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Original Article

Exhaustive swimming differentially inhibits P2X$_1$ receptor- and α$_1$-adrenoceptor-mediated vasoconstriction in isolated rat arteries

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Aim: To investigate the effects of exhaustive swimming exercise on P2X1 receptor- and α1-adrenoceptor-mediated vasoconstriction of different types of arteries in rats.

Methods: Male Wistar rats were divided into 2 groups: the sedentary control group (SCG) and the exhaustive swimming exercise group (ESEG). The rats in the ESEG were subjected to a swim to exhaustion once a day for 2 weeks. Internal carotid, caudal, pulmonary, mesenteric arteries and aorta were dissected out. Isometric vasoconstrictive responses of the arteries to α,β-methylene ATP (α,β-MeATP) or noradrenaline (NA) were recorded using a polygraph.

Results: The exhaustive swimming exercise did not produce significant change in the EC$_{50}$ values of α,β-MeATP or NA in vasoconstrictive response of most of the arteries studied. The exhaustive swimming exercise inhibited the vasoconstrictive responses to P2X1 receptor activation in the internal carotid artery, whereas it reduced the maximal vasoconstrictive responses to α1-adrenoceptor stimulation in the caudal, pulmonary, mesenteric arteries and aorta. The rank order of the reduction of the maximal vasoconstriction was as follows: mesenteric, pulmonary, caudal, aorta.

Conclusion: Exhaustive swimming exercise differentially affects the P2X1 receptor- and α1-adrenoceptor-regulated vasoconstriction in internal carotid artery and peripheral arteries. The ability to preserve purinergic vasoconstriction in the peripheral arteries would be useful to help in maintenance of the basal vascular tone during exhaustive swimming exercise.

Keywords: exhaustive exercise; swimming; α$_1$-adrenoceptor; P2X$_1$ receptor; vasoconstriction; artery

Introduction

Regularly engaging in moderate exercise provides many well-established health benefits, including the prevention of, or a reduction in, the deleterious effects of pathological conditions such as hypertension, coronary artery disease, atherosclerosis, diabetes mellitus, and osteoporosis[1–3]. However, the beneficial effects of exercise are lost with overexertion, which causes the production of free radicals[4] and subsequent damage to lipids, proteins, and DNA. Exhaustive exercise has been shown to lead to tissue damage in animals[5, 6]. It has also been reported that exhaustive exercise resulted in significant impairment of regional left ventricular systolic and diastolic function in rats[7]. In the right ventricle of the human heart, tissue Doppler measurements of systolic and early diastolic function have been found to decrease significantly after prolonged strenuous exercise compared to pre-training values[8].

Functional changes in the vasculature of animals subjected to physical exercise have attracted considerable attention for many years. Acute exercise significantly attenuates α-adrenoceptor-mediated vasoconstriction of the thoracic aorta isolated from rabbits[9], and chronic exercise also decreases adrenergic agonist-induced vasoconstriction in the isolated thoracic aorta and carotid artery of spontaneously hypertensive rats[10]. It has also been reported that sedentary (control) and exercise-trained rats show no difference in the maximum levels of vasoconstriction induced by noradrenaline (NA) or phenylephrine in the isolated thoracic aorta[11]. Moderate levels of exercise did not alter either the myogenic regulation of the arterial diameter stimulated by increased transmural pressure or the smooth muscle responses to a thromboxane agonist in control and *db/db* mice[12]. Chronic treadmill running sig-

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nificantly enhanced myogenic vasoconstriction in the coronary resistance arteries isolated from female pigs[13]. Potential reasons for this discrepancy might be differences among arteries studied, the laboratory animals used and the exercise modes employed.

It is well known that the sympathetic and purinergic co-transmission involving NA and adenosine 5'-triphosphate (ATP) exists in a variety of blood vessels. The neurogenic vasoconstriction induced by electrical field stimulation consists of a purinergic (prazosin-resistant) component and an adrenergic (prazosin-sensitive) component in different blood vessels. P2X and P2Y receptors are widely distributed throughout the cardiovascular system and are important in the regulation of vascular tone[14]. The $P2X_1$ purinoceptor is the primary P2X subtype expressed on most vascular smooth muscle cells[15], and is responsible for purinergic arterial contraction[16, 17]. α,β-Methylene ATP (α,β-MeATP) is considered to be a useful reagent to investigate $P2X_1$ receptor-mediated vasoconstriction[18]. It has been reported that rats with diet-induced obesity have enhanced sympathetic nerve-mediated vasoconstriction via upregulation of purinergic and adrenergic neurotransmission[19]. Vidal *et al*[20], however, have indicated that α,β-MeATP significantly inhibits vasoconstrictive responses to electrical field stimulation in the tail arteries of spontaneously hypertensive rats, but does not inhibit these responses in the tail arteries of normal rats. Therefore, the purpose of this study was to examine whether there is similar or differential inhibition of $α_1$-adrenoceptor- and $P2X_1$ purinoceptor-mediated vasoconstriction in the aorta and the internal carotid, caudal, pulmonary and mesenteric arteries in healthy rats after exhaustive swimming exercise.

Materials and methods
Animals
Male Wistar rats weighing 300–350 g (aged 12–13 weeks) were housed one per cage in a temperature-controlled room (24±1 °C) with a 12 h light/dark cycle and received approximately 50% of their daily food intake[6] and tap water *ad libitum*. The commercial standard chow was purchased from Hebei Medical University. Rats were randomly divided into two groups: a sedentary control group (SCG) and an exhaustive swimming exercise group (ESEG). All animals used in this study received humane care in compliance with institutional animal care guidelines. All procedures performed were approved by the Local Institutional Committee.

Chemicals
[–]-Noradrenaline bitartrate (NA), α,β-methylene adenosine 5'-triphosphate lithium salt (α,β-MeATP), desmethylimipramine hydrochloride, deoxycorticosterone acetate, yohimbine hydrochloride, propranolol hydrochloride and acetylcholine hydrochloride were obtained from the Sigma Chemical Company, USA. The above reagents were dissolved in distilled water except for deoxycorticosterone acetate which 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.

Training protocols
To familiarize the rats with water immersion and reduce water-induced stress, the rats of the ESEG were made to swim in an apparatus holding no less than a water depth of 60 cm for 15 min daily at 8:30 am for 6 days/week during the first week. After the rats had adapted to the swimming exercise, the animals were subjected to a swim to exhaustion with a weight equivalent to 3% of their body weight tied to their tails[21] for two weeks. The training, began from 8:30 am to 11:30 am[22], was conducted daily for 6 days/week by the same person. Exhaustion was defined by two criteria: the rats remained below the water surface for 10 s, and the rats showed a lack of a "righting reflex" when they were placed on a flat surface[21]. Simultaneously, the rats of the SCG were kept in a small chamber holding a water depth of 10 cm. The water temperature was maintained at 34–36 °C[23].

Arterial preparations
Rats of the ESEG and SCG were anesthetized by subcutaneous injection of urethane (1.5 g/kg) 24 h after the last exhaustive swimming session then sacrificed by cutting the femoral artery, resulting in exsanguination. After the chest and abdomen were opened by a midline incision, the thoracic aorta, right pulmonary artery and superior mesenteric artery were carefully removed. An anterior midline incision was made on the neck to expose the carotid artery. The left and right internal carotid arteries were identified from their origin at the common carotid artery bifurcation to their entry points into the skull and were isolated. The caudal artery was surgically exposed from the ventral side then dissected from surrounding tissues and removed. These isolated arteries were maintained in ice-cold oxygenated Krebs-Henseleit (K-H) solution (133 mmol/L NaCl, 4.7 mmol/L KCl, 1.35 mmol/L NaH_2PO_4, 16.3 mmol/L $NaHCO_3$, 0.61 mmol/L $MgSO_4$, 7.8 mmol/L glucose and 2.52 mmol/L $CaCl_2$). The vascular endothelium of each artery was removed by gently rubbing the lumen with a scored polythene cannula, the external diameter of which was slightly smaller than the internal diameter of the blood vessel. A ring segment (4 mm long) without endothelium was mounted horizontally in a 10-mL organ bath, and the isometric tension was recorded by a polygraph (ERT-884, Youlin Electron Co, Kaifeng, China). Preloads were applied to the preparations of internal carotid artery (1.0 g), caudal artery (0.75 g), pulmonary artery (1.0 g), mesenteric artery (1.0 g), and aorta (2.0 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% O_2 and 5% CO_2 (pH 7.4). Successful removal of the arterial endothelium was confirmed by the loss of the relaxation response to acetylcholine (ACh, 1 μmol/L) in precontracted arterial rings treated with NA.

Experimental protocols
Before performing the following procedures, a cumulative dose-response curve for NA (0.0001–100 μmol/L) was con-

structed for each of the arterial preparations to observe the vasoconstrictive responsiveness, followed by further equilibration for 1 h. After the isolated arterial experiments were performed, a dose-response curve for KCl (10–120 mmol/L) was constructed in the arterial preparations, and the wet weight of each preparation was recorded.

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

In the arterial preparations used to construct a second cumulative dose-response curve for NA, desmethylimipramine (0.1 µmol/L), deoxycorticosterone (5 µmol/L), yohimbine (0.3 µmol/L) and propranolol (1 µmol/L) were added to the organ bath for 30 min to block neuronal or extra-neuronal uptake of NA and to block α_2- and β-adrenoceptors, respectively. The second cumulative dose-response curve for NA (0.0001–100 µmol/L) was constructed for each arterial preparation of the aorta and the internal carotid, caudal, pulmonary, and mesenteric arteries to observe α_1-adrenoceptor-regulated vasoconstriction.

Vasoconstrictive responses to α,β-MeATP in the aorta and the internal carotid, caudal, pulmonary, and mesenteric arteries

Because α,β-MeATP rapidly desensitizes its own receptors, a single concentration of α,β-MeATP at 0.1, 1.0, 10, or 100 µmol/L was added to the organ bath (each arterial preparation was exposed to α,β-MeATP only once) for each arterial preparation, and the resultant responses of several preparations exposed to different concentrations were grouped together to form a dose-response curve[25].

Statistical analysis

Vasoconstrictive responses to NA and α,β-MeATP were expressed as the maximal changes in tension (g) and were further normalized to wet tissue weight (g/mg tissue). Values presented here are the mean±SEM. Two-way ANOVA was used to evaluate any differences between the two sets of dose-response curves. If the F statistic was significant, it was compared to the individual datum with its respective control value by Bonferroni's test. We compared the EC_{50} values of the agonists, the maximal vasoconstriction to KCl and the wet tissue weight of arterial preparations between the two groups using an unpaired t-test. P values less than 0.05 were considered

to be statistically significant. The data were analyzed using GraphPad Prism version 5.00 (San Diego, California, USA).

Results

Effect of exhaustive swimming exercise on wet tissue weight and the vasoconstrictive response to KCl

The wet weight of the mesenteric arterial ring segments in the SCG was 0.73±0.02 mg, which was significantly lower than that in the ESEG (0.85±0.02 mg) (P<0.01, Figure 1A). The values of the wet weights of the internal carotid, caudal, pulmonary, and aortic arterial ring segments in the SCG were not significantly different from those in the ESEG (P>0.05, Figure 1A). The maximal vasoconstrictive responses to KCl in the mesenteric and caudal arteries in the SCG were 1.72±0.05 g/mg tissue and 3.27±0.10 g/mg tissue, respectively. These responses were much greater than those of the ESEG; the vasoconstrictive responses in the mesenteric and caudal arteries were 1.46±0.06 g/mg tissue and 2.85±0.10 g/mg tissue, respectively (P<0.01, Figure 1B). There were no significant differences in the maximal vasoconstrictive responses to KCl of the internal carotid artery, pulmonary artery and the aorta between the SCG and the ESEG rats (P>0.05, Figure 1B). However, the range of EC_{50} values for KCl in the 5 types of arteries subsequently exposed to the second administration of NA ranged from 17.23 to 31.74 mmol/L, and the EC_{50} values of KCl in the SCG were not significantly different from those in the ESEG (Table 1).
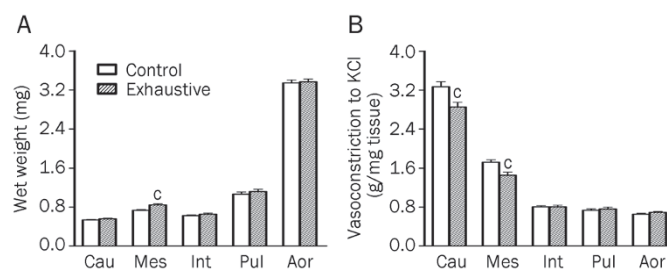

Figure 1. Tissue wet weight (A) and vasoconstriction to 120 mmol/L KCl (B) in the rat mesenteric (Mes), caudal (Cau), pulmonary (Pul), internal carotid (Int) arteries and aorta (Aor) from the sedentary control group (SCG, n=29−34) and the exhaustive swimming exercise group (ESEG, n=28−35). Data were expressed as mean±SEM. ^{c}P<0.01 vs SCG.

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. NA group, n=8−15; α,β-MeATP group, n=20−24; KCl group, n=8−15. ^{c}P<0.01 vs SCG.

Artery	SCG			ESEG		
	NA	α,β-MeATP	KCl	NA	α,β-MeATP	KCl
Internal carotid	7.15±0.07	5.36±0.22	20.20±1.17	7.36±0.18	4.71±0.22	21.38±0.64
Caudal	6.70±0.05	6.19±0.18	29.25±1.45	6.78±0.06	6.09±0.15	31.74±1.45
Mesenteric	6.98±0.08	5.70±0.18	23.25±0.64	7.00±0.11	5.51±0.23	21.36±1.86
Aorta	7.43±0.04	4.97±0.14	20.71±1.39	7.11±0.10^c	4.90±0.22	21.10±1.58
Pulmonary	7.58±0.07	5.40±0.34	17.23±1.46	7.54±0.07	5.62±0.21	20.22±0.85

The vasoconstrictive responsiveness of the aorta and the internal carotid, caudal, pulmonary, and mesenteric arteries to NA

Before analyzing the difference in the vasoconstrictive responses to either α_1-adrenoceptor stimulation or $P2X_1$ purinoceptor stimulation between the two rat groups, we examined the vasoconstrictive responsiveness of the selected arteries individually. In the rats of the SCG, there were no significant differences in the vasoconstrictive responses to the first exposure to NA in the internal carotid, caudal, pulmonary, mesenteric artery or aorta between the preparations subsequently exposed to the second administration of NA and those subsequently exposed to α,β-MeATP ($P>0.05$, Figure 2A–6A). The same results were observed in the rats from the ESEG ($P>0.05$, Figure 2B–6B).
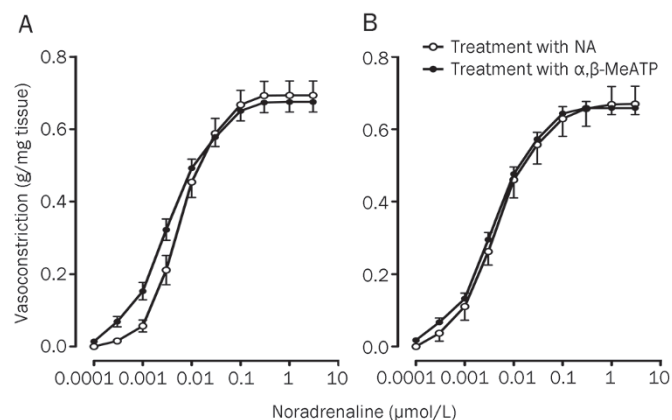

Figure 2. A comparison of the vasoconstrictive responses to noradrenaline between the preparations exposing to the second administration of noradrenaline (treatment with NA; n=10, left; n=9, right) and those exposing to α,β-methylene ATP (treatment with α,β-MeATP; n=24, left; n=24, right) in the rat pulmonary arteries from the sedentary control group (A) and the exhaustive swimming exercise group (B). Data were expressed as mean±SEM.

Effect of exhaustive swimming exercise on the vasoconstrictive responses to NA in the aorta and the internal carotid, caudal, pulmonary, and mesenteric arteries

A second exposure of the 5 selected arteries to NA (0.0001–100 µmol/L) produced vasoconstrictive responses in a dose-dependent manner in the rats of the SCG and the ESEG. The exhaustive swimming exercise significantly decreased the vasoconstrictive response to NA in the pulmonary and caudal arterial preparations ($P<0.01$, Figure 7A and 8A), reaching a maximal inhibition of 13.68% in the pulmonary artery and 9.48% in the caudal artery. In the mesenteric arterial preparation, the inhibition of vasoconstrictive response to NA by exhaustive swimming exercise was more potent, reaching a maximal inhibition of 21.02% ($P<0.01$, Figure 9A). Exhaustive swimming exercise significantly inhibited the vasoconstrictive responses to NA in the aorta, but the maximal response was not affected (Figure 10A). The vasoconstrictive response
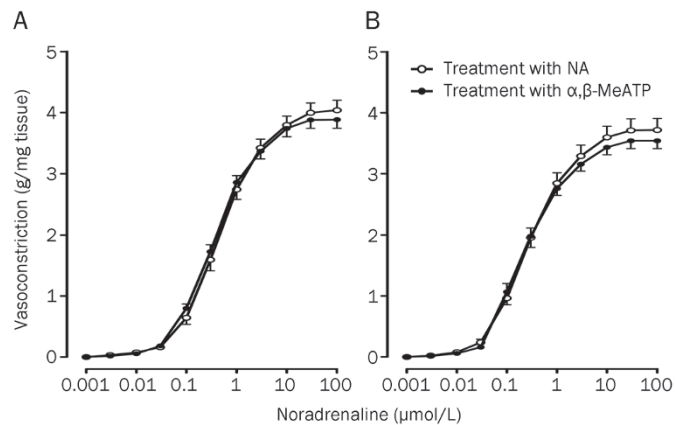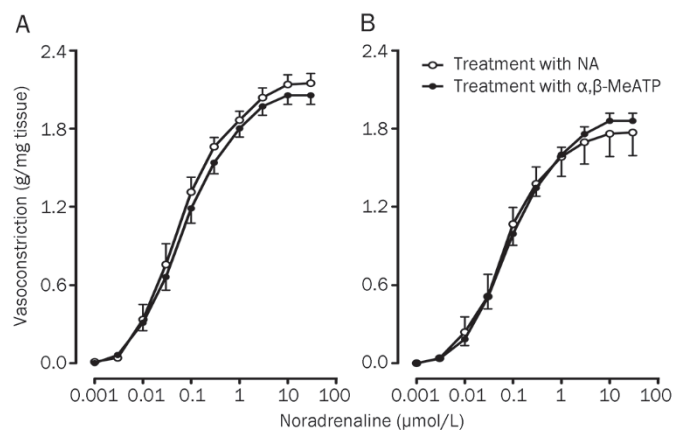

Figure 3. A comparison of the vasoconstrictive responses to noradrenaline between the preparations exposing to the second administration of noradrenaline (treatment with NA; n=11, left; n=15, right) and those exposing to α,β-methylene ATP (treatment with α,β-MeATP; n=20, left; n=20, right) in the rat caudal arteries from the sedentary control group (A) and the exhaustive swimming exercise group (B). Mean±SEM.

Figure 4. A comparison of the vasoconstrictive responses to noradrenaline between the preparations exposing to the second administration of noradrenaline (treatment with NA; n=11, left; n=9, right) and those exposing to α,β-methylene ATP (treatment with α,β-MeATP; n=22, left; n=22, right) in the rat mesenteric arteries from the sedentary control group (A) and the exhaustive swimming exercise group (B). Data were expressed as mean±SEM.

to NA in the internal carotid arterial preparation was not significantly affected by exhaustive swimming exercise ($P>0.05$, Figure 11A). The range of the negative $\log(EC_{50})$ values (where the EC_{50} values are expressed as mol/L) for the 5 types of arteries given a second treatment with NA was 6.70–7.58. In the thoracic aorta, the negative $\log(EC_{50})$ value of NA in the SCG (7.43±0.04) was slightly larger than that in the ESEG (7.11±0.10) ($P<0.01$); however, the EC_{50} values of NA in the SCG were not significantly different from those in the ESEG in the other 4 types of arteries (Table 1).
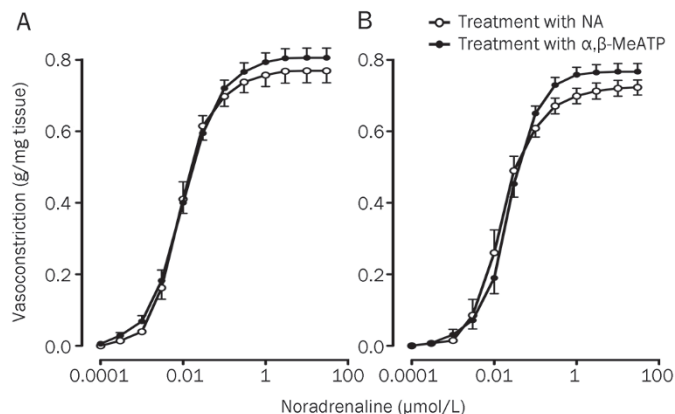
Figure 5. A comparison of the vasoconstrictive responses to noradrenaline between the preparations exposing to the second administration of noradrenaline (treatment with NA; *n*=11, left; *n*=8, right) and those exposing to α,β-methylene ATP (treatment with α,β-MeATP; *n*=23, left; *n*=22, right) in the rat thoracic aorta from the sedentary control group (A) and the exhaustive swimming exercise group (B). Data were expressed as mean±SEM.
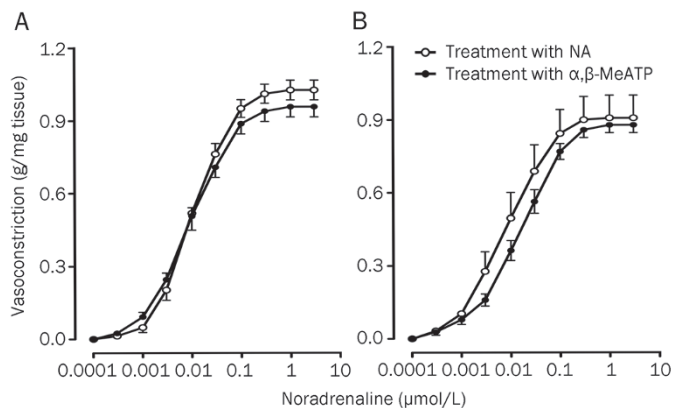

Figure 6. A comparison of the vasoconstrictive responses to noradrenaline between the preparations exposing to the second administration of noradrenaline (treatment with NA; *n*=9, left; *n*=8, right) and those exposing to α,β-methylene ATP (treatment with α,β-MeATP; *n*=20, left; *n*=20 right) in the rat internal carotid arteries from the sedentary control group (A) and the exhaustive swimming exercise group (B). Data were expressed as mean±SEM.

Effect of exhaustive swimming exercise on vasoconstrictive responses to α,β-MeATP in the aorta and the internal carotid, caudal, pulmonary, and mesenteric arteries

α,β-MeATP (0.1–100 μmol/L) produced vasoconstriction in the internal carotid, caudal, pulmonary, and mesenteric arteries, as well as the aorta, in a dose-dependent manner in the rats of the SCG and the ESEG. The vasoconstrictive responses to α,β-MeATP in the caudal artery, pulmonary artery, mesenteric artery and the aorta were not significantly affected by exhaustive swimming exercise (*P*>0.05, Figure 7B–10B). However, exhaustive swimming exercise significantly decreased
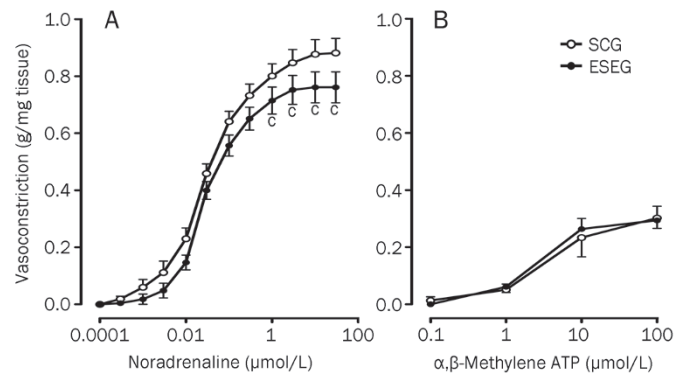

Figure 7. A comparison of the vasoconstrictive responses to the second administration of noradrenaline (A) or to α,β-methylene ATP (B) in the rat pulmonary arteries between the sedentary control group (SCG; *n*=10, A; *n*=5−7, B) and the exhaustive swimming exercise group (ESEG; *n*=9, A; *n*=5−7, B). Mean±SEM. Statistical significance was analyzed by two-way ANOVA in A: ^{c}P<0.01 *vs* SCG.
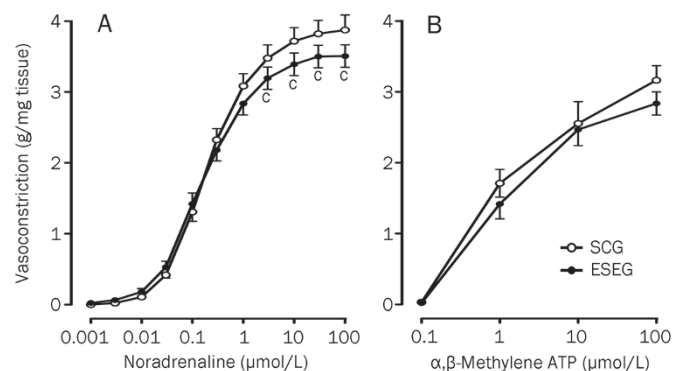

Figure 8. A comparison of the vasoconstrictive responses to the second administration of noradrenaline (A) or to α,β-methylene ATP (B) in the rat caudal arteries between the sedentary control group (SCG; *n*=11, A; *n*=5, B) and the exhaustive swimming exercise group (ESEG; *n*=15, A; *n*=5, B). Data were expressed as mean±SEM. Statistical significance was analyzed by two-way ANOVA in A: ^{c}P<0.01 *vs* SCG.

the vasoconstrictive response to α,β-MeATP in the internal carotid arterial preparations, reaching a maximum inhibition of 50.38% (*P*<0.01, Figure 11B). The range of the negative log(EC$_{50}$) values for α,β-MeATP in the 5 types of arteries was 4.71–6.19, and the EC$_{50}$ values of α,β-MeATP in the SCG were not significantly different from those in the ESEG (Table 1).

Discussion

The co-transmission of NA and ATP is well established in the sympathetic innervation of a variety of blood vessels in animals. However, in this study, we discovered that exhaustive swimming exercise did not affect the EC$_{50}$ values of NA or α,β-MeATP, but this type of exercise significantly decreased the P2X$_1$ receptor-mediated vasoconstriction in the rat internal carotid artery. Conversely, the vasoconstrictive response to
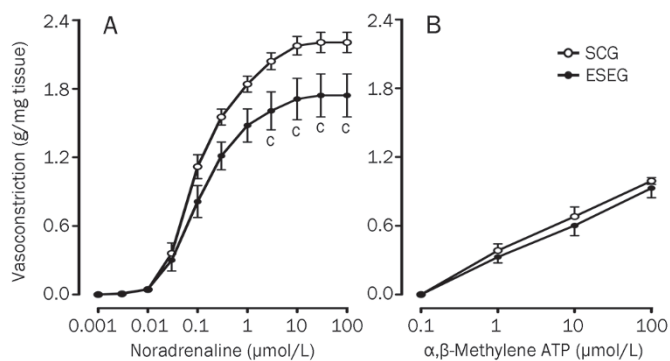
Figure 9. A comparison of the vasoconstrictive responses to the second administration of noradrenaline (A) or to α,β-methylene ATP (B) in the rat mesenteric arteries between the sedentary control group (SCG; *n*=11, A; *n*=5–6, B) and the exhaustive swimming exercise group (ESEG; *n*=9, A; *n*=5–6, B). Data were expressed as mean±SEM. $^c P < 0.01$ *vs* SCG.

Figure 10. A comparison of the vasoconstrictive responses to the second administration of noradrenaline (A) or to α,β-methylene ATP (B) in the rat thoracic aorta between the sedentary control group (SCG; *n*=11, A; *n*=5–6, B) and the exhaustive swimming exercise group (ESEG; *n*=8, A; *n*=5–6, B). Data were expressed as mean±SEM. $^c P < 0.01$ *vs* SCG.
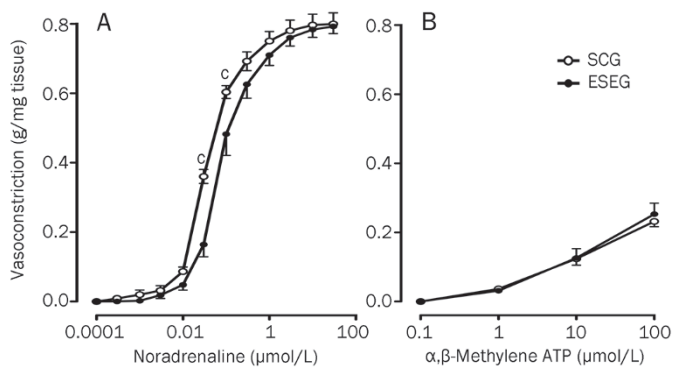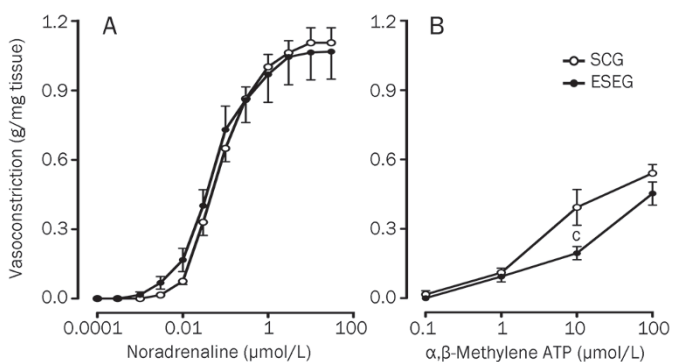

Figure 11. A comparison of the vasoconstrictive responses to the second administration of noradrenaline (A) or to α,β-methylene ATP (B) in the rat internal carotid arteries between the sedentary control group (SCG; *n*=9, A; *n*=5, B) and the exhaustive swimming exercise group (ESEG; *n*=8, A; *n*=5, B). Data were expressed as mean±SEM. $^c P < 0.01$ *vs* SCG.

α_1-adrenoceptor, but not P2X$_1$ receptor, activation was inhibited in the caudal artery, pulmonary artery, mesenteric artery and the aorta obtained from rats subjected to the exhaustive swimming exercise.

Before observing the vasoconstrictive responses to α_1-adrenoceptor or P2X$_1$ receptor stimulation, we examined the vasoconstrictive responsiveness of each artery. In the rats of the SCG and the ESEG, there were no significant differences in the vasoconstrictive responses to the first exposure to NA (Figure 2–6) in the internal carotid, caudal, pulmonary, and mesenteric artery along with the aorta. No significant differences in vasoconstrictive responsiveness between the preparations that were either exposed to a second administration of NA or to α,β-MeATP were observed, suggesting that the 5 types of arteries obtained from the SCG rats and the ESEG rats were comparable in the present study. Because the EC$_{50}$ values of KCl treatment were significantly greater in different types of arteries treated with α,β-MeATP than NA[24], and the maximum vasoconstrictive response to KCl in the rat mesenteric and caudal arteries in the SCG were greater than the response in the ESEG, we expressed the vasoconstrictive responses to NA and α,β-MeATP as g/mg tissue. Concomitantly, we found the exhaustive swimming exercise did not alter the EC$_{50}$ values of KCl.

It has been reported that long-term swimming exercise significantly reduces the vasoconstrictive response to phenylephrine in the rat mesenteric artery and thoracic aorta when the endothelium is intact, but not in the endothelium-denuded arteries[26, 27]. Chronic exercise also enhances endothelium-mediated vasodilatation in the endothelium-intact aorta and mesenteric artery, but this exercise does not affect NA-induced vasoconstriction in the endothelium-denuded arteries isolated from spontaneously hypertensive rats[28]. Moreover, exercise training increases acetylcholine-induced relaxation and eNOS protein levels in the porcine pulmonary artery[29, 30], but not in the pulmonary artery from hypertensive rats[31]. Therefore, we removed the vascular endothelium of the isolated mesenteric, pulmonary, caudal and internal carotid arteries from the rat to directly observe the changes in α_1-adrenoceptor- and P2X$_1$ receptor-mediated vascular smooth muscle contractions because several studies suggest that exercise affects not only the vasoconstriction of vascular smooth muscles[32, 33], but also the function of the vascular endothelium[26, 27].

NA induces vasoconstriction and vasodilatation via α-adrenoceptors and β-adrenoceptors. Furthermore, Carter *et al*[34] reported that though α_2-adrenoceptors do not play a large role in NA-mediated vasoconstriction of the thoracic aorta in normotensive rats, there is an increased role of these receptors in hypertensive rats. Prior to measuring vasoconstrictive responsiveness after a second treatment with NA in this study, we added several reagents (propranolol, yohimbine, desmethylimipramine and deoxycorticosterone) to the organ bath to block β-adrenoceptors and α_2-adrenoceptors, as well as neuronal and extra-neuronal uptake of NA. This allows for the measurement of vasoconstriction after a second exposure of each arterial preparation to NA to be attributed to

α₁-adrenoceptors alone.

Though it is widely accepted that the P2X$_1$ receptor seems to be the most important P2X subtype in the vascular smooth muscle, this concept is not observed in functional studies. Nori *et al*[35] observed the coexpression of three P2X receptor mRNAs (P2X$_1$, P2X$_2$, and P2X$_4$) in the rat vascular smooth muscle. However, it was reported that the P2X$_4$ receptor did not couple to a vasomotor response[36], and the P2X$_2$ receptor was mainly expressed in nerves and arterial endothelial cells and only found at low levels in smooth muscle cells[37]. Recently, Wallace *et al*[38] investigated the expression of P2X receptors in the tail and mesenteric arteries of rats aged 4, 6, and 12 weeks by using immunohistochemistry. P2X$_1$ receptor-specific immunoreactivity was associated with the smooth muscle layer of both arteries from all rats of all three ages, and the P2X$_4$ receptor was weakly expressed in the smooth muscle layer of the tail artery in 4- and 6-week-old rats along with the mesenteric artery of 4- and 12-week-old rats. Immunoreactivity of the other subtypes of the P2X receptor family was not detected in the smooth muscle layer of arteries in 6- and 12-week-old rats[38]. In our study, we used 12–13-week-old rats (300–350 g), and the vascular endothelium of the regional arteries was removed. This age of rats combined with the use of α,β-MeATP (which is inactive as an agonist at the recombinant P2X$_2$ purinoceptor[39]) allows us to assume that the P2X$_1$ purinoceptors are primarily involved in the vasoconstrictive response to α,β-MeATP.

This study was the first investigation of measuring the changes in the P2X$_1$ receptor-regulated vasoconstriction of regional arteries from rats subjected to the exhaustive swimming exercise. We also compared the effects of exhaustive swimming exercise on both α₁-adrenoceptor- and P2X$_1$ receptor-mediated vasoconstriction in the isolated internal carotid, caudal, pulmonary and mesenteric arteries along with the aorta. Our study clearly showed that the vasoconstrictive responses to α₁-adrenoceptor stimulation in the aorta and the caudal, pulmonary and mesenteric arteries from the exhaustively exercised rats were significantly smaller than those from the normal rats, and a rank order of the decrease in the maximal vasoconstriction was determined as follows: mesenteric artery, pulmonary artery, caudal artery, aorta. Similar results were reported in the endothelium-denuded aorta[32] and mesenteric artery[33] from normal rats subjected to physical exercise. Moreover, we found that the P2X$_1$ receptor-mediated vasoconstriction in all 5 arterial subtypes was not affected by exhaustive exercise. Using an isolated perfused splenic artery, Yang *et al*[40] observed two peaks of vasoconstriction in response to periarterial nerve stimulation: an initial transient constriction induced by the purinergic ligand ATP and a second peak response consisting primarily of an adrenergic component. Our recent study demonstrated that the maximal vasoconstriction mediated by P2X$_1$ receptors reached at least 40% of those mediated by α₁-adrenoceptors in the rat internal carotid, mesenteric and pulmonary arteries. P2X$_1$ receptor-mediated vasoconstriction even reached 80% in the caudal artery[24], which suggested that purinergic transmission can be useful to maintain the basal vascular tone and could be important to the hemodynamic control in rats subjected to exhaustive swimming exercise. Sugawara *et al*[41] also suggested that a reduction in α-adrenoceptor-mediated vascular tone contributes to improved arterial compliance in healthy adults who engage in endurance exercise.

In contrast to the rat caudal artery, pulmonary artery, mesenteric artery and the aorta, the vasoconstrictive response to α₁-adrenoceptor stimulation in the rat internal carotid artery was not significantly affected by exhaustive swimming exercise. However, the vasoconstrictive response to P2X$_1$ receptor stimulation in the internal carotid artery from the exhaustively exercised rats was significantly reduced. The reason for this difference is still unclear based on the data from the present study. Normally, the brain is entirely dependent upon glucose as an energy substrate as implied by cerebral metabolic ratio, which is close to 6.0[42]. After exhaustive exercise, the cerebral metabolic ratio drops to 1.7, suggesting a link between brain metabolism and central fatigue[43].

It was reported recently that the ratio of ischemic-to-nonischemic blood flow decreases from 6 to 24 months of age, and the capillary density of nonischemic and ischemic muscle also decreases in an age-dependent manner in wild-type mice[44]. Swimming training was shown to reduce the formation of abnormal vessels and promoted collateral artery formation in the ischemic limbs of aged wild-type mice[44]. We plan to observe and clarify the effect of exhaustive swimming on vasoconstriction mediated by purinoceptors or adrenoceptors in aged rats in the near future. Maiorana *et al*[45] reported that chronic heart failure patients engaging in aerobic exercise had no changes in the wall thickness and the wall-to-lumen ratio of the brachial artery, while patients engaging in resistance exercise had a significantly reduced brachial artery wall thickness and wall-to-lumen ratio. The method of exhaustive swimming used in our study does not fall within the categories of either aerobic exercise or resistance exercise. It is unclear whether aerobic exercise affects the vasoconstriction mediated by purinoceptors and adrenoceptors in the same manner as resistance exercise does in normal or heart failure model animals.

Overall, the results of the present study showing the different effects of exhaustive swimming exercise on the vasoconstriction mediated by α₁-adrenoceptors and P2X$_1$ receptors between rat internal carotid artery and the peripheral arteries could help to explain that stress or injury can mediate different vascular responses in animals and humans and could be associated with tissue or organ functions. In conclusion, the exhaustive swimming exercise differentially affects the vasoconstriction regulated by P2X$_1$ receptors and α₁-adrenoceptors in the rat internal carotid artery and the peripheral arteries, and the ability to preserve purinergic vasoconstriction of the peripheral arteries is useful in maintaining the basal vascular tone during exhaustive swimming exercise.

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Author contribution

Lei-ming REN and Yi-ling WU designed the research. Lu LI, Tao WU, Cong WEI, and Jian-ke HAN performed the research. Zhen-hua JIA analyzed the data.

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