Experimental Physiology

Influence of heat stress on the reactivity of isolated chicken carotid artery to vasoactive agents

Yeshavanth K. Banasavadi Siddegowda[1], Marie Dennis Marcus Leo[1], Dinesh Kumar[1], Om Kumar Hooda[2], Vellanki Ravi Prakash[1] and Santosh K. Mishra[1]

[1]*Division of Pharmacology and Toxicology and* [2]*Division of Physiology and Climatology, Indian Veterinary Research Institute, Izatnagar-243122, Uttar Pradesh, India*

Cerebral ischaemia is considered to be an important cause of central nervous system dysfunction in heat stress. We hypothesized that heat stress would alter the reactivity of isolated carotid artery to vasoactive agents. Carotid arteries were isolated from broiler chickens maintained either at 23–24°C with 55–65% humidity (control conditions) or exposed to 40 ± 1°C with 35% humidity for 4 h (heat stress). Contractions were elicited with vasoconstrictors such as 5-HT, phenylephrine, guanfacine and $CaCl_2$ (K^+-depolarized) in endothelium-denuded arterial rings. Heat stress significantly increased the potency of 5-HT, but had no effect on the sensitivity of the vessel to phenylephrine or guanfacine. In contrast, it markedly decreased the potency and efficacy of $CaCl_2$. Vasodilator responses to ACh (endothelium-intact) and sodium nitroprusside (endothelium-denuded), however, were unaffected. Although cyclopiazonic acid (10 μM) significantly decreased 5-HT responses in both the conditions, the agonist was still more potent in heat stress. Extracellular Ca^{2+} removal had no effect on contractions caused by 5-HT in control conditions, but it significantly decreased the agonist potency in heat stress. Interestingly, nifedipine (1 μM) markedly inhibited 5-HT-induced contractions both in control conditions and in heat stress, implying an inhibitory effect on both Ca^{2+} influx and release. Thus, nifedipine had a markedly greater inhibitory effect on 5-HT-induced contractions in heat stress compared with control conditions. The results suggest that heat stress increased the vasoconstrictor responses to 5-HT by a mechanism that involved extracellular Ca^{2+} influx through nifedipine-sensitive L-type calcium channels.

(Received 5 June 2007; accepted after revision 23 August 2007; first published online 24 August 2007)
Corresponding author S. K. Mishra: Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar-243122, Uttar Pradesh, India. Email: smishraivri@rediffmail.com

Birds, being homeotherms like mammals, regulate their body temperature through a variety of physiological mechanisms. However, during heat stress due to high ambient temperature, failure of thermoregulatory mechanisms leads to high morbidity and mortality. Although high temperature has an effect on every organ of the body, the brain appears to be especially vulnerable to thermal damage (Baker, 1982; Simon, 1993). In mammals, for example, cerebral ischaemia is considered to be one of the important causes of central nervous system dysfunction in heat stroke (Lin & Lin, 1992). In comparison to other vascular beds, where small arteries and arterioles regulate blood flow, large arteries such as the vertebral and carotid arteries make an important contribution to total cerebral vascular resistance and are major determinants of microvascular pressure in the cerebral circulation (Faraci & Heistad, 1990). In a recent study, heating-induced constriction of isolated rabbit carotid artery and its responses to exogenously applied noradrenaline (NA) and potassium chloride (KCl) have been demonstrated (Mustafa *et al.* 2004). Based on these findings, the authors have postulated that hyperthermia *in vivo* may lead to a decrease in cerebral blood flow, leading to cerebral ischaemia and brain tissue damage. 5-Hydroxytryptamine (5-HT) and catecholamines are potent endogenous vasoconstrictors of carotid arteries in different species of animals (Lin & Lin, 1992; Vhora & Chiba, 1994; Mustafa *et al.* 2004). Although cerebral

 DOI: 10.1113/expphysiol.2007.038844

capillary blood flow in chickens is increased during hyperthermia (Wolfenson *et al.* 1981), there are no reports on the reactivity of any of the cerebral blood vessels in general and carotid artery in particular to acute heat stress in birds. The goal of the present study was therefore to examine the influence of short-term heat stress on the reactivity of isolated chicken carotid artery to vasoconstrictors such as 5-HT and to selective α_1- and α_2-adrenoceptor agonists such as phenylephrine (PE) and guanfacine, respectively.

Heat stress has been shown to increase endothelium-dependent relaxation in rat aorta, mesenteric and coronary arteries (Richard *et al.* 2002). Since endothelium plays an important role in regulating the vascular tone in the cerebral circulation, we examined the influence of heat stress on the reactivity of chicken carotid artery to acetylcholine (ACh) and sodium nitroprusside (SNP), which have been shown to dilate blood vessels through endothelium-dependent and -independent mechanisms, respectively.

Methods

Induction of heat stress

Experiments were performed in accordance with the guidelines approved by the Institutional Animal Care and Use Committee. Healthy male chickens of CSML broiler line, brought from the Central Avian Research Institute, Izatnagar were reared up to the age of 6–8 weeks under normal housing and feeding conditions. The birds, housed individually, were divided into two groups: (1) birds maintained at ambient temperature of 23–24°C with 55–65% humidity (control); and (2) birds exposed to heat stress for 4 h at 40 ± 1°C with 35% humidity (Bogin *et al.* 1996) at the Psychrometric Chamber of the Climatology Laboratory of the Indian Veterinary Research Institute.

Assessment of heat stress

Heterophil/lymphocyte ratio. Blood smear examination for the assessment of heat stress was done according to the method described previously (Altan *et al.* 2003). In brief, two drops of blood were collected from each bird from the brachial vein using a sterile syringe. Blood smears were immediately prepared on clean glass slides and air dried. A maximum of six blood smears were prepared before and after heat stress induction. The blood smears were stained immediately using Wright stain for 15 min. Heterophil/lymphocyte ratio was determined for each bird before and after heat stress induction.

Rectal temperature. Rectal temperature of individual birds was recorded using a digital thermometer (Medicare, Chandigarh, India), once before and once immediately after heat stress.

Malondialdehyde concentration. Erythrocytes are susceptible to oxidative stress as a result of the high polyunsaturated fatty acid content of their membranes (Cicha *et al.* 1999). Lipid peroxidation in erythrocytes was estimated in terms of malondialdehyde (MDA), the last product of lipid breakdown caused by oxidative stress. Malondialdehyde concentration was estimated by the thiobarbituric acid method (Ohkawa *et al.* 1979).

Tissue preparation and mechanical recording

Immediately after 4 h of heat exposure, birds were administered ketamine hydrochloride (20–100 mg kg^{-1}) intravenously and common carotid arteries were dissected out and transferred to oxygenated modified Krebs–Henseleit solution (MKHS) with the following composition (mM): 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.1 D-glucose, 11.9 $NaHCO_3$ and 2.5 $CaCl_2$, pH 7.4. Connective tissue and fat were carefully removed. The arteries were cut into 3-mm-long ring segments. These segments were mounted on two stainless-steel hooks made from 37 gauge stainless-steel wire and suspended in 10 ml organ baths containing MKHS, maintained at 37°C, and aerated continuously with 95% O_2–5% CO_2. Tension was continuously recorded using a high-sensitivity isometric force transducer and recorded in a computer using Chart version 5.4.1 software program (Powerlab, AD Instruments, Bella Vista, NSW, Australia). The segments were initially loaded to the optimum tension of 1.5 g, which was previously determined by using high-K^+ (80 mM) physiological saline solution (Composition mM: 39.2 NaCl, 78.8 KCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 11.1 D-glucose, 11.9 $NaHCO_3$ and 2.5 $CaCl_2$, pH 7.4.) as the contracting agent after applying different passive tensions. The arterial rings were allowed to equilibrate for 90 min, during which time they were washed every 10 min. The time interval between removing the birds from the psychrometric chamber, following 4 h of heat exposure, and the first assessment of the reactivity to vasoactive agents in isolated carotid arteries was about 2 h.

The endothelium was routinely removed from the arterial segments to avoid the influence of endothelial factors in modifying the reactivity of the vessels to exogenously applied vasoconstrictor agents. This was done by gently abrading the endothelial surface using a rough object such as horse tail hair. Some experiments were done in endothelium-intact rings to examine the influence of heat stress in endothelium-dependent relaxation. The presence or absence of the endothelium was verified by examining the response to ACh. In endothelium-intact rings, ACh caused > 80% relaxation. Arterial rings were considered endothelium denuded when no relaxation was evident in response to ACh.

Experimental protocol for tension experiments

Assessment of vasoconstrictors. After the equilibration period, viability of arterial rings was examined by stimulating them with high-K^+ (80 mM) depolarizing solution (similar to MKHS except that KCl replaced NaCl on equimolar basis) repeatedly until two identical contractions were obtained. The peak tension achieved with K^+ depolarizing solution was set as 100% for expressing the tension generated by vasoconstrictor agonists. Agonists, such as 5-HT, phenylephrine (α_1-adrenoceptor agonist) and guanfacine (α_2-adrenoceptor agonist), were added cumulatively to elicit concentration-dependent contractions in isolated carotid artery rings, taken from control and heat-stressed broiler chickens. For eliciting contraction with $CaCl_2$ in K^+-depolarized carotid artery rings, tissues were washed several times with Ca^{2+}-free (EGTA, 1.0 mM) MKHS before exposing them to nominally Ca^{2+}-free (no EGTA), K^+ (80 mM) depolarizing solution. Thirty minutes after equilibration in K^+ depolarizing medium, $CaCl_2$ was added cumulatively to elicit concentration-dependent contractions. The maximal concentration of $CaCl_2$ used in these experiments was 10 mM. Concentrations > 10 mM of $CaCl_2$ caused precipitation and were therefore not used.

In order to examine the influence of extracellular Ca^{2+} on agonist-induced contraction, two different protocols were used, as follows: (1) agonist-induced contractions were elicited either in Ca^{2+}-free (1 mM EGTA) medium; or (2) in the presence of nifedipine (1 μM) in normal (2.5 mM $CaCl_2$) MKHS. The role of intracellular Ca^{2+} store depletion in carotid artery vasoconstriction was assessed by using the selective inhibitor of the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA), cyclopiazonic acid (CPA).

Assessment of vasodilators. A submaximal concentration of phenylephrine (3 μM, causing ~60% of KCl [80 mM] contraction) was added to the bath to preconstrict endothelium-intact carotid artery rings obtained from control or heat-stressed birds. After the contraction reached steady state, cumulative concentration–response curves for ACh were determined. The influence of heat stress on the reactivity of carotid artery rings to SNP was investigated using endothelium-denuded preparations. The arterial rings were preconstricted with phenylephrine (3 μM), and when the contraction reached a steady state, cumulative concentration–response curves for SNP were elicited.

Drugs

5-Hydroxytryptamine, phenylephrine, guanfacine, cyclopiazonic acid, nifedipine, acetylcholine chloride and sodium nitroprusside were from Sigma Chemical (St. Louis, MO, USA). Cyclopiazonic acid was dissolved in DMSO and nifedipine was dissolved in absolute alcohol. All other chemicals were dissolved in distilled water.

Statistics

Responses to vasoconstrictor agonists were expressed as a percentage of K^+ (80 mM)-induced contraction. Vasodilator responses were expressed as the percentage reversal of contraction induced by 3 μM phenylephrine. The EC_{50} (the concentration producing half maximal response) and E_{max} (the maximal response) of different agonists were determined by non-linear regression using Graphpad Prism 4.0 (San Diego, CA, USA). Two-way ANOVA followed by Bonferroni *post hoc* test was used for statistical comparison between groups. The pD_2 (mean negative logarithm of molar concentration producing 50 percent of maximal response) values were determined by the formula: $pD_2 = -\log EC_{50}$. Student's unpaired t test was used to determine statistical significance at $P < 0.05$.

Results

Effect of heat stress on rectal temperature and leucocyte count

The mean rectal temperature of the chickens before induction of heat stress was 41.5 ± 0.20°C. Exposure of the chickens to high ambient temperature (40 ± 1°C with 35% humidity) for 4 h increased the rectal temperature significantly ($P < 0.05$) to 43.75 ± 0.17°C ($n = 6$). Heat-stressed birds exhibited significantly ($P < 0.05$) reduced lymphocyte (70.00 ± 0.44%) and increased heterophil count (21.5 ± 0.41%), compared with the lymphocyte (79.13 ± 0.41%) and heterophil counts (13.91 ± 0.28%) before heat-stress induction. As a result, the heterophil/lymphocyte ratio showed a significant ($P < 0.05$) increase from 0.17 ± 0.004 to 0.30 ± 0.004 ($n = 6$).

Effect of heat stress on malondialdehyde concentration

Heat stress significantly ($P < 0.05$) increased lipid peroxidation, as indicated by a rise in malondialdehyde concentration from 3.01 ± 0.26 nmol ml^{-1} before heat exposure to 4.22 ± 0.15 nmol ml^{-1} after heat treatment ($n = 6$).

Effect of heat stress on vasoconstrictor responses

Cumulatively added 5-HT (10^{-9}–10^{-5} M) at increments of 0.5 log unit caused concentration-dependent contractions in endothelium-denuded carotid artery rings both from control and from heat-stressed chickens (Fig. 1*A*). Heat stress significantly ($P < 0.05$) increased the potency (pD_2),

but had no effect on the efficacy (E_{max}) of 5-HT, when compared with the control arteries (Table 1).

The α_1-adrenergic agonist phenylephrine (10^{-9} to 3×10^{-5} M) and α_2-adrenergic agonist guanfacine (10^{-9} to 3×10^{-5} M) caused concentration-dependent contractions of endothelium-denuded carotid artery rings (Fig. 1*B* and *C*, respectively). Heat stress had no significant effect on the potency and efficacy of these two adrenergic agonists (Table 1).

The influence of heat stress on contraction in response to $CaCl_2$ in K^+ (80 mM)-depolarized carotid artery rings is shown in Fig. 1*D* and Table 1. $CaCl_2$ (10^{-5}–10^{-2} M), added cumulatively, caused concentration-dependent contraction of endothelium-denuded carotid artery rings. Heat stress significantly ($P < 0.05$) decreased the potency and efficacy of vasoconstrictor responses to $CaCl_2$. Pre-incubation with the L-type Ca^{2+} channel blocker nifedipine (1 μM) abolished contractions elicited by $CaCl_2$ in K^+-depolarized tissues obtained either from control or from heat-stressed chickens (Fig. 2).

In order to examine the contribution of intracellular Ca^{2+} release to the increased potency of 5-HT in carotid arteries from heat-stressed animals, CPA, an inhibitor of SERCA, was employed. In endothelium-denuded carotid arteries, application of CPA (10 μM) in the continuous presence of extracellular Ca^{2+} had variable effects on the

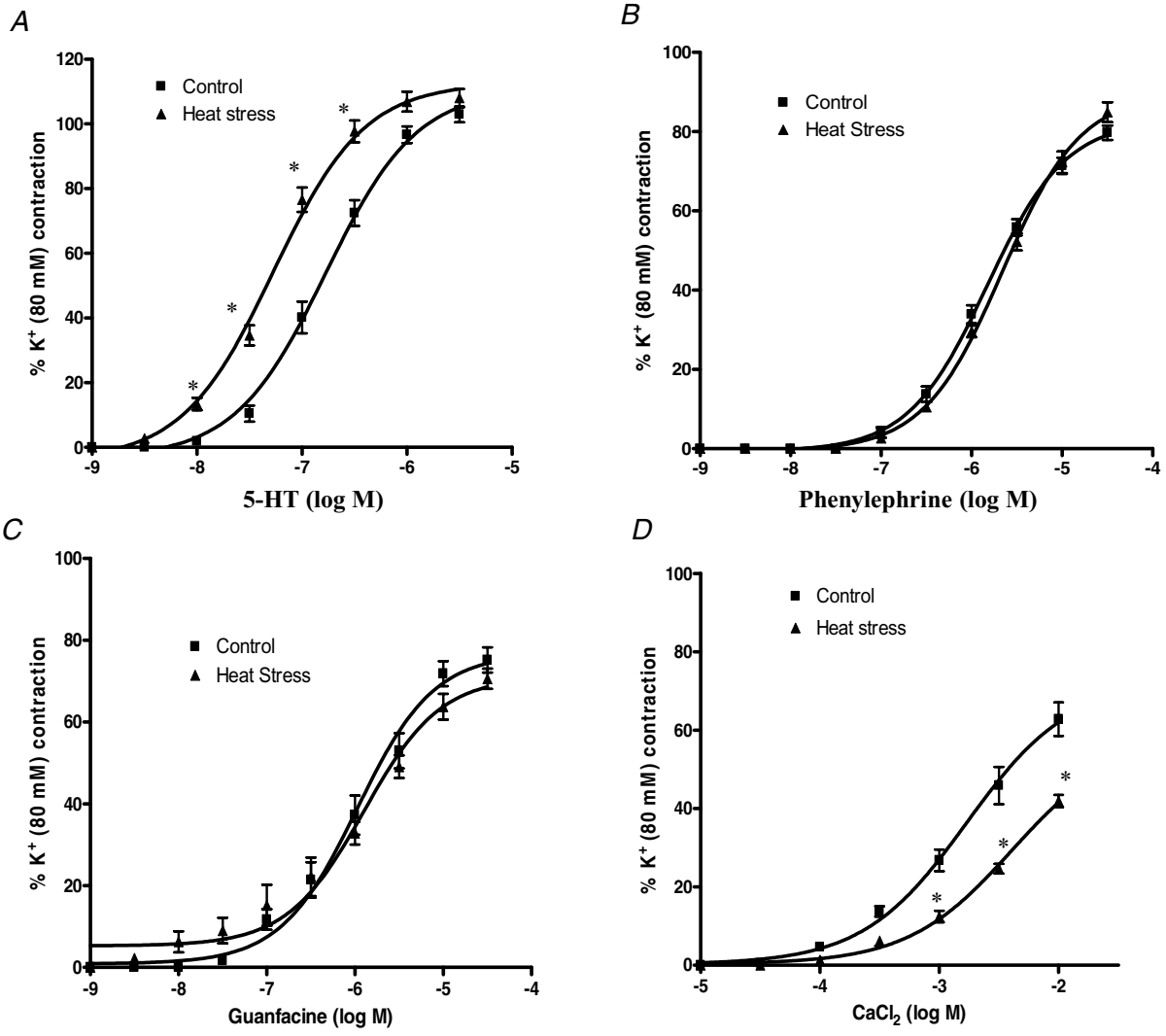

Figure 1. Effect of heat stress on concentration-dependent contractions elicited by 5-HT (10^{-9} to 3×10^{-6} M; *A*), phenylephrine (10^{-9} to 3×10^{-5} M; *B*), guanfacine (10^{-9} to 3×10^{-5} M) and $CaCl_2$ (10^{-5}–10^{-2} M in 80 mM K^+-depolarized tissues), added cumulatively on endothelium-denuded, isolated carotid arteries

Agonist-induced contractions were expressed as a percentage of the maximal contraction induced by KCl (80 mM). Vertical bars represent S.E.M. Data were analysed by two-way ANOVA, followed by Bonferroni *post hoc* test. *$P < 0.05$ in comparison to control conditions.

Table 1. Influence of heat stress on pD_2 and E_{max} values of vasoactive agents

	pD_2		E_{max} (%)	
Vasoactive agents	Control	Heat stress	Control	Heat stress
Vasoconstrictors				
5-HT	6.77 ± 0.05 ($n = 12$)	7.27 ± 0.04* ($n = 11$)	111.0 ± 2.71	112.7 ± 2.00
Phenylephrine	5.83 ± 0.03 ($n = 10$)	5.66 ± 0.03 ($n = 9$)	82.83 ± 1.38	89.64 ± 1.57
Guanfacine	5.97 ± 0.07 ($n = 8$)	5.90 ± 0.09 ($n = 6$)	76.81 ± 2.53	71.12 ± 2.88
$CaCl_2$	2.77 ± 0.09 ($n = 15$)	2.39 ± 0.07* ($n = 8$)	72.52 ± 4.47	58.27 ± 3.47*
Vasodilators				
ACh(+endothelium)	6.65 ± 0.07 ($n = 9$)	6.78 ± 0.12 ($n = 12$)	139.6 ± 5.03	143.2 ± 9.61
SNP(−endothelium)	6.63 ± 0.07 ($n = 6$)	6.68 ± 0.07 ($n = 6$)	116.6 ± 4.17	125.2 ± 5.30

Concentration–response curves for vasoconstrictors were elicited in endothelium-denuded carotid arteries. Potassium chloride (80 mM) contraction was used as the reference and set at 100% for calculation of the percentage contraction elicited by different contractile agonists. The E_{max} values for vasodilators were calculated from the percentage reversal of phenylephrine (3 μM) contraction. The pD_2 and E_{max} values were calculated from sigmoidal dose–response curves fitted with non-linear regression using GraphPad Prism 4.0. *$P < 0.05$; numbers in parentheses refer to the number of carotid artery rings from at least three or four chickens.

basal tone, such that no change in tone was observed in control tissues (Fig. 3*A*), while a slow and sustained rise in tension, reaching ∼20% of K^+ (80 mM)-induced contraction, was evident in arterial rings from heat-stressed birds (Fig. 3*B*). Cyclopiazonic acid significantly ($P < 0.05$) decreased vasoconstrictor responses to 5-HT in arteries from both control and heat-stressed birds (Fig. 3*A*, *B* and *C*). Removal of extracellular Ca^{2+} or pretreatment of the tissues with nifedipine (1 μM) in the continued presence of CPA abolished 5-HT-induced contractions (data not shown). In the presence of CPA alone, however, arterial rings from the heat-stress conditions were more sensitive to the contractile action of 5-HT in comparison to control rings (Table 2).

The influence of Ca^{2+}-free solution (Ca^{2+}-free MKHS containing 1.0 mM EGTA) and nifedipine (1 μM) on contraction elicited with cumulative concentrations of 5-HT (10^{-9}–10^{-5} M) on carotid artery rings from control and heat-stress conditions is presented in Fig. 4. Removal of extracellular Ca^{2+} had no significant effect on contraction induced by 5-HT when compared with that observed in the presence of 2.5 mM Ca^{2+} in control rings (Fig. 4*A*). In the heat-stress conditions, however, extracellular Ca^{2+} removal caused a significant rightward shift in the concentration–response curve of 5-HT in comparison to that obtained in the presence of Ca^{2+} (2.5 mM) in the medium (Fig. 4*B*). Interestingly, in the absence of Ca^{2+}, 5-HT was equipotent in contracting arteries from normothermic control, as well as heat-stressed birds (Table 2).

Pre-incubation of tissues with nifedipine (1 μM) for 30 min markedly decreased the vasoconstrictor responses of 5-HT (10^{-9}–10^{-5} M) in carotid artery rings from control, as well as heat-stressed birds (Fig. 4). In the presence of nifedipine, however, the potency and efficacy of 5-HT to contract the arteries from heat-stressed birds were significantly ($P < 0.05$) reduced in comparison to control rings (Table 2).

Effect of heat stress on vasodilator responses

The influence of heat stress on endothelium-dependent relaxation induced by ACh and endothelium-independent relaxation induced by SNP on carotid artery rings is illustrated in Fig. 5. Acetylcholine (10^{-9} to 3×10^{-5} M) caused concentration-dependent relaxation of carotid artery rings preconstricted with phenylephrine (3 μM). Endothelium removal abolished the vasodilator responses to ACh. Further, pretreatment of endothelium-intact tissues with L-NAME (1 mM) or 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (10 μM) caused almost 1 log unit rightward shift in the concentration–response curve elicited with ACh (data not shown). In arterial rings preconstricted with phenylephrine (3 μM), SNP (10^{-9} to 3×10^{-6} M), a nitrovasodilator, caused concentration-dependent relaxation of endothelium-denuded arterial rings. Heat stress had no significant effect on the efficacy and potency of either ACh or SNP in dilating these vessels (Table 1).

Discussion

The goal of the present study was to analyse the effect of acute heat stress on the constrictor and dilator responses of isolated chicken carotid artery to a variety of vasoactive agents. The results with the constrictors suggest that acute heat stress significantly increased the sensitivity of isolated arterial rings to 5-HT, but had no effect on the reactivity of the vessels to α_1- and α_2-adrenergic agonists such as phenylephrine

and guanfacine, respectively. Further, the potency and efficacy of $CaCl_2$ in causing contraction of high-K^+-depolarized arterial preparations were markedly reduced in heat-stress conditions. However, vasodilator responses to ACh (endothelium-dependent vasodilatation) and SNP (endothelium-independent vasodilatation) were not influenced by heat stress.

5-Hydroxytryptamine is a potent vasoconstrictor in different blood vessels, and has been specifically implicated in vascular disorders such as stroke and cerebral vasospasm (Saxena & Villalon, 1990; Vanhoutte, 1990). Interestingly, the increased reactivity of carotid artery to 5-HT in heat stress, as observed in the present study, may indicate that this endogenous amine may play a crucial role in decreasing cerebral blood flow. Intracellular Ca^{2+} is one of the important determinants of the altered reactivity of the vascular smooth muscle to constrictor agents. Therefore, we investigated smooth muscle Ca^{2+} handling by 5-HT as influenced by heat stress. 5-Hydroxytryptamine-induced contractions in vascular smooth muscles are known to be dependent on either sarcoplasmic reticulum IP_3-sensitive Ca^{2+} channels or Ca^{2+} influx from the extracellular fluid through sarcolemmal voltage-dependent Ca^{2+} channels (Yuan *et al.* 1997; Nazarov *et al.* 2000; Wilson *et al.* 2005). Removal of extracellular Ca^{2+} had virtually no effect on contraction elicited by 5-HT in control arterial rings. However, extracellular Ca^{2+} removal significantly decreased the potency of 5-HT in arterial rings from heat-stress conditions. These results suggest that heat stress alters 5-HT-mediated Ca^{2+} handling by the tissue, such that contraction elicited by the agonist is primarily dependent on intracellular Ca^{2+} release in control conditions, while an additional mechanism involving extracellular Ca^{2+} influx is operational in heat-stress conditions. The contribution of intracellular Ca^{2+} release to the vasoconstrictor action of 5-HT in this arterial smooth muscle is further evident from the observation that cyclopiazonic acid, a selective inhibitor of SERCA, markedly reduced vasoconstrictor responses to the agonist. The results of the present study are consistent with observations made on several blood vessels (Ratz & Flaim, 1984; Nakaki *et al.* 1985; Cohen & Wittenauer, 1987). Intriguingly, the concentration of nifedipine that abolished Ca^{2+}-induced contractions in K^+-depolarized carotid arteries significantly decreased the contraction caused by 5-HT both in control and in heat-stress conditions. There are some reports showing that dihydropyridine calcium channel blockers interact with targets other than L-type calcium channels (Corsini *et al.* 1996; Hirasawa & Pittman, 2003; Orth *et al.* 1996). Amlodipine, for instance, inhibited thapsigargin-sensitive Ca^{2+} stores in thrombin-stimulated vascular smooth muscle cells (Stepien & Marche, 2000). The role of Ca^{2+} influx through L-type calcium channels is evident when $[Ca^{2+}]_i$ release is inhibited by CPA. Thus, we observed that CPA-resistant contractile responses to 5-HT were abolished either in Ca^{2+}-free medium or in the presence of nifedipine. These results suggest that Ca^{2+} release by itself is enough to fully support the contraction, and that the Ca^{2+} influx may be surplus to requirements (hence the Ca^{2+}-free/EGTA data), thereby supporting the hypothesis that 5-HT is stimulating both Ca^{2+} influx and release in chicken carotid artery.

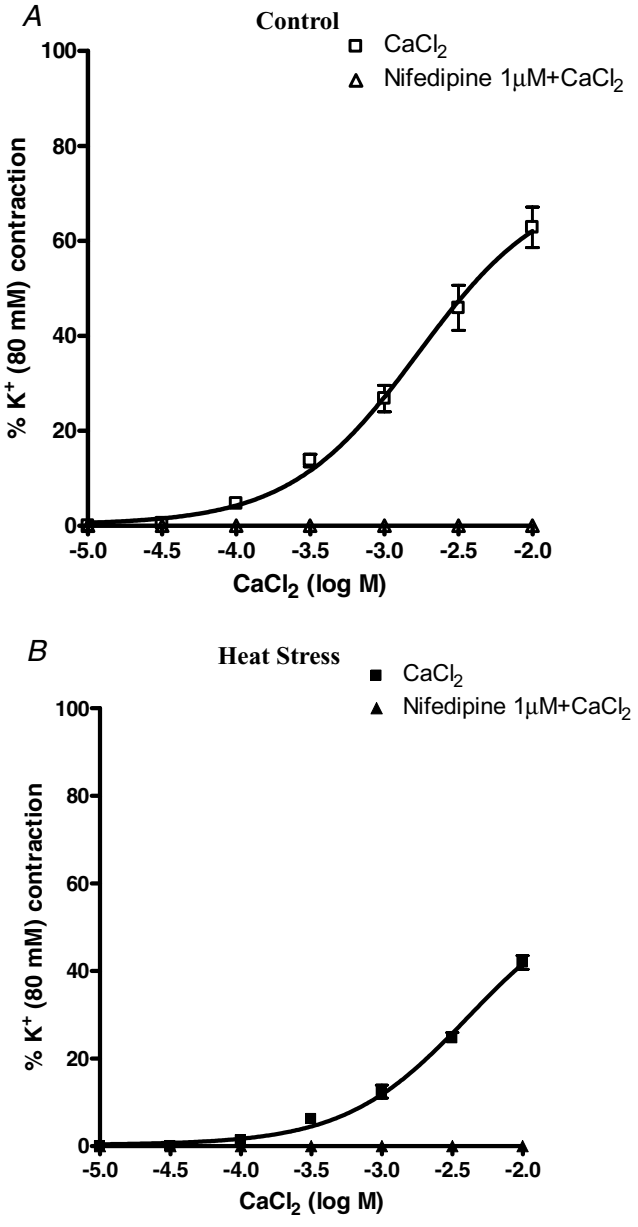

Figure 2. Effect of nifedipine (1 μM) on contractions elicited by $CaCl_2$ (10^{-5}–10^{-2} M) in 80 mM K^+-depolarized carotid arteries of control (*A*) and heat-stressed birds (*B*)
Note the complete inhibition of $CaCl_2$-induced contractions by nifedipine. Vertical bars represent S.E.M.

Removal of extracellular Ca^{2+} abolished the increased vasoconstrictor potency of 5-HT, thereby implying a role for extracellular Ca^{2+} in determining the agonist

Table 2. Influence of heat stress on Ca^{2+} handling by 5-HT in carotid arteries

	pD_2		E_{max} (%)	
Treatments	Control	Heat stress	Control	Heat stress
5-HT	6.77 ± 0.05 ($n = 12$)	7.27 ± 0.04^{b} ($n = 11$)	111.0 ± 2.71	112.7 ± 2.00
Ca^{2+} free +1 mM EGTA + 5-HT	6.81 ± 0.11 ($n = 6$)	6.86 ± 0.06^{a} ($n = 10$)	106.7 ± 6.16	108.5 ± 3.5
Cyclopiazonic acid (10 μM) + 5-HT	6.48 ± 0.12^{a} ($n = 6$)	$7.04 \pm 0.05^{a,b}$ ($n = 7$)	73.83 ± 5.48^{a}	78.48 ± 1.73^{a}
Nifedipine (1 μM) + 5-HT	6.57 ± 0.05^{a} ($n = 6$)	$6.28 \pm 0.08^{a,b}$ ($n = 7$)	78.04 ± 2.14^{a}	$61.19 \pm 3.61^{a,b}$

The pD_2 and E_{max} values for 5-HT in different conditions were calculated from sigmoidal dose–response curves fitted with non-linear regression using GraphPad Prism 4.0. $^{a}P < 0.05$ in comparison to 5-HT; $^{b}P < 0.05$ in comparison to control arterial rings; numbers in parentheses refer to the number of carotid artery rings from at least three or four chickens.

sensitivity in the heat-stress conditions. Interestingly, nifedipine significantly decreased the sensitivity as well as the efficacy of 5-HT in arterial rings from heat-stressed birds in comparison to control birds. This observation suggests that nifedipine decreases intracellular Ca^{2+} release as well as Ca^{2+} influx in carotid arteries during heat stress. In contrast to agonist-stimulated Ca^{2+} influx, the Ca^{2+} influx (probably through L-type calcium channels) stimulated by K^{+}-induced depolarization appears to be reduced in heat stress, which is evident from decreased

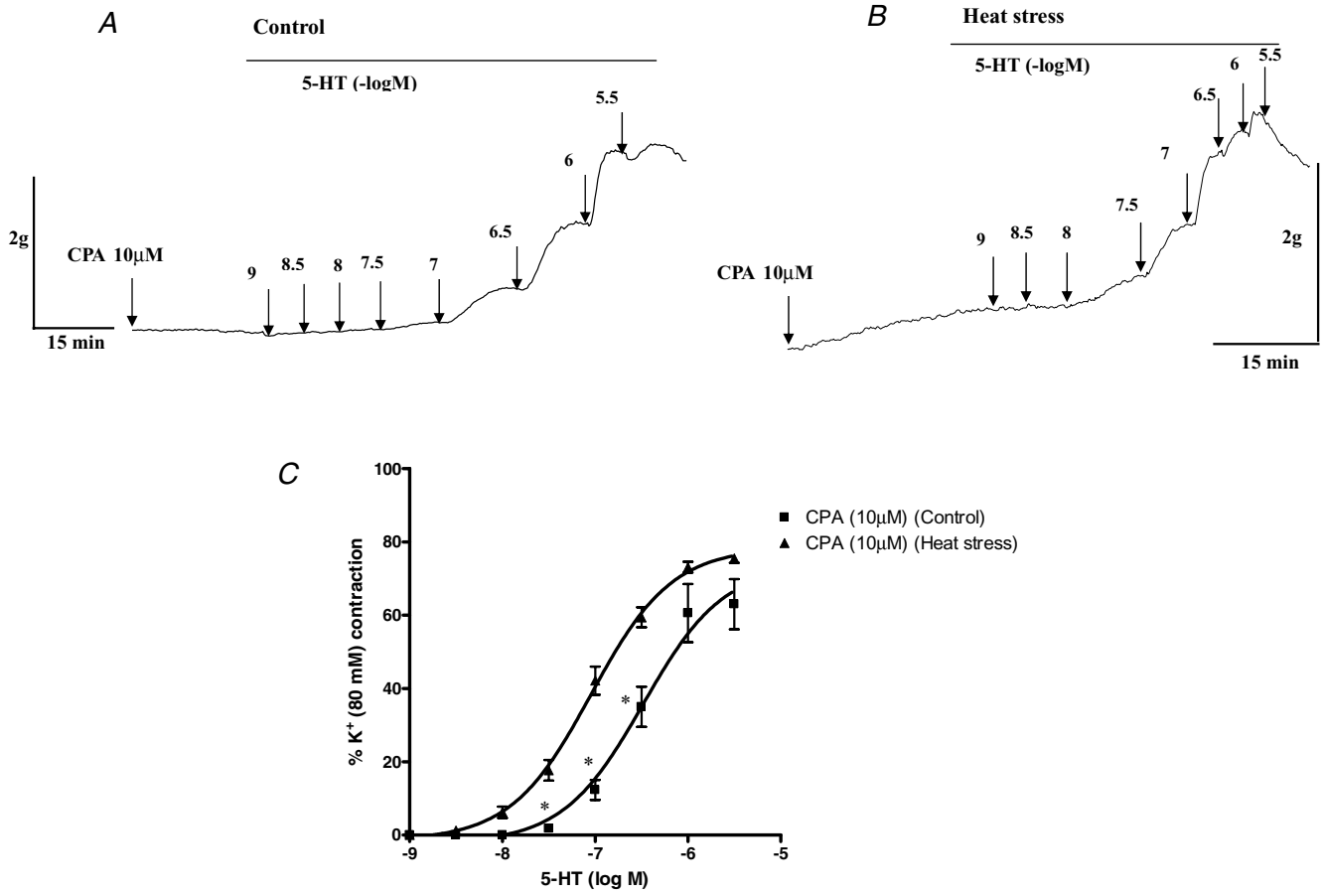

Figure 3. Effect of CPA (10 μM) on the sensitivity of 5-HT (10^{-9} to 3×10^{-6} M)-induced contractions in heat-stress conditions compared with control conditions

Raw traces show the effect of 30 min pretreatment of the endothelium-denuded carotid artery rings with CPA on basal tone and concentration-dependent contractions produced by 5-HT in control (*A*) and heat-sress conditions (*B*). *C* shows the mean concentration–response curves for 5-HT elicited in the presence of CPA. The 5-HT-induced contractions were expressed as a percentage of the maximal contraction induced by KCl (80 mM). Vertical bars represent S.E.M. Data were analysed by two-way ANOVA, followed by Bonferroni *post hoc* test. $^{*}P < 0.05$ in comparison to control conditions.

reactivity of carotid arteries to $CaCl_2$ in the K^+-depolarized preparation. This finding contradicts the observation made in rabbit carotid artery, where heating the blood vessel to 41–43°C enhanced the contractile responses to KCl (Mustafa *et al.* 2004). This discrepancy can be explained on the basis of the different experimental protocols followed to assess vascular reactivity to KCl. For example, reactivity of chicken carotid artery was determined following short-term heat exposure (40°C for 4 h) of birds, while the effect of *in vitro* hyperthermia (temperatures of 41–43°C) was studied in rabbit carotid artery. Another important difference is that we studied the vasoconstrictor responses in endothelium-denuded arterial rings, while in the case of rabbit carotid artery,

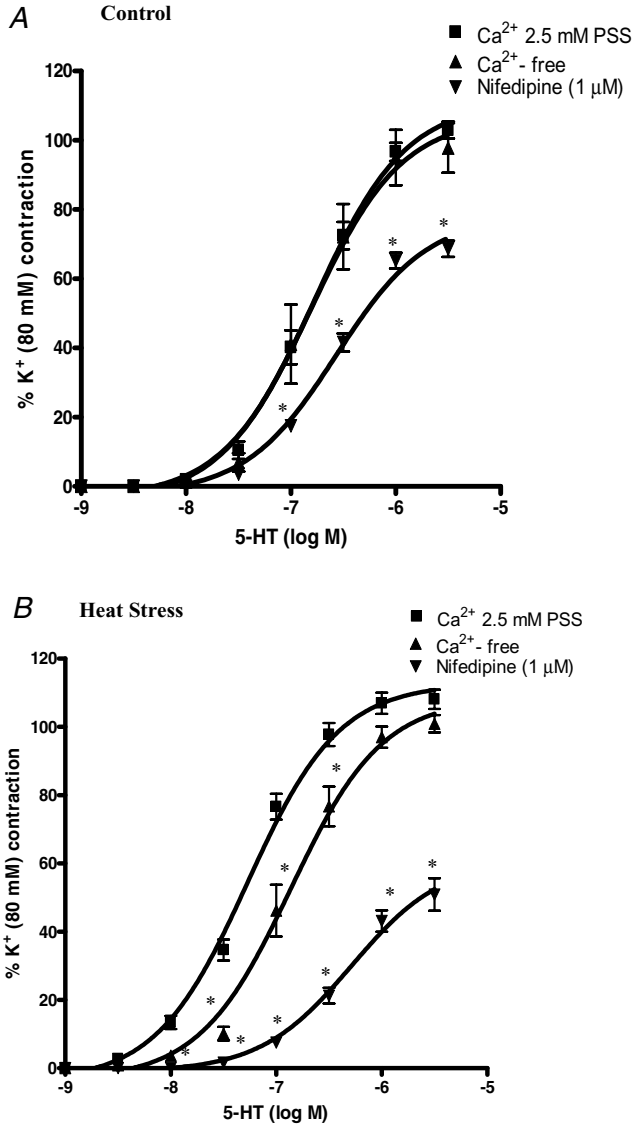

Figure 4. Effect of extracellular Ca^{2+} removal or nifedipine (1 μM) on the sensitivity of 5-HT (10^{-9} to 3×10^{-6} M)-induced contractions in control (*A*) and heat-stress conditions (*B*)
The effect of 30 min exposure to Ca^{2+}-free (EGTA 1 mM) solution or pretreatment with nifedipine for 30 min on concentration-dependent contractions elicited by cumulatively added 5-HT in endothelium-denuded carotid arteries from control and heat-stress conditions. The 5-HT-induced contractions were expressed as a percentage of the maximal contraction induced by KCl (80 mM). Vertical bars represent S.E.M. PSS, physiological salt solution. Data were analysed by two-way ANOVA, followed by Bonferroni *post hoc* test. *$P < 0.05$ in comparison to Ca^{2+} 2.5 mM PSS.

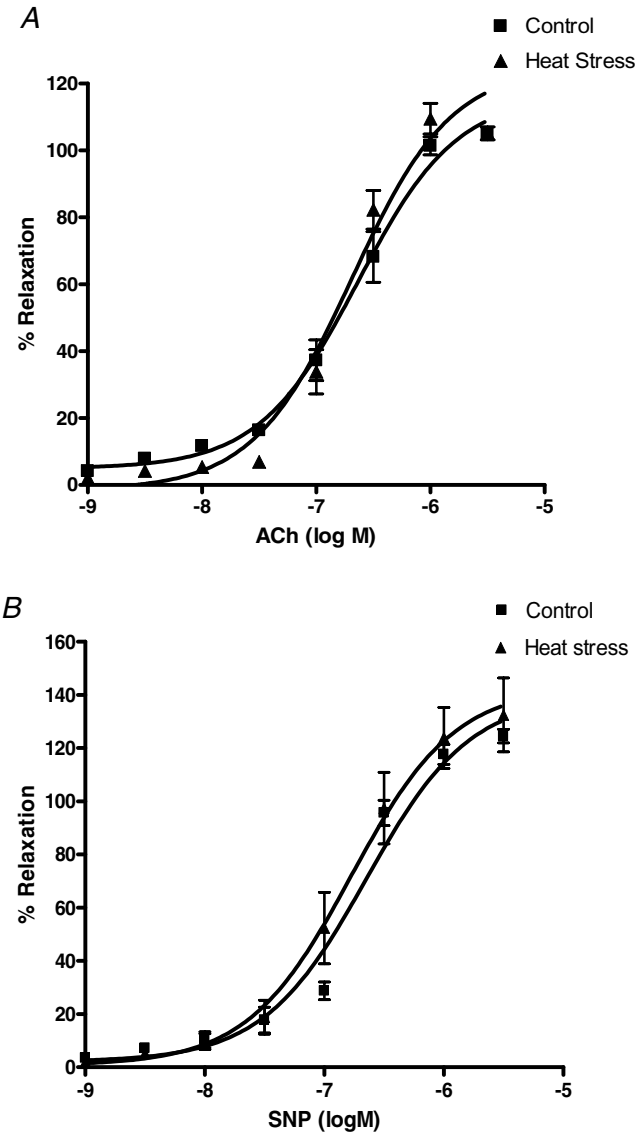

Figure 5. Effect of heat stress on the vasodilator responses to ACh (10^{-9} to 3×10^{-5} M; *A*) and SNP (10^{-9} to 3×10^{-6} M; *B*) in endothelium-intact (*A*) and endothelium-denuded (*B*) carotid artery rings, respectively
Arterial rings were preconstricted with phenylephrine (3 μM), and different concentrations of vasodilators were added cumulatively to the sustained phase of contraction. Reversal of phenylephrine contraction was used to calculate the percentage relaxation. Vertical bars represent S.E.M. Data were analysed by two-way ANOVA, followed by Bonferroni *post hoc* test.

contractile responses to vasoactive agents were examined in the presence of vascular endothelium. The role of endothelium in determining the potency of KCl to heating has been demonstrated in rat mesenteric artery. For example, heating to 41°C significantly enhanced the contractile response of KCl in endothelium-intact but not in endothelium-denuded rat mesenteric artery (Massett *et al.* 1998*b*). The contribution of $[Ca^{2+}]_i$ to increased reactivity is ruled out by the finding that 5-HT was more potent in contracting carotid arteries from heat-stressed birds in comparison to control birds in the presence of CPA, which depletes $[Ca^{2+}]_i$. It is therefore suggested that extracellular Ca^{2+} influx involving a nifedipine-sensitive mechanism is crucial in increasing the reactivity of carotid artery to 5-HT in heat stress.

Heating has been reported to decrease (Mustafa *et al.* 2004) or have no effect (Massett *et al.* 1998*b*) on the contractile responses to α-adrenergic agonists, such as noradrenaline and phenylephrine. Further, dose-dependent increase in mean arterial pressure in response to noradrenaline, phenylephrine and adrenaline was attenuated by increasing the core temperature of rats to 41.5°C (Massett *et al.* 1998*a*). We observed that the reactivity of chicken carotid artery to the α_1-adrenergic agonist phenylephrine and the α_2-adrenergic agonist guanfacine were not influenced by heat stress. It is therefore suggested that adrenergic agonists may not have a significant role in determining carotid blood flow during heat stress.

Hyperthermia has been reported to have variable influence on the endothelium-dependent relaxation in different vascular beds. For example, increasing the core temperature to 41.5°C in rats had no significant effect on the haemodynamic responses (mean arterial blood pressure, mesenteric artery resistance and hindlimb blood flow) to ACh, but ACh-evoked relaxation of renal artery was enhanced (Massett *et al.* 1998*a*). In rabbit femoral artery, *in vitro* hyperthermia had no effect on ACh-induced endothelium-dependent relaxation, but relaxation caused by SNP was enhanced (Padilla *et al.* 1998). We observed that carotid artery relaxation caused by either ACh (endothelium-dependent relaxation) or SNP (endothelium-independent relaxation) was not altered by heat stress. It is therefore suggested that endothelium/NO signalling may have little contribution in the heat-stress-induced alteration of carotid artery reactivity.

In summary, the results of the present study demonstrate that the reactivity of chicken carotid artery to 5-HT, but not that to α-adrenergic agonists, is enhanced in acute heat-stress conditions. Removal of extracellular Ca^{2+} abolished the increased arterial sensitivity to 5-HT in heat stress. Interestingly, in the presence of nifedipine, the sensitivity as well as the efficacy of 5-HT was markedly decreased in arteries from heat-stress conditions in comparison to control conditions. Intracellular Ca^{2+} depletion with CPA had no significant effect on the increased vasoconstrictor potency of 5-HT in heat-stress conditions, thereby implying little role for $[Ca^{2+}]_i$ in determining the altered vascular reactivity. The inhibition of responses to $CaCl_2$ in K^+-depolarized tissues implicates a decrease in L-type calcium channel function in heat-stress conditions. Vascular endothelium appears to have no significant role in carotid artery blood flow in heat stress. Further studies are required to elucidate the role of endogenous 5-HT in determining cerebral circulation in hyperthermia.

References

Altan O, Pabuccuoglu A, Altan A, Konyalioglu S & Bayraktar H (2003). Effect of heat stress on oxidative stress, lipid peroxidation and some stress parameters in broilers. *Br Poult Sci* **44**, 545–550.

Baker MA (1982). Brain cooling in endotherms in heat and exercise. *Ann Rev Physiol* **44**, 85–96.

Bogin E, Avidar Y, Pech-Waffenschmidt V, Doron Y, Israeli BA & Kevkhayev E (1996). The relationship between heat stress, survivability and blood composition of the domestic chicken. *Eur J Clin Chem Clin Biochem* **34**, 463–469.

Cicha I, Suzuki Y, Tateishi N & Maeda N (1999). Rheological changes in blood in human red blood cells under oxidative stress. *Pathophysiology* **6**, 103–110.

Cohen ML & Wittenauer LA (1987). Serotonin receptor activation of phosphoinositide turnover in uterine, fundal, vascular, and tracheal smooth muscle. *J Cardiovasc Pharmacol* **10**, 176–181.

Corsini A, Bonfatti M, Quarato P, Accomazzo MR, Raiteri M, Sartani A, Testa R, Nicosia S, Paoletti R & Fumagalli R (1996). Effect of the new calcium antagonist lercanidipine and its enantiomers on the migration and proliferation of arterial myocytes. *J Cardiovasc Pharmacol* **28**, 687–694.

Faraci FM & Heistad DD (1990). Regulation of large cerebral arteries and cerebral microvascular pressure. *Circ Res* **66**, 8–17.

Hirasawa M & Pittman QJ (2003). Nifedipine facilitates neurotransmitter release independently of calcium channels. *Proc Natl Acad Sci U S A* **100**, 6139–6144.

Lin MT & Lin SZ (1992). Cerebral ischemia is the main cause for heat stroke syndrome in rabbits. *Experientia* **48**, 225–227.

Massett MP, Lewis SJ, Bates JN & Kregel KC (1998*b*). Effect of heating on vascular reactivity in rat mesenteric arteries. *J Appl Physiol* **85**, 701–708.

Massett MP, Lewis SJ & Kregel KC (1998*a*). Effect of heating on the hemodynamic responses to vasoactive agents. *Am J Physiol Regul Integr Comp Physiol* **275**, R844–R853.

Mustafa S, Thulesius O & Ismael HN (2004). Hyperthermia-induced vasoconstriction of the carotid artery, a possible causative factor of heatstroke. *J Appl Physiol* **96**, 1875–1878.

Nakaki T, Roth BL, Chuang DM & Costa E (1985). Phasic and tonic components in 5-HT_2 receptor-mediated rat aorta contraction: participation of Ca^{++} channels and phospholipase C. *J Pharmacol Exp Ther* **234**, 442–446.

Nazarov V, Aquino-DeJesus J & Apkon M (2000). Extracellular pH, Ca^{2+} influx, and response of vascular smooth muscle cells to 5-hydroxytryptamine. *Stroke* **31**, 2500–2507.

Ohkawa H, Ohishi N & Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **95**, 351–358.

Orth SR, Nobiling R, Bonisch S & Ritz E (1996). Inhibitory effect of calcium channel blockers on human mesangial cell growth: evidence for actions independent of L-type Ca^{2+} channels. *Kidney Int* **49**, 868–879.

Padilla J, Garcia-Villalon AL, Fernandez N, Monge L, Gomez B & Dieguez G (1998). Effects of hyperthermia on contraction and dilatation of rabbit femoral arteries. *J Appl Physiol* **85**, 2205–2212.

Ratz PH & Flaim SF (1984). Mechanism of 5-HT contraction in isolated bovine ventricular coronary arteries. Evidence for transient receptor-operated calcium influx channels. *Circ Res* **54**, 135–143.

Richard V, Laude K, Artigues C, Kaeffer N, Henry JP & Thuillez C (2002). Heat stress increases endothelium-dependent relaxations and prevents reperfusion-induced endothelial dysfunction. *Clin Exp Pharmacol Physiol* **29**, 956–962.

Saxena PR & Villalon CM (1990). Cardiovascular effects of serotonin agonists and antagonists. *J Cardiovasc Pharmacol* **15**, S17–S34.

Simon HB (1993). Hyperthermia. *N Engl J Med* **329**, 483–487.

Stepien O & Marche P (2000). Amlodipine inhibits thapsigargin-sensitive Ca^{2+} stores in thrombin-stimulated vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* **279**, H1220–H1227.

Vanhoutte PM (1990). The endothelium and the role of 5-hydroxytryptamine in vascular disease. In *Cardiovascular Pharmacology of 5-Hydroxytryptamine, Prospective Therapeutic Applications*, ed. Saxena PR, Wallis DI, Wouters W & Bevan P, pp. 379–390. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Vhora S & Chiba S (1994). Functional 5-HT receptor subtypes in the isolated canine common carotid artery. *Heart Vessels* **9**, 235–240.

Wilson SM, Mason HS, Ng LC, Montague S, Johnston L, Nicholson N, Mansfield S & Hume JR (2005). Role of basal extracellular Ca^{2+} entry during 5-HT-induced vasoconstriction of canine pulmonary arteries. *Br J Pharmacol* **144**, 252–264.

Wolfenson D, Frei YF, Snapir N & Berman A (1981). Heat stress effects on capillary blood flow and its redistribution in the laying hen. *Pflugers Arch* **390**, 86–93.

Yuan XJ, Bright RT, Aldinger AM & Rubin LJ (1997). Nitric oxide inhibits serotonin-induced calcium release in pulmonary artery smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* **272**, L44–L50.