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Research Paper

Regional variations of vasomotion to G-protein coupled receptor agonists following heat stress in rats

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Abstract

Objectives This study was designed to compare vascular contractile and relaxing responses to G-protein coupled receptor agonists among the different regions of arteries following heat stress in rats.

Methods Heat exposure was performed by increasing the internal temperature of the rats to 42°C for 15 min. After heat stress for 48 h, a myograph system was used to monitor the contractile responses in rat renal, femoral and mesenteric arteries to agonists of endothelin type B (ET_B) receptor, endothelin type A (ET_A) receptor, serotonin receptor and α-adrenoceptor, respectively. In addition, calcitonin gene-related peptide (CGRP)-induced vasodilation was studied.

Key findings The results showed that heat stress induced decreased contractions mediated by α-adrenoceptors and serotonin receptors (at lower concentration), while it increased contraction mediated by endothelin ET_B receptors and enhanced relaxation mediated by CGRP receptors in the renal artery. Heat stress increased contractions mediated by endothelin ET_B receptors, endothelin ET_A receptors and α-adrenoceptors in the femoral artery. In the mesenteric artery, heat stress increased contractions mediated by endothelin ET_B and serotonin receptors and relaxation mediated by CGRP receptors.

Conclusions The vasomotor responses to the G-protein coupled receptor agonists with altered vascular contractions and relaxations were different in rat renal, femoral and mesenteric arteries after heat stress. This might have contributed to the redistribution of blood flow and aids understanding of the preconditioning phenomenon.

Keywords artery; G-protein coupled receptor; heat stress rat; vasomotion

Introduction

The preconditioning phenomenon is most likely to have benefits for patients suffering from repeated ischaemic episodes or at reperfusion following ischaemia. Heat stress has similar characteristics to delayed ischaemic preconditioning to protect the myocardium against infarction, stunning and arrhythmias.[1–6] It is well documented that endothelial cells can secrete and metabolize multiple active substances and participate in the regulation of cardiovascular functions. A number of vascular agonists that act on the receptors of a large family of G-protein coupled receptors on vascular smooth muscle cells contribute to maintaining vascular tone.

The alteration of the G-protein coupled receptor-mediated vascular contraction or relaxation after heat stress may be a key point for cardiovascular regulation. We have demonstrated that endothelium-dependent relaxation and the structure of endothelium in mesenteric artery of rats could be altered after heat stress.[7] This study was designed to use endothelium-denuded vessels to investigate the regional variation in functions of G-protein coupled receptors after heat stress. We hypothesized that there may be variations in vasoconstrictions and vasodilations in different regions of arteries after heat stress. We have examined and compared vascular contractile and relaxing responses to the G-protein coupled receptor agonists among renal, femoral and mesenteric arteries after heat stress.

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Materials and Methods

Reagents

Phenylephrine, sarafotoxin 6c, endothelin-1, serotonin (5-hydroxytryptamine), calcitonin gene-related peptide (CGRP), and acetylcholine chloride were purchased from Sigma (St Louis, MO, USA). The peptides were dissolved in 0.1% w/v bovine serum albumin in double-distilled water. Other substances (NaCl, $NaHCO_3$, KCl, $MgCl_2$, NaH_2PO_4, $CaCl_2$ and glucose) were dissolved in buffer solution. All drugs were further diluted in buffer solution and added just before the experiment. The concentrations were expressed as the final molar concentration in the tissue bath.

Animal preparation

Sixty-eight male Sprague-Dawley rats (250–300 g) were purchased from the Animal Center of Xi'an Jiaotong University College of Medicine, China. All animals were handled according to the guidelines provided by the Animal Care and Use Committee at Shaanxi Province. The experimental protocols for using the animals had been reviewed and approved by the animal ethics committee at Xi'an Jiaotong University. After being anaesthetized through intraperitoneal injection of 40 mg/kg sodium pentobarbital, the rats were placed in an infrared heating environmental chamber. The rectal temperature was recorded by means of a probe. Heat exposure was performed to increase the internal temperature to 42°C for 15 min. The sham group was anaesthetized and rectal temperature was measured, but without hyperthermia. Forty-eight hours after heat stress, the rats were anaesthetized and exsanguinated. The renal, femoral and mesenteric arteries were removed gently and dissected free of adhering tissue under a microscope.

Vasomotor responses

Each renal, femoral and mesenteric artery from the rats was cut into 1 mm long ring segments. The arterial segments were placed in a buffer solution (containing in mM: NaCl 119, $NaHCO_3$ 15, KCl 4.6, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.5 and glucose 5.5) and were mounted on two L-shaped metal prongs in a myograph (Danish Myo Technology A/S, Aarhus, Denmark). One prong was connected to a force displacement transducer for continuous recording of the isometric tension (ADInstruments, Oxford, UK) and the other to a displacement device. The mounted arterial segments were immersed in temperature controlled (37°C) tissue baths containing the buffer solution. The solution was continuously gassed with 5% CO_2 in O_2 resulting in a pH of 7.4. A passive tension of

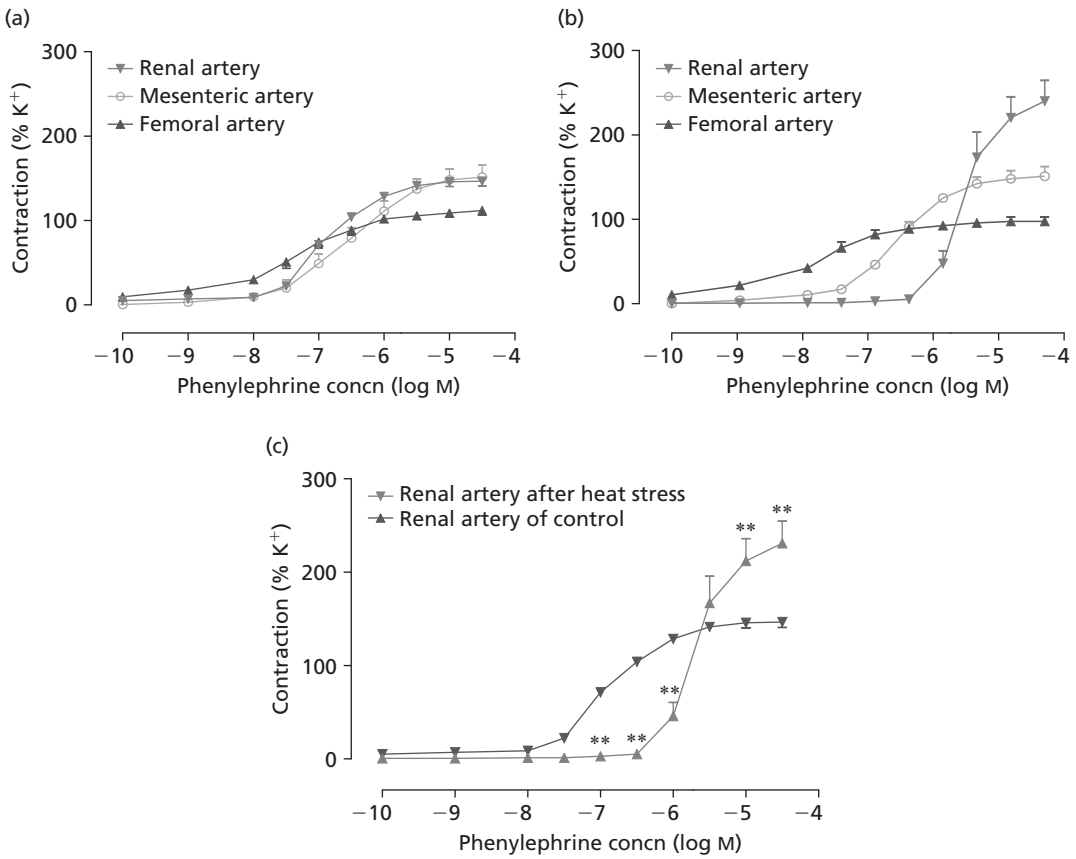

Figure 1 Concentration–contractile curves of endothelium-denuded artery segments induced by phenylephrine in rats. Renal, mesenteric and femoral arteries were used. (a) Control rats; (b) after heat stress. (c) Concentration–contractile curves of endothelium-denuded renal artery. Data are given as mean ± SEM. Number of animals examined, n = 7–9. $^{**}P < 0.01$ compared with control

2–4 mN was applied to the arterial segments. The tension was chosen based on the diameter of individual segments and was allowed to stabilize for 1 h before the experiments were started. The contractile capacity of each vessel segment was examined by exposure to a K^+-rich (60 mM) Kreb's buffer solution, in which NaCl was exchanged for an equimolar concentration of KCl. When two reproducible contractions over 1 mN had been achieved, the vessels were used for further experiments. The arterial endothelium was denuded with a 10-s perfusion of 0.1% Triton-X 100. Removal of the endothelium was confirmed by loss of relaxant response to 10^{-5} M acetylcholine. Endothelium-denuded arterial segments were used to examine the systolic or diastolic function of endothelin ET_A receptor, endothelin ET_B receptor, serotonin receptor, CGRP receptor, and α-adrenoceptor by administration of their agonists. Concentration–response curves were obtained by cumulative addition of the agonists to the tissue baths. To study endothelin ET_A receptor-mediated contraction, a sarafotoxin 6c concentration–effect curve was performed first, and the segments remained in contact with the highest concentration of sarafotoxin 6c (1 μM) for a further 1 h to desensitize the endothelin ET_B receptor.[8,9] During this period, the contractile response to sarafotoxin 6c faded to the baseline levels even though the sarafotoxin 6c still remained in contact with the segments. After endothelin ET_B receptor had been desensitized, the concentration–effect curve induced by endothelin-1 was performed. Thus, the contractile response to endothelin-1 was only mediated by the endothelin ET_A receptor.[10]

Statistical analysis

Statistics were based on one measurement per rat. When more than one vessel in an individual had been used, the average was used for that individual. Contractile responses in each vessel

Table 1 Overview of E_{max} or R_{max} and pEC50 or pIC50 of phenylephrine-, sarafotoxin 6c-, endothelin-1-, serotonin-, and calcitonin gene-related peptide-induced contraction or relaxation on endothelium-denuded arteries of heat stress rats

Agonist	*n*	K^+ (mN)	E_{max} or R_{max} (%)	pEC50 or pIC50
Phenylephrine				
MA Control	9	3.8 ± 0.6	151 ± 14	6.70 ± 0.09
Heat stress	7	3.5 ± 0.4	145 ± 11	6.72 ± 0.10
FA Control	8	3.8 ± 0.5	112 ± 6	7.40 ± 0.09
Heat stress	8	3.6 ± 0.8	94 ± 5	7.96 ± 0.16[b]
RA Control	8	2.9 ± 0.6	146 ± 6	6.97 ± 0.05
Heat stress	8	2.4 ± 0.4	230 ± 24[b]	5.68 ± 0.08[b]
Sarafotoxin 6c				
MA Control	9	3.3 ± 0.7	–	–
Heat stress	7	3.1 ± 0.4	15 ± 3	7.31 ± 0.26
FA Control	8	3.1 ± 0.5	–	–
Heat stress	8	3.7 ± 0.7	9 ± 3	9.33 ± 0.87
RA Control	8	3.2 ± 0.7	–	–
Heat stress	8	3.8 ± 0.5	6 ± 2	7.40 ± 0.17
Endothelin-1				
MA Control	9	2.9 ± 0.7	141 ± 10	8.09 ± 0.11
Heat stress	7	3.4 ± 0.3	133 ± 9	8.02 ± 0.07
FA Control	8	2.9 ± 0.5	108 ± 4	7.18 ± 0.12
Heat stress	8	3.0 ± 0.4	128 ± 7[b]	7.77 ± 0.04[b]
RA Control	8	3.7 ± 0.5	110 ± 14	7.92 ± 0.04
Heat stress	8	3.5 ± 0.6	181 ± 10[b]	7.49 ± 0.08[a]
Serotonin				
MA Control	9	3.3 ± 0.4	155 ± 12	6.42 ± 0.13
Heat stress	7	3.6 ± 0.8	148 ± 9	7.29 ± 0.11[b]
FA Control	8	3.7 ± 0.7	146 ± 10	7.51 ± 0.24
Heat stress	8	4.0 ± 0.7	158 ± 9	7.27 ± 0.15
RA Control	8	3.3 ± 0.6	149 ± 10	7.19 ± 0.18
Heat stress	8	3.8 ± 0.6	246 ± 22[b]	6.29 ± 0.09[b]
CGRP				
MA Control	9	3.8 ± 0.8	7.4 ± 3.3	9.68 ± 0.11
Heat stress	7	3.4 ± 0.6	33.4 ± 4.7[b]	9.72 ± 0.13
FA Control	8	2.7 ± 0.5	10.1 ± 1.7	8.17 ± 0.19
Heat stress	8	3.3 ± 0.7	16.3 ± 3.1	8.47 ± 0.58
RA Control	8	3.9 ± 0.8	9.8 ± 2.1	9.24 ± 0.16
Heat stress	8	3.4 ± 0.7	42.9 ± 3.7[b]	8.63 ± 0.21[b]

Mesenteric (MA), femoral (FA) and renal (RA) arteries were used. E_{max} (maximum contractile effect) is expressed as percent of 60 mM K^+-induced contraction and R_{max} (maximum relaxant effect) is expressed as percent of 20 μM serotonin-induced contraction. pEC50 or pIC50 values are the negative logarithm of the molar concentration that produced half maximum contraction or relaxation. Data are given as mean ± SEM, *n* = number of animals examined. [a]$P < 0.05$, [b]$P < 0.01$ compared with control. CGRP, calcitonin gene-related peptide.

segment were expressed as percentage of the contraction induced by 60 mM K^+. Relaxation responses were expressed as percentage of the precontraction induced by 20 μM serotonin. E_{max} and R_{max} values refer to the maximum contractile effect and the maximum relaxant effect, respectively. The pEC50 or pIC50 (negative logarithm of the molar concentration that produced half maximum contraction or relaxation) was calculated from the straight-line equation between the concentration above and below the midpoint of the concentration–response curves.

Data were given as mean ± SEM. One-way analysis of variance followed by Dunnett's test was applied for comparisons for more than two groups. An unpaired *t*-test was used when two sets of data were compared. A two-tailed test with a *P* value less than 0.05 was considered to be significant.

Results

Comparison of G-protein coupled receptor-mediated contractions in different regions of the arteries after heat stress

α-*Adrenoceptor*

In the control group, phenylephrine induced a strong concentration-dependent contraction of all vessels tested with the following efficacy order (the order was according to the sequence of the E_{max}, and the following contractile effects were similar): mesenteric artery (151 ± 14%) > renal artery (146 ± 6%) > femoral artery (112 ± 6%). After heat stress, the efficacy order was altered: renal artery (230 ± 24%) > mesenteric artery (145 ± 11%) > femoral artery (94 ± 5%).

Compared with the control, the concentration–contractile curve in renal artery after heat stress caused a significant rightward shift, and the pEC50 value was decreased from 6.97 ± 0.05 to 5.68 ± 0.08. In femoral artery, the contractile curve was leftward shifted, and the pEC50 was markedly increased from 7.40 ± 0.09 to 7.96 ± 0.16, compared with the control. After heat stress, as our previous study had demonstrated, phenylephrine-induced contraction in mesenteric artery was almost the same as in control (Figure 1, Table 1).[7]

ET receptors

Sarafotoxin 6c did not cause significant contraction in any of the tested arteries of control rats. However, after heat stress, sarafotoxin 6c induced minor vasoconstriction in tested vessels with the following efficacy order: mesenteric artery (15 ± 3%) > femoral artery (9 ± 3%) > renal artery (6 ± 2%), with the pEC50 values being 7.31 ± 0.26, 9.33 ± 0.87 and 7.40 ± 0.17, respectively (Figure 2, Table 1).

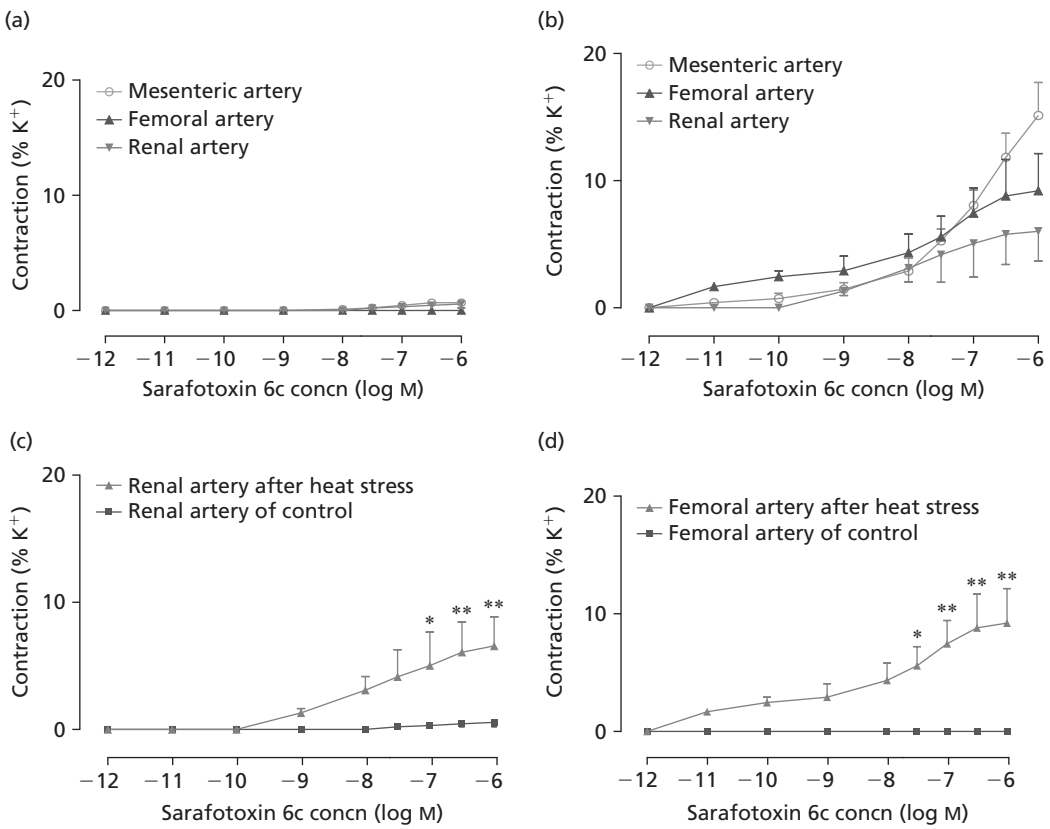

Figure 2 Concentration-contractile curves of endothelium-denuded artery segments induced by sarafotoxin 6c in rats. Renal, mesenteric and femoral arteries were used. (a) Control rats; (b) after heat stress. Concentration–contractile curves of endothelium-denuded renal artery (c) and femoral artery (d). Data are given as mean ± SEM. Number of animals examined, n = 7–9. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control

Endothelin-1 induced a concentration-dependent contraction in all tested vessels in control rats with the following efficacy order: mesenteric artery (141 ± 10%) > renal artery (110 ± 14%) > femoral artery (108 ± 4%). After heat stress, the efficacy order was changed as follows: renal artery (181 ± 10%) > mesenteric artery (133 ± 9%) > femoral artery (128 ± 7%). In renal artery, compared with the control, the E_{max} of the concentration–contractile curve was markedly increased (from 110 ± 14 to 181 ± 10%) and the pEC50 was significantly decreased (from 7.92 ± 0.04 to 7.49 ± 0.08). In femoral arteries, the concentration–contractile curve after heat stress was leftward shifted with significantly increased E_{max} (from 108 ± 4 to 128 ± 7%) and pEC50 (from 7.18 ± 0.12 to 7.77 ± 0.04), compared with the control (Figure 3, Table 1). In mesenteric arteries, as was reported previously, the contractile curve induced by endothelin-1 in heat stress rats was almost the same as that in control rats.[7]

Serotonin receptors

Serotonin induced a strong concentration-dependent contraction in all tested vessels. The concentration–contractile curves were biphasic in both the renal and femoral arteries of control groups and in the femoral artery of heat stress rats.

In control rats, the efficacy of the contractile curves induced by serotonin in renal, femoral and mesenteric arteries were similar (149 ± 10%, 146 ± 10% and 155 ± 12%, respectively). After heat stress, the efficacy order of contractile curves was changed as follows: renal artery (246 ± 22%) > femoral artery (158 ± 9%) > mesenteric artery (148 ± 9%). In renal arteries, the contractile curve was changed from biphasic in the control to simple sigmoidal after heat stress, with a significantly decreased pEC50. In femoral artery, compared with the control, the contractile curve in heat stress rats was almost the same as that in the control (Figure 4, Table 1). In mesenteric artery, as we reported previously, the concentration–contraction curve was leftward shifted without a statistically changed E_{max} after heat stress.[7]

Comparison of CGRP-mediated relaxations in different regions of the arteries after heat stress

CGRP induced a minor relaxation on endothelium-denuded artery after precontraction with 20 μM serotonin in renal, femoral and mesenteric arteries of control rats (9.8 ± 2.1, 10.1 ± 1.7 and 7.4 ± 3.3%, respectively). After heat stress, the relaxation effects induced by CGRP were changed and the R_{max} order of relaxation curves was as follows: renal artery (42.9 ± 3.7%) > mesenteric artery (33.4 ± 4.7%) > femoral

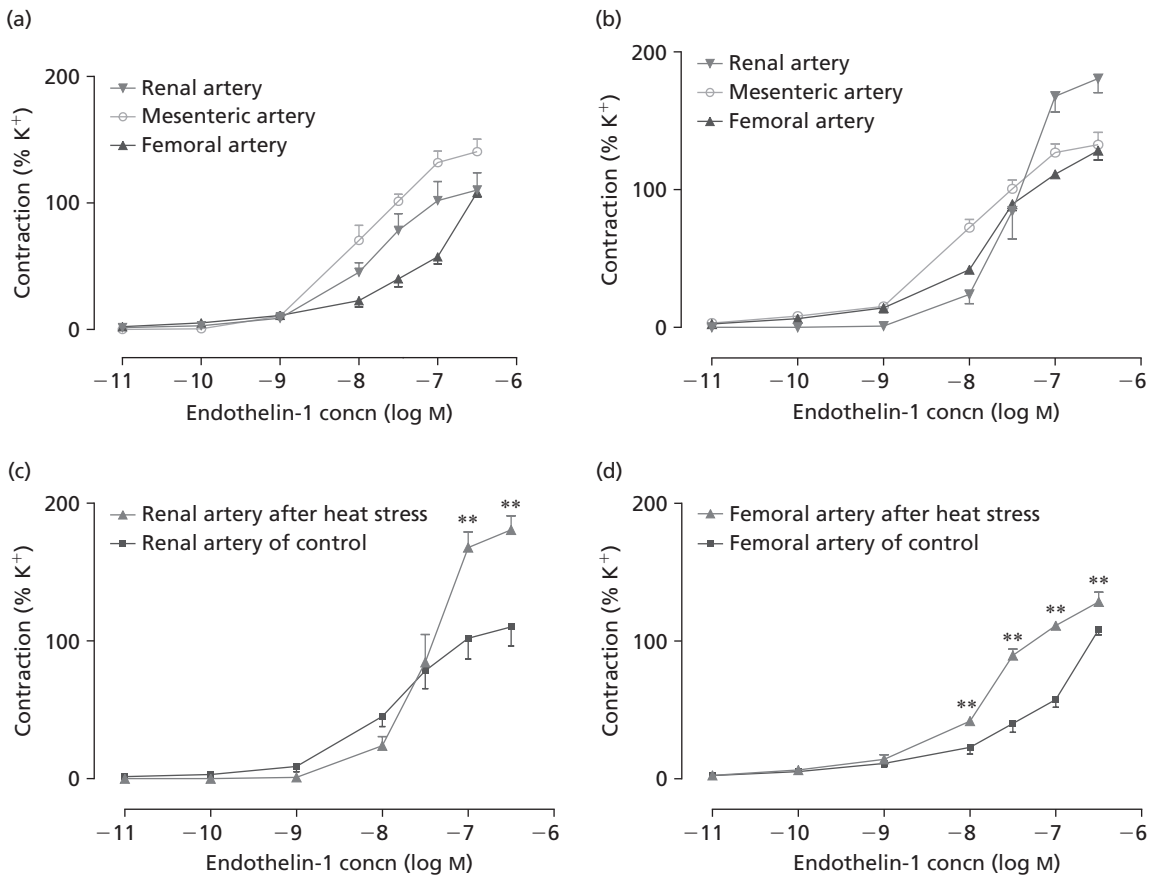

Figure 3 Concentration–contractile curves of endothelium-denuded artery segments induced by endothelin-1 in rats. Renal, mesenteric and femoral arteries were used. (a) Control rats; (b) after heat stress. Concentration–contractile curves of endothelium-denuded renal artery (c) and femoral artery (d). Data are given as mean ± SEM. Number of animals examined, n = 7–9. $^{**}P < 0.01$ compared with control

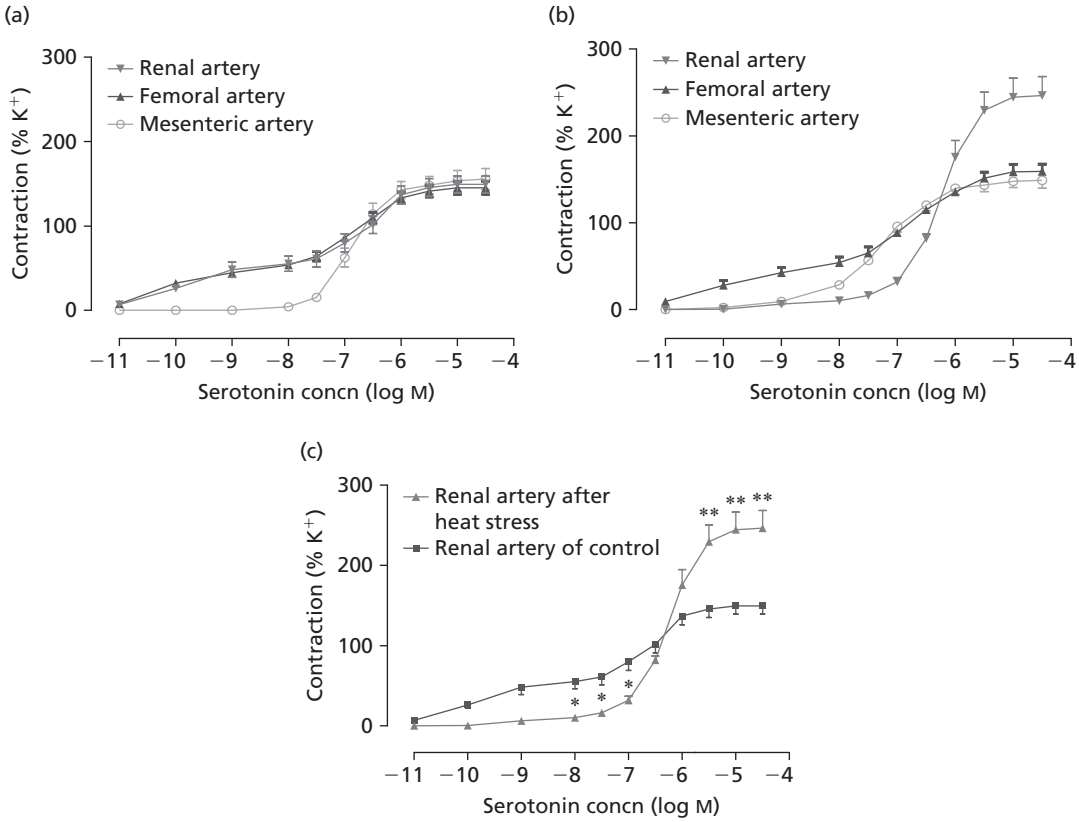

Figure 4 Concentration-contractile curves of endothelium-denuded artery segments induced by serotonin in rats. Renal, mesenteric and femoral arteries were used. (a) Control rats; (b) after heat stress. Concentration–contractile curves of endothelium-denuded renal artery (c). Data are given as mean ± SEM. Number of animals examined, n = 7–9. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control

artery (16.3 ± 3.1%). After heat stress, the relaxation curves of renal and mesenteric arteries were obviously leftward shifted, with increased R_{max} values. However, there were no significant changes in the relaxation curves of femoral artery between the control and heat stress groups (Figure 5, Table 1).

Discussion

Heat stress is able to enhance the viability of isolated cardiomyocytes in the metabolic condition-induced ischaemia *in vitro* and it evokes the release of neurotransmitters and autacoids.[11–14]

The α-adrenoceptor plays an important role in cardiovascular diseases. In the conscious rat, it is reported that an increase was observed in plasma catecholamine concentrations and myocardial noradrenaline turnover during heat stress, and α-adrenoceptor-mediated vasoconstriction in experimental ischaemia was enhanced.[15–17] This study has shown that phenylephrine induced a rightward-shifted contraction curve after heat stress in renal artery, with a hyporesponsiveness at lower concentrations and a hyperresponsiveness at higher concentrations. Phenylephrine (10^{-8}–$10^{-6.5}$ M) hardly induced any contraction. However, it elicited obvious contraction in the control at 10^{-8} M, as the physiological noradrenaline concentration in the plasma was approximately 10^{-8}–10^{-7} M, implying that the contractility of the renal artery mediated by adrenergic nerve was decreased obviously after heat stress, and the blood flow was enhanced. The contractile curve of femoral artery markedly shifted leftward after heat stress, showing that the vasomotor response mediated by α-adrenoceptor was increased and the blood flow should be decreased.

The endothelin ET_B receptor in the smooth muscle cells mediates vasoconstriction.[18,19] Most of the vessels have the ability to develop contractile effects mediated by endothelin ET_B receptors and this plasticity differs in vascular regions.[20] This study has shown that concentration–contractile curves mediated by ET_B receptors after heat stress were leftward shifted with a slightly increased E_{max} in the renal, femoral and mesenteric arteries. However, ET_A receptor-mediated contractile curve was unchanged in mesenteric artery after heat stress. In renal artery, the contractile curve induced by endothelin-1 after heat stress was almost the same as the control group, at 10^{-10}–$10^{-7.5}$ M, while the contractile effect was significantly increased at $10^{-7.5}$–$10^{-6.5}$ M. In femoral artery, the concentration–contractile curve induced by endothelin-1 after heat stress was leftward shifted, and the contraction increased from 10^{-9} M. As the physiological endothelin-1 concentration in the plasma is approximate to 10^{-9} M, the results showed that the contractile effect mediated by ET_A receptor was enhanced in femoral artery after heat stress and the blood flow should be decreased.

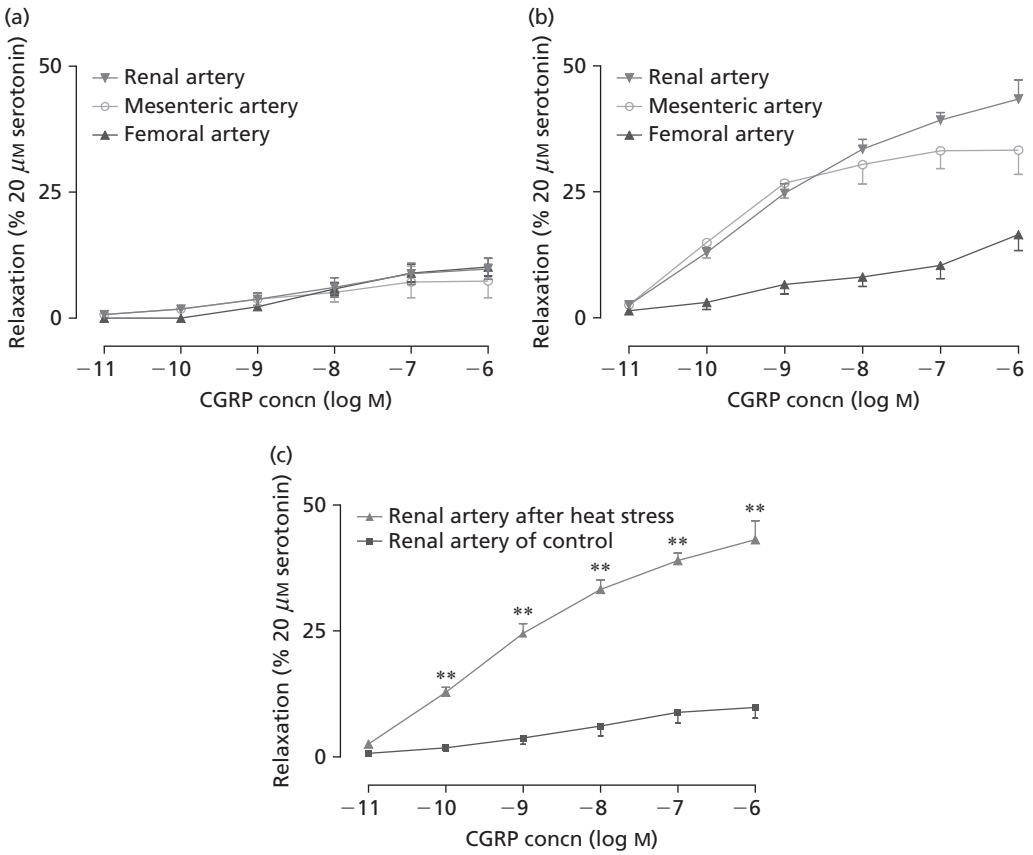

Figure 5 Concentration–relaxation curves induced by calcitonin gene-related peptide on endothelium-denuded artery segments precontracted with serotonin in rats. Renal, mesenteric and femoral arteries were used. (a) Control rats; (b) after heat stress. Concentration–relaxation curves induced by calcitonin gene-related peptide (CGRP) on endothelium-denuded renal artery (c). Data are given as mean ± SEM. Number of animals examined, n = 7–9. $^{**}P < 0.01$ compared with control

Serotonin, an autacoid, has been found to elicit enhanced contractile effects in some vascular disorders, and the physiological concentration in the plasma is approximately 10^{-7} M. This study demonstrated that there was a significantly increased contraction induced by serotonin at 10^{-8}–10^{-7} M in mesenteric artery after heat stress, revealing that the contractile effect was increased and the blood flow may have been decreased. In renal artery, the concentration–contractile curve induced by serotonin was changed from biphasic responses in the control to a sigmoidal curve after heat stress, suggestive of a subtype of serotonin receptor which was downregulated. The contractile curve induced by serotonin after heat stress was rightward shifted with the significantly increased E_{max} at a higher concentration, while the contractile effect was weak at the concentration of 10^{-10}–10^{-7} M, indicating the contractile effect mediated by serotonin receptor was decreased and the blood flow should have increased.

CGRP is a potent vasodilator and widely distributed in cardiovascular tissues. CGRP possesses protection of post-conditioning. Most blood vessels are surrounded by a dense perivascular CGRPergic neural network, which is found at the adventitia layer and the junction of the adventitia and the media.[21] Previous studies showed that heat stress evoked the release of CGRP.[22,23] The results showed that the relaxation curves induced by CGRP after heat stress were significantly leftward shifted on renal and mesenteric arteries with significantly increased $R_{max.}$ The efficacy of relaxation in renal artery was higher than in mesenteric artery. The relaxation curve was almost the same on femoral artery after heat stress. The results showed the vascular relaxation effects mediated by CGRP receptors were enhanced in renal and mesenteric arteries after heat stress and suggested the blood flow should be increased.

Conclusions

In renal artery, after heat stress, the contractile responses to the adrenoceptor agonist and serotonin (at lower concentration) were decreased while the CGRP-induced relaxation was enhanced. In femoral artery, the contractile effects mediated by endothelin ET_B-receptor, endothelin ET_A-receptors and α-adrenoceptors after heat stress were increased. In mesenteric artery, the contractile effects mediated by ET_B-receptors and serotonin receptors and the relaxation effect induced by CGRP after heat stress were enhanced. The alterations of the vascular contraction and relaxation in different regional

arteries were different. This might lead to a new balance of vascular resistances and the redistribution of blood flow in the different regions.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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