Physiological significance of P2X receptor-mediated vasoconstriction in five different types of arteries in rats

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Received: 29 October 2010 / Accepted: 1 March 2011 / Published online: 11 May 2011
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Abstract P2X₁ receptors, the major subtype of P2X receptors in the vascular smooth muscle, are essential for α,β -methylene adenosine 5'-triphosphate (α,β -MeATP)-induced vasoconstriction. However, relative physiological significance of P2X₁ receptor-regulated vasoconstriction in the different types of arteries in the rat is not clear as compared with α_1 -adrenoceptor-regulated vasoconstriction. In the present study, we found that vasoconstrictive responses to noncumulative administration of α,β -MeATP in the rat isolated mesenteric arteries were significantly smaller than those to single concentration administration of α,β -MeATP. Therefore, we firstly reported the characteristic of α,β -MeATP-regulated vasoconstrictions in rat tail, internal carotid, pulmonary, mesenteric arteries, and aorta using single concentration administration of α,β -MeATP. The rank order of maximal vasoconstrictions for α,β -MeATP ($E_{\max,\alpha,\beta\text{-MeATP}}$) was the same as that of maximal vasoconstrictions for noradrenaline ($E_{\max,\text{NA}}$) in the internal carotid, pulmonary, mesenteric arteries, and aorta. Moreover, the value of $(E_{\max,\alpha,\beta\text{-MeATP}}/E_{\max,\text{KCl}})/(E_{\max,\text{NA}}/E_{\max,\text{KCl}})$ was 0.4 in each of the four arteries, but it was 0.8 in the tail artery. In conclusion, P2X₁ receptor-mediated vasoconstrictions are equally important in rat internal carotid,

pulmonary, mesenteric arteries, and aorta, but much greater in the tail artery, suggesting its special role in physiological function.

Keywords α,β -Methylene ATP · Noradrenaline · P2X₁ receptor · α_1 -Adrenoceptor · Isolated artery · Vasoconstriction · Rat

Introduction

The sympathetic purinergic co-transmission involving noradrenaline (NA) and adenosine 5'-triphosphate (ATP) is present in a variety of blood vessels. Both purinergic (prazosin-resistant) component and adrenergic (prazosin-sensitive) component were shown to involve the neurogenic vasoconstriction induced by electrical field stimulation in different types of blood vessels including the rabbit ear [1], saphenous [2], hepatic [3], and splenic artery [4]. However, the ratio of NA:ATP released by the sympathetic nerves supplying different blood vessels is variable. The purinergic component is relatively minor (~10%) in rat tail artery [5], whereas nerve-mediated responses are largely purinergic in rabbit splenic artery [4]. In rabbit mesenteric artery ATP is the sole excitatory transmitter, although NA is released and contributes to feedback inhibition [6].

P2 receptors are widely distributed in the cardiovascular system and are important in the regulation of vascular tone [7]. The P2X₁ receptor is the principal P2X receptor subtype expressed on most vascular smooth muscles [8, 9] and responsible for the purine nucleotide-induced arterial contraction [10, 11]. α,β -MeATP, a stable analogue of ATP, evoked transient constrictions of the femoral, tail, uterine, and large mesenteric arteries taken from the wild-type mice, but these responses were abolished in the arteries taken

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from P2X₁ receptor-deficient mice [11]. Thus, α,β -MeATP has been considered as a useful agent to investigate P2X₁ receptor-mediated vasoconstriction [12]. However, an appropriate administration regime of α,β -MeATP in vitro using isolated arteries has not been yet evaluated carefully, because the agent causes profound desensitization of P2X₁ receptors. Previous studies showed the noncumulative concentration–response curve for α,β -MeATP when α,β -MeATP was repetitively applied to the rat mesenteric artery and thoracic aorta at the time interval of 10–20 min [10, 13]. However, some investigators used a single concentration of α,β -MeATP (each arterial segment was only exposed to α,β -MeATP once) in the rat mesenteric artery and thoracic aorta to obtain the concentration–response curve [14, 15]. Moreover, α,β -MeATP was even added to the organ bath in a cumulative manner to obtain a concentration–response curve in the rat thoracic aorta and pulmonary artery [16, 17].

In pilot experiments using isolated rat mesenteric artery, we noted that vasoconstrictive response to a single concentration of α,β -MeATP was much bigger than that during noncumulative administration of α,β -MeATP, indicating a possible pharmacological artifact reported in the published studies where cumulative or noncumulative administration of α,β -MeATP was applied. In this study, we compared the vasoconstrictive response to a single concentration of α,β -MeATP with that to noncumulative administration of α,β -MeATP in rat mesenteric artery rings. In addition, we investigated the differences in α,β -MeATP-induced vasoconstrictions in rat-isolated thoracic aorta, pulmonary, internal carotid, mesenteric, and caudal arteries, and we compared the differences between P2X₁ receptor-induced and α_1 -adrenoceptor-induced vasoconstrictive responses in above rat arteries.

Materials and methods

Animals

Male Wistar rats (300–350 g) were provided by the Laboratory Animal Center of Hebei Medical University (China). Rats were given the standard laboratory chow and tap water ad libitum and were kept in 12 h light/12 h dark cycle in the animal care facility. All animals used in the present study received humane care in compliance with institutional animal care guidelines. All procedures were approved by the Local Institutional Committee.

Chemicals

[–]-Noradrenaline (NA) bitartrate, α,β -MeATP (lithium salt), desmethylinipramine hydrochloride, deoxycorticoster-

terone acetate, yohimbine hydrochloride, propranolol hydrochloride, and acetylcholine hydrochloride were obtained from Sigma Chemical. Deoxycorticosterone acetate was dissolved in 1,2-propanediol. The final concentration of 1,2-propanediol in the tissue bath did not affect the vascular responses to NA. Other agents were dissolved in distilled water.

Arterial preparations

Rats were anesthetized by hypodermic injection of urethane (1.5 g/kg), then killed by cutting femoral artery, resulting in exsanguination. After chest and abdomen were opened by a midline incision, thoracic aorta, right pulmonary arteries, and superior mesenteric artery were carefully removed. An anterior midline incision was made on the neck, and the carotid arteries were exposed. The left and right internal carotid artery was isolated and exposed from its origin at the common carotid artery bifurcation to its entry point into the skull. The caudal artery was surgically exposed from the ventral side, then dissected free from surrounding tissues and removed. Isolated arteries were maintained in ice-cold Krebs–Henseleit (K–H) solution containing (mmol/l) [4]: NaCl 133, KCl 4.7, NaH₂PO₄ 1.35, NaHCO₃ 16.3, MgSO₄ 0.61, glucose 7.8, and CaCl₂ 2.52. The vascular endothelium was removed by gently rubbing the lumen with a scored polythene cannula (outside diameter 0.2–2.5 mm), the external diameter of which was slightly smaller than the internal diameter of the blood vessel [18]. Ring segment (4 mm long) without endothelium was mounted horizontally in a 10 ml organ bath by carefully inserting tungsten wire through the lumen of the arterial ring preparation and anchoring it to a stationary support. Another tungsten wire similarly inserted was connected to an isometric tension transducer coupled to a polygraph (ERT-884, Youlin Electron, Kaifeng, China) [19, 20] to record a change in tension of the preparation. Preloads were applied to the preparations of aorta (2.0 g) [21], pulmonary artery (1.0 g) [22], internal carotid artery (1.0 g), mesenteric artery (1.0 g) [23], and tail artery (0.75 g) [24]. The preparations were allowed to equilibrate for 1 h in K–H solution. The solution was maintained at 37°C and aerated with 95% O₂ and 5% CO₂ (pH 7.4). A successful removal of the arterial endothelium was confirmed by the loss of relaxation response to acetylcholine (ACh, 1 μ mol/l) in precontracted arterial rings with NA [25].

Experimental protocols

In each of the arterial preparations, the following three procedures were performed. A cumulative concentration–response curve for NA (0.0001–100 μ mol/l) was firstly constructed to test the vasoconstrictor responsiveness of the

preparation. Then a further equilibration for 1 h was needed. At the end of the isolated arterial experiments, a cumulative concentration–response curve for KCl (10–120 mmol/l) was constructed, and the wet weight of each preparation was measured.

Vasoconstrictive responses to α,β -MeATP administered in different manners in the mesenteric artery

To avoid the rapid desensitization of P2X₁ receptors by α,β -MeATP [26], we tested the two administration regimes in the following experiments as previously described [4, 27]. In five mesenteric arterial preparations, α,β -MeATP (0.1, 1.0, 10, and 100 μ mol/l) was added noncumulatively to the organ bath at 1 h intervals, and a concentration–response curve for α,β -MeATP was generated per preparation [4, 27]. When the vasoconstrictive response to the last dose reached the maximum, the preparation was thoroughly washed. In other 20 mesenteric arterial preparations, a single concentration of α,β -MeATP at 0.1, 1.0, 10, or 100 μ mol/l was added to the organ bath, respectively (each arterial preparation was only exposed to α,β -MeATP once), and the resultant responses of several preparations exposed to different concentrations were grouped together to form a concentration–response curve [14, 15]. When each vasoconstrictive response to α,β -MeATP reached its maximal height, the preparation was thoroughly washed.

Vasoconstrictive responses to NA in the thoracic aorta and pulmonary, internal carotid, mesenteric, and caudal arteries

In all arterial preparations, desmethylinipramine (0.1 μ mol/l), deoxycorticosterone (5 μ mol/l), yohimbine (0.3 μ mol/l), and propranolol (1 μ mol/l) were added to the bath solution for 30 min to block neuronal and extra neuronal uptake of NA, and α_2 - and β -adrenoceptors, respectively [28]. Then, a second cumulative concentration–response curve for NA (0.0001–100 μ mol/l) was constructed in each arterial preparation of the pulmonary, internal carotid, mesenteric, and caudal arteries, and aorta to observe α_1 -adrenoceptor-induced vasoconstriction.

Vasoconstrictive responses to single concentration of α,β -MeATP in the thoracic aorta and pulmonary, internal carotid, and caudal arteries

In each arterial preparation of the four different arteries, a single concentration of α,β -MeATP at 0.1, 1.0, 10, or 100 μ mol/l was added to the organ bath (each arterial preparation was only exposed to α,β -MeATP once), and the responses of several preparations exposed to different

concentrations were grouped together to form a concentration–response curve.

Statistics

Vasoconstrictive responses to NA and α,β -MeATP were expressed as the maximal change in tension (g), as a value normalized to tissue wet weight (g/mg tissue), or as a percentage of the maximal vasoconstriction to KCl (% KCl). Values presented here are the mean $\pm$ S.E. mean. Two-way analysis of variance was used to compare any differences between two sets of concentration-dependent response curves. If *F* statistic was significant, we further compared the individual datum with its respective control value using Bonferroni's test. The log EC₅₀ values of NA and α,β -MeATP producing concentration-dependent vasoconstrictions were analyzed and calculated by nonlinear regression (curve fit) of Prism 5.00. In order to estimate EC₅₀ values of KCl, we drew a line graph with mmol/l of KCl as *X*-axis and percent (responses) as *Y*-axis in each of the preparations and estimated a value of EC₅₀ on the *X*-axis (intercept on *X*-axis) for the concentration–response curve of KCl in each preparation. We compared the maximal vasoconstriction to KCl, the tissue wet weight of arterial preparations and log EC₅₀ between two groups using unpaired *t* test. *P* values < 0.05 were considered statistically significant. The data were analyzed using GraphPad Prism version 5.00 (San Diego, CA, USA).

Results

Vasoconstrictive responses to α,β -MeATP administered in different manners in the mesenteric artery

Before exposing the mesenteric artery to α,β -MeATP, the concentration–response curve for NA in preparations used in the group where single concentration of α,β -MeATP (single concentration administration group, SCAG) was subsequently applied was not significantly different from that in the group of noncumulative administration of α,β -MeATP (noncumulative administration group, NCAG) (*P* > 0.05, Fig. 1). In addition, no significant differences in wet weight of the mesenteric arterial ring segments were observed in the two groups (SCAG, 0.8 $\pm$ 0.02 mg, *n* = 20; NCAG, 0.8 $\pm$ 0.1 mg, *n* = 5; *P* > 0.05). However, the concentration–response curve for α,β -MeATP in the preparations exposed to a single concentration of the agent was significantly different from that exposed to a noncumulative administration of the agent (*P* < 0.05, Fig. 2). When the vasoconstrictive responses were normalized to tissue wet weight, vasoconstrictive responses to 100 μ mol/l α,β -

Fig. 1 Vasoconstrictive responses of the mesenteric arteries to noradrenaline in single concentration administration [α, β -methylene ATP (α, β -MeATP)] group [SCAG (α, β -MeATP), $n=20$] and in noncumulative administration (α, β -MeATP) group [NCAG (α, β -MeATP), $n=5$]. Points represent the mean values, and vertical bars show S.E. mean

MeATP administered in single concentration manner and noncumulative manner were 0.7 ± 0.04 g/mg tissue and 0.4 ± 0.1 g/mg tissue, respectively ($P < 0.05$, Fig. 2a). However, when the vasoconstrictive responses were normalized to the maximal contraction of 120 mmol/l KCl, α, β -MeATP (100 $\mu\text{mol/l}$)-induced vasoconstrictive responses were not significantly different between SCAG ($48.6 \pm 5.2\%$) and NCAG ($38.0 \pm 2.5\%$; $P > 0.05$, Fig. 2b). The $-\log \text{EC}_{50}$ (mol/l) value of α, β -MeATP in SCAG (5.5 ± 0.2 ; $n=20$) was not significantly different from 5.2 ± 0.2 in NCAG ($n=5$; $P > 0.05$).

At the end of the experiments, the vasoconstrictive responses to KCl in preparations used in the group of single concentration of α, β -MeATP were significantly greater than that of noncumulative administration of α, β -MeATP ($P < 0.05$ and $P < 0.01$, Fig. 3). The EC_{50} (mmol/l) value of KCl in NCAG was 38.1 ± 3.1 ($n=5$), significantly greater than 30.1 ± 0.8 in SCAG ($n=20$, $P < 0.01$). Moreover, both the EC_{50} values of KCl were significantly greater than EC_{50} value of KCl in the preparations exposed to a second cumulative administration of NA ($P < 0.01$, Table 1).

Vasoconstrictive responses to NA in the thoracic aorta and pulmonary, internal carotid, mesenteric, and caudal arteries

The second cumulative concentration–response curves for NA (0.0001–100 $\mu\text{mol/l}$) were constructed in each arterial preparation of the thoracic aorta and pulmonary, internal carotid, mesenteric, and caudal arteries. NA produced

Fig. 2 Vasoconstrictive responses to α, β -methylene ATP expressed as g/mg tissue (a) or as percentage of the maximal vasoconstriction to 120 mmol/l KCl (b) in the mesenteric arteries used in the groups of single concentration administration ($n=20$) and noncumulative administration ($n=5$). Points represent the mean values, and vertical bars show S.E. mean. * $P < 0.05$ and ** $P < 0.01$ vs. single concentration

Fig. 3 Vasoconstrictive responses to KCl in the mesenteric arteries used in single concentration administration [α,β -methylene ATP (α,β -MeATP)] group [SCAG (α,β -MeATP), $n=20$] and in noncumulative administration (α,β -MeATP) group [NCAG (α,β -MeATP), $n=5$]. Points represent the mean values, and vertical bars show S.E. mean. * $P<0.05$ and ** $P<0.01$ vs. SCAG (α,β -MeATP)

vasoconstrictive responses in a concentration-dependent manner in these different types of arteries. When the vasoconstrictive responses to NA were normalized to the tissue wet weight (g/mg tissue), the rank order of the maximal vasoconstrictive responses ($E_{\max}$) was caudal > mesenteric > internal carotid > pulmonary = aorta (Fig. 4a). In addition, when the vasoconstrictive responses to NA were normalized to the maximal vasoconstriction induced by KCl (% KCl $E_{\max}$), the rank order of the $E_{\max}$ was internal carotid > aorta > pulmonary = mesenteric > caudal (Fig. 5a). The range of $-\log EC_{50}$ (mol/l) values for NA in the five types of arteries was 6.9–7.8, and the rank order was caudal = mesenteric < internal carotid < aorta = pulmonary (Table 1).

Table 1 The $-\log EC_{50}$ (mol/l) for NA and α,β -MeATP as well as EC_{50} (mmol/l) for KCl in various isolated arterial preparations

Artery	NA group ($n=8$)		α,β -MeATP group ($n=20$)	
	NA	KCl	α,β -MeATP	KCl
Caudal (C)	6.9 ± 0.1	29.9 ± 1.4	5.9 ± 0.2	$41.8 \pm 0.4^{@@}$
Mesenteric (M)	7.0 ± 0.1	$24.1 \pm 0.4^{**}$	5.5 ± 0.2	$30.1 \pm 0.8^{@@}$
Internal carotid (I)	$7.4 \pm 0.1^{***\#}$	$22.7 \pm 1.2^{***\#}$	$5.1 \pm 0.2^{**}$	$28.4 \pm 1.3^{@}$
Aorta (A)	$7.6 \pm 0.1^{***\Delta}$	$19.3 \pm 0.6^{***\Delta}$	$4.9 \pm 0.1^{***\Delta}$	$22.7 \pm 0.4^{@@}$
Pulmonary (P)	$7.8 \pm 0.1^{***\Delta}$	$16.8 \pm 1.0^{***\Delta\Delta}$	$5.1 \pm 0.1^{***\Delta}$	$23.2 \pm 0.3^{@@}$

* $P<0.05$, ** $P<0.01$ vs. C;

Δ $P<0.05$, $\Delta\Delta$ $P<0.01$ vs. M;

$P<0.05$ vs. P; @ $P<0.05$,

@@ $P<0.01$ vs. NA group

Vasoconstrictive responses to a single concentration of α,β -MeATP in the thoracic aorta and pulmonary, internal carotid, mesenteric, and caudal arteries

α,β -MeATP produced a concentration-dependent vasoconstrictive responses in the thoracic aorta and pulmonary, internal carotid, mesenteric, and caudal arteries (each arterial preparation was only exposed to α,β -MeATP once). There were no significant differences for the wet weight of preparations used between NA and α,β -MeATP experiments ($P>0.05$, Fig. 6a). The maximal vasoconstrictive response to KCl in each type of the preparations used in the NA experiments was not significantly different from that used in the α,β -MeATP experiments ($P>0.05$, Fig. 6b). The rank order of the $E_{\max}$ was caudal > mesenteric > internal carotid > pulmonary = aorta when the vasoconstrictive responses to α,β -MeATP were normalized to tissue wet weight (g/mg tissue; Fig. 4b), whereas it was internal carotid = caudal > aorta > pulmonary = mesenteric when normalized to maximal vasoconstriction induced by KCl (% KCl $E_{\max}$; Fig. 5b). The range of $-\log EC_{50}$ (mol/l) values for α,β -MeATP in the five types of arteries was 4.9–5.9, and their rank order was pulmonary = aorta = internal carotid < mesenteric = caudal (Table 1). The values of $(E_{\max \cdot \alpha,\beta\text{-MeATP}}/E_{\max \cdot \text{KCl}})/(E_{\max \cdot \text{NA}}/E_{\max \cdot \text{KCl}})$ were 0.8, 0.4, 0.4, 0.4, and 0.4 for the caudal, internal carotid, mesenteric, pulmonary arteries, and aorta, respectively. The range of EC_{50} (mmol/l) values for KCl in the five types of arteries used in NA group was 16.8–29.9, and their rank order was caudal > mesenteric = internal carotid > aorta = pulmonary (Table 1). The EC_{50} values of KCl in the five types of arteries used in NA group were significantly smaller than those in α,β -MeATP group (Table 1).

Discussion

Isometric vasoconstrictive responses to α,β -MeATP were often expressed as a percentage of the contractile response to high KCl solution in order to investigate pharmacological characterization in different types of arteries [13, 29]. However, our present study found that the vasoconstrictive

Fig. 4 Vasoconstrictive responses to noradrenaline (**a** $n=8$) and α,β -methylene ATP (**b** $n=20$, each preparation exposed to α,β -methylene ATP once) expressed as g/mg tissue in the caudal, mesenteric, internal carotid, pulmonary and aortic arteries. Points represent the mean values, and vertical bars show S.E. mean

responses to KCl in the rat isolated mesenteric arteries in noncumulative administration (α,β -MeATP) group were significantly smaller than that in single concentration administration (α,β -MeATP) group or in NA control group. Although the maximal vasoconstrictions induced by KCl in tail, internal carotid, mesenteric, pulmonary arteries, and aorta used in single concentration administration (α,β -MeATP) group were not different from that used in NA control group, EC_{50} values of KCl became significantly

greater in these five types of arteries in single concentration administration (α,β -MeATP) group compared to NA control group. Additionally, the vasoconstrictive responses to α,β -MeATP expressed as g/mg tissue in single concentration administration group were significantly greater than that in noncumulative administration group in the rat-isolated mesenteric arteries. Since the vasoconstrictive responsiveness tested by NA at the beginning of the study in the group of single concentration of α,β -MeATP was not

Fig. 5 Vasoconstrictive responses to noradrenaline (**a** $n=8$) and α,β -methylene ATP (**b** $n=20$, each preparation exposed to α,β -methylene ATP once) expressed as percentage of the maximal vasoconstriction to 120 mmol/l KCl in the internal carotid, aortic, pulmonary, mesenteric and caudal arteries. Points represent the mean values, and vertical bars show S.E. mean

Fig. 6 Tissue wet weight (a) and maximal vasoconstriction to 120 mmol/l KCl (b) of the internal carotid (*Int*), pulmonary (*Pul*), aortic (*Aor*), mesenteric (*Mes*) and caudal (*Cau*) arteries treated with single concentration of α,β -methylene ATP (α,β -MeATP, $n=20$) or with noradrenaline (NA, $n=8$). Columns represent the mean values, and vertical bars show S.E. mean

significantly different from that in the group of noncumulative administration of α,β -MeATP, and there was no significant difference in the wet weight of the mesenteric arterial preparations between these two groups, we suggest that repeated exposure of the rat isolated mesenteric artery to higher concentration of α,β -MeATP obviously suppresses not only $P2X_1$ receptor-mediated vasoconstriction but also KCl-induced vasoconstriction. Noncumulative administration regime is not suitable for α,β -MeATP-induced vasoconstriction, at least in the study of the rat-isolated mesenteric artery. It should be noted that the vasoconstrictive response to α,β -MeATP administered in noncumulative manner was artificially more enlarged when the responses were normalized to the maximal contraction induced by 120 mmol/l KCl compared to the responses expressed as g/mg tissue (Fig. 2). Our experimental results clearly showed that $P2X_1$ receptor-mediated vasoconstrictive responses largely desensitized during noncumulative administration of α,β -MeATP to the rat mesenteric artery. This administration regime also affected KCl-related vasoconstrictive responses.

In this study, we employed a single concentration administration of α,β -MeATP to avoid the profound desensitization of $P2X_1$ receptors and firstly reported the regional characteristic of $P2X_1$ receptor-regulated vasoconstriction in the rat isolated thoracic aorta and pulmonary, internal carotid, mesenteric, and caudal arteries, in comparison with α_1 -adrenoceptor-regulated vasoconstriction. We showed that wet weight of each type preparation of the five regional arteries used in the NA group was not significantly different from that used in the α,β -MeATP group, and there was no significant difference in the vasoconstrictive

responsiveness tested by NA at the beginning of the study in each type of the arteries between the two groups. Moreover, the maximal vasoconstrictive response to KCl of each type preparation of the five regional arteries used in the NA group was not significantly different from that used in the α,β -MeATP group, although the EC_{50} values of KCl became significantly greater in the α,β -MeATP group. The vasoconstrictive response to KCl (120 mmol/l) is usually used to assess the contractile ability of the vascular smooth muscle. Agonist-induced vasoconstrictions are often standardized by $E_{\max\text{-KCl}}$ in order to eliminate an influence of the different thickness of muscle layer in larger and smaller arteries [30–32]. Therefore, it was reasonable to compare the magnitude of the vasoconstriction induced by α,β -MeATP with that induced by NA, regardless of the responses expressed as g/mg tissue or as a percentage of the maximal vasoconstriction to KCl in this study.

When the vasoconstrictive responses to α,β -MeATP and NA in the five regional arteries were expressed as g/mg tissue, both the rank orders of the $E_{\max\text{-}\alpha,\beta\text{-MeATP}}$ and $E_{\max\text{-NA}}$ were the same, i.e., caudal > mesenteric > internal carotid > pulmonary = aorta. In addition, when we normalized the vasoconstrictive response to a maximal contraction of KCl, the rank order of $E_{\max\text{-}\alpha,\beta\text{-MeATP}}$ was caudal = internal carotid > aorta > pulmonary = mesenteric, and the rank order of $E_{\max\text{-NA}}$ was internal carotid > aorta > pulmonary = mesenteric > caudal. Obviously, the rank order of $E_{\max\text{-NA}}$ was the same as that of $E_{\max\text{-}\alpha,\beta\text{-MeATP}}$ in four different arteries of the internal carotid, pulmonary, mesenteric arteries, and aorta, regardless of the vasoconstrictions expressed as g/mg tissue or as a percentage of the maximal vasoconstriction to KCl. Moreover, the

values of $(E_{\max \cdot \alpha, \beta\text{-MeATP}}/E_{\max \cdot \text{KCl}})/(E_{\max \cdot \text{NA}}/E_{\max \cdot \text{KCl}})$ in the internal carotid, mesenteric, pulmonary arteries, and aorta were about 0.4, indicating that α, β -MeATP-mediated vasoconstriction reaches at least 40% of the maximal vasoconstriction to NA in these arteries.

On the other hand, it is widely accepted that P2X₁ receptor seems to be the most important P2X subtype in vascular smooth muscle [8], although this concept is not a result from functional study. Nori et al. [33] observed the coexpression of mRNAs of three P2X receptor subtypes (P2X₁, P2X₂, and P2X₄) in rat vascular smooth muscle. Some previous studies found that P2X₄ subtype did not couple to a vasomotor response [34] and that P2X₂ receptor was mainly located on nerves and arterial endothelial cells, but with low density on the smooth muscle cells [35]. α, β -MeATP was inactive as an agonist at the recombinant P2X₂ receptor [36]. Recently, Wallace et al. [37] investigated the expression of P2X receptor subtypes by immunohistochemistry in the tail and mesenteric arteries of rats aged 4, 6, and 12 weeks. P2X₁ receptor-specific immunoreactivity was associated with the smooth muscle layer of both arteries from rats aged 4, 6, and 12 weeks, and P2X₄ receptor subtype was weakly expressed in the smooth muscle layer of 4-week and 6-week tail artery and 4-week and 12-week mesenteric artery, but other subtypes of P2X receptors were not detected in the smooth muscle layer of 6-week and 12-week arteries [37]. In our study, 12- to 13-week-old rats (300–350 g) were used, and the vascular endothelium of the regional arteries was removed. Therefore, it is likely that P2X₁ receptors are mainly involved in the vasoconstrictive responses to α, β -MeATP in the present study.

Since there was a very high level of consistency between the vasoconstrictions induced by α, β -MeATP and NA in the rat-isolated internal carotid, mesenteric, pulmonary arteries, and aorta, we suggest that the activation of P2X₁ receptor plays an important role in physiological regulation of the vascular tone of these arteries, and the maximal vasoconstrictions mediated via P2X₁ receptors reach at least 40% of those mediated via α_1 -adrenoceptors in the internal carotid, mesenteric, pulmonary arteries, and aorta of the rat. Furthermore, the value of $(E_{\max \cdot \alpha, \beta\text{-MeATP}}/E_{\max \cdot \text{KCl}})/(E_{\max \cdot \text{NA}}/E_{\max \cdot \text{KCl}})$ in the tail artery was 0.8, which was much bigger than the values of other four arteries, indicating much more functional P2X₁ receptors distributed in the rat tail artery. Tail heat loss and blood flow increase with ambient temperature [38] as well as with increasing body temperature in the rat [39]. Directing blood into the tail causes heat loss, while conversely restricting blood flow to the tail reduces heat loss in the cold. The presence of a greater purinergic vasoconstriction in the rat tail artery than other regional arteries might suggest that extracellular nucleotides play an important role in the normal physiological functions such as thermoregulation and balance. In

contrast to other regions of arteries, P2X₁ receptor-mediated vasoconstriction in rat tail artery is unique not only in its potent vasoconstrictive feature but also in its higher affinity to α, β -MeATP.

In the isolated vascular experiments, vasoconstrictive responses were often normalized to the vasoconstriction induced by KCl which was applied at the beginning of experiments in previous studies [13, 29, 40, 41]. The main issue in present study is whether KCl administration at the beginning of experimentation affects vasoconstrictive responses to α, β -MeATP. However, our present study did not show any evidence about the influence of an initial administration of KCl on responses to α, β -MeATP, even though we found that initial administration of α, β -MeATP significantly changed the EC₅₀ values of KCl in the tail, internal carotid, mesenteric, pulmonary arteries, and aorta of the rat.

In conclusion, P2X₁ receptor-mediated vasoconstrictions are equally important in the rat internal carotid, pulmonary, mesenteric arteries, and aorta, but they are much greater in the rat tail artery, suggesting a special role in its physiological function.

Acknowledgements This work was supported by a grant from the National Program on Key Basic Research Project of China (973 Program; No. 2005CB523301).

Conflicts of interest None

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