the first exposure to α,β-meATP 3 μmol/L, which was added to the organ bath before constructing the concentration-response curve, were 4.9±0.7, 2.8±0.6, 3.0±0.3, 1.6±0.4, 2.4±0.5, and 2.35±0.06 in the Re, Fe, Sa, Me, Sp, and Ea arteries, and which were not significantly different from the vascular responses (g) induced by the second exposure to α,β-meATP 3 mmol/L in the values of 4.9±0.5, 3.0±0.6, 3.2±0.4, 1.7±0.4, 2.4±0.5, and 2.33±0.05 (*n*=4, *P*>0.05).

Contractile responses to KCl in the arteries without endothelium for both groups of NA and a , b -meATP After constructing the concentration-dependent response curves for NA or α,β-meATP in each experiment with six kinds of arteries, the contractile responses to KCl 120 mmol/L were observed in order to standardize the vascular responses to agonists NA or α,β-meATP. The vasoconstrictive responses (g) induced by KCl (120 mmol/L) between the two experimental groups (NA and α,β-meATP) in the six kinds of arteries were not significantly different from each other (*P*>0.05), and which were 5.4±0.4, 4.7±0.7, 3.9±0.4, 2.6 ±0.5, 2.35±0.23, and 2.3±0.4 (*n*=6) in NA group; and 5.9±0.4, 4.9±0.6, 3.8±0.6, 2.0±0.4, 2.5±0.7, and 2.4±0.1 (*n*=4) in α,β-meATP group.

Contractile responses to a , b -meATP in the arteries before and after desensitizing $P2X_1$-like receptor In the preparations of the Re, Fe, Sa, Me, Sp and Ea arteries without endothelium, α,β-meATP 1 mmol/L caused obvious contractile responses (g) in the values of 4.4±0.3, 2.3±0.4, 2.5±0.3, 1.5±0.4, 2.0±0.6, and 1.70±0.22 (*n*=4), respectively. The contractile responses to α,β-meATP diminished rapidly in the unwashed-preparations continually exposed to the first administration of α,β-meATP for 20 min, and the second administration of the same concentration of α,β-meATP did not produce any responses (*n*=4).

DISCUSSION

It is widely accepted that the $P2X_1$-like receptor seems to be the most important P2X subtype in the vascular smooth muscle[11], though this concept is not a result from functional study. Nori *et al*[12] observed the coexpression of mRNAs of three P2X receptor subtypes ($P2X_1$, $P2X_2$, and $P2X_4$) in the rat vascular smooth muscle. However, it was reported that $P2X_4$ subtype did not couple to a vasomotor response[13], and that $P2X_2$ receptor was mainly located on nerves and arterial endothelial cells, only low density on the smooth muscle cells[14]. Moreover, α,β-meATP was inactive as an agonist at the recombinant $P2X_2$ receptor[15]. EC_{50} val-

ues of α,β-meATP to activate the $P2X_1$ receptor were reported to be approximately 0.5–5 mmol/L[13]. In the present study, the EC_{50} values of α,β-meATP in the six kinds of arteries were 0.23–0.77 mmol/L which were consistent with the reported EC_{50} values of α,β-meATP, and the vasoconstrictive responses induced by α,β-meATP in the Re, Fe, Sa, Me, Sp, and Ea arteries were subject to tachyphylaxis, indicting that the functional P2X receptors in the smooth muscle of the rabbit regional arteries were dominantly $P2X_1$-like subtype.

The vasoconstrictive responses to KCl (120 mmol/L) are usually used to assess the contractile ability of the vascular smooth muscle[9,10,16], and agonists-induced vasoconstrictions are often standardized by $E_{max\cdot KCl}$ in order to eliminate an influence of the different thickness of muscle layer in larger and smaller arteries. In the present experiments, because the vasoconstriction induced by KCl 120 mmol/L in the Sa, Sp, and Ea arteries without endothelium were much bigger than that with endothelium (Fig 1), we used the arterial ring preparations without endothelium. In the end of experiments constructing the concentration-dependent response curves for both NA and α,β-meATP, KCl-induced vasoconstrictive responses ($E_{max\cdot KCl}$) of the same kind of artery in the two experimental groups were not significantly different from each other. Therefore, it was reasonable to standardize the responses to NA or α,β-meATP with $E_{max\cdot KCl}$ in the present study.

The values of $E_{max\cdot NA}$ or $E_{max\cdot \alpha\beta\text{-meATP}}$ varied significantly among the regional arteries, ie, Re>Fe>Sa>Me>Sp>Ea arteries for NA or Re>Sa=Fe>Ea>Sp=Me arteries for α,β-meATP. When the vascular responses were standardized by $E_{max\cdot KCl}$, however, the values of $E_{max\cdot NA}/E_{max\cdot KCl}$ were almost the same in the six kinds of arteries (Tab 1). On the other hand, the values of $E_{max\cdot \alpha\beta\text{-meATP}}/E_{max\cdot KCl}$ were still different (Fe<Re<Sa=Ea=Sp=Me).

It has been known that the releases of NA induced by electrical field stimulation from Ea, Fe, Re, and pulmonary (Pu) arteries of the rabbit are different in characteristics from the releases of ATP[5]. The amounts of NA released by electrical stimulation from the four arteries were almost equal, but the amounts of ATP released by electrical stimulation varied significantly, Ea>Re>Fe>>Pu[5]. Moreover, it is well known that the density of sympathetic innervation increases with the decrease in lumen diameter of artery[17]. In the present experiments, the standardized maximal responses to α,β-meATP in the large arteries (Fe and Re) were significantly smaller than those in smaller arteries (Sa, Ea, Sp, and Me), which was consistent with the experimental results obtained from both transmitter release study[5] and histological study[17].

In contrast with the EC_{50} values of α,β-meATP, those of NA in the rabbit regional arteries were not the same, eg, the EC_{50} values in Me artery was 54 times that of Fe artery. Satoh *et al*[18] reported that there were different EC_{50} values of NA to produce vasoconstrictive responses in the rabbit aortic, mesenteric, renal, and iliac arteries, indicating an existence of different α_1 adrenoceptor subtypes, and they further confirmed that in addition to α_{1A} and α_{1B} subtypes, other subtypes such as α_{1D} existed in the rabbit arteries. Similar results had been obtained from canine arteries[19] and veins[20]. Hence, functional $P2X_1$-like receptor holds an important action in the control of blood pressure, especially in the rapid and powerful vasoconstriction of the defense reaction.

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功能性 $P2X_1$ 样受体在离体兔动脉的分布[1]

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关键词 动脉；嘌呤能 P2 受体；兔；肌收缩

目的：研究功能性 $P2X_1$-like 受体在兔 6 种动脉平滑肌的分布. 方法：观察兔离体肾动脉(Re)、股动脉(Fe)、隐动脉(Sa)、肠系膜动脉(Me)、脾动脉(Sp)和耳动脉(Ea)环的收缩反应. 结果：NA 的最大收缩反应($E_{max\cdot NA}$)值为 Re>Fe>Sa>Me=Sp>Ea；经氯化钾最大收缩反应($E_{max\cdot KCl}$)标准化后，NA 的标准化最大收缩反应($E_{max\cdot NA}/E_{max\cdot KCl}$)值，在 6 种血管基本一致. α,β-Methylene ATP 最大收缩反应($E_{max\cdot \alpha\beta\text{-meATP}}$)值为 Re>Sa=Fe>Ea=Sp=Me；经 $E_{max\cdot KCl}$ 标准化后，α,β-methylene ATP 的标准化最大收缩反应($E_{max\cdot \alpha,\beta\text{-meATP}}/E_{max\cdot KCl}$)数值各血管仍不同，Fe<Re<其他四种动脉. α,β-Methylene ATP 在六种动脉诱发的收缩反应具有快速耐受性. 结论：结果提示 6 种动脉中，功能性 P2X 受体为 $P2X_1$-like 受体亚型. 该受体调节的收缩反应强度为，Fe<Re<其他 4 种动脉.

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