British Journal of Pharmacology (2000) 130, 345–350

www.nature.com/bjp

Heat stress-induced protection of endothelial function against ischaemic injury is abolished by ATP-sensitive potassium channel blockade in the isolated rat heart

[1]Marie Joyeux, [2]Jean-François Bouchard, [2]Daniel Lamontagne, [1]Diane Godin-Ribuot & *,[1]Christophe Ribuot

[1]Laboratoire du Stress Cardiovasculaire et Pathologies Associées, Faculté de Pharmacie, Université Joseph Fourier, Domaine de la Merci, F38706 La Tronche, France and [2]Faculté de Pharmacie, Université de Montréal, Québec H3C 3J7, Canada

1 The protection conferred by heat stress (HS) against myocardial ischaemia-reperfusion injury, in terms of mechanical function preservation and infarct size reduction, is well documented and mechanisms underlying these effects have been extensively explored. However, the effect of HS on coronary circulation is less known. The aim of this study was thus to investigate the role of ATP-sensitive potassium (K_{ATP}) channels in the protection against ischaemic injury afforded by HS to the coronary endothelial function.

2 Twenty-four hours after whole body hyperthermia (42°C for 15 min, H groups) or sham anaesthesia (Sham groups), isolated perfused rat hearts were subjected to a 15 min stabilization period followed by a 30 min infusion of either 0.3 μM glibenclamide (Gli, a K_{ATP} channel blocker) or its vehicle (V). Hearts were then exposed to a low-flow ischaemia (30 min)-reperfusion (20 min) (I/R) or normally perfused (50 min), after which coronaries were precontracted with 0.1 μM U-46619. Finally, the response to the endothelium-dependent vasodilator, 5-hydroxytryptamine (5-HT, 10 μM) was compared to that of the endothelium-independent vasodilator, sodium nitroprusside (SNP, 3 μM).

3 In hearts from Sham-V and Sham-Gli groups, I/R selectively diminished 5-HT-induced vasodilatation without affecting the vasodilatation to SNP. In V-treated groups, prior HS preserved the vasodilatation produced by 5-HT. This HS-induced protection was abolished by Gli treatment.

4 In conclusion, these results suggest that K_{ATP} channel activation contributes to the preservation of coronary endothelial function conferred by heat stress against ischaemic insult.

British Journal of Pharmacology (2000) **130,** 345–350

Keywords: Heat stress; coronary endothelial function; K_{ATP} channels; glibenclamide

Abbreviations: Gli, glibenclamide; HS, heat stress; 5-HT, 5-hydroxytryptamine; IP, ischaemic preconditioning; I/R, ischaemia-reperfusion; K_{ATP} channel, ATP-sensitive potassium channel; SNP, sodium nitroprusside; V, vehicle

Introduction

Heat stress (HS) is known to protect the myocardium against ischaemia-reperfusion injury by preserving mechanical function (Currie *et al*., 1988) and reducing myocardial necrosis (Donnelly *et al*., 1992; Joyeux *et al*., 1997a). Mechanisms involved in these protective effects have been explored and different potential end-effectors have been identified. Among them, cardiac heat stress proteins (HSPs) have been proposed. Indeed, a direct correlation between the amount of HSP72 expression and the degree of HS-induced ischaemic tolerance has been observed in the rat (Hutter *et al*., 1994) and in the rabbit (Marber *et al*., 1994). ATP-sensitive potassium (K_{ATP}) channels may represent another end-effector of the HS response since it has been demonstrated that K_{ATP} channel blockade abolishes HS-induced resistance to myocardial infarction in the rat (Joyeux *et al*., 1998) and in the rabbit (Hoag *et al*., 1997; Pell *et al*., 1997). In addition to myocyte damage, ischaemia-reperfusion also alters endothelial function in large coronary vessels (Van Benthuysen *et al*., 1987) and in coronary microvessels (DeFily & Chilian, 1993). Little is known about the effect of heat stress on the endothelium function of coronary arteries and the mechanisms involved in this endothelial protection remain to be determined. Amrani *et al*. (1994) have shown in the isolated working rat heart model that prior HS improves the endothelium-dependent vasodilatation after a cardioplegic arrest at 4°C. The aim of this study was thus to test whether heat stress could afford protection to coronary vessels against the consequences of a normothermic ischaemia-reperfusion sequence and to explore the role of K_{ATP} channels in this heat stress response. For that, we have investigated endothelium-independent and dependent vasodilatation in the isolated rat heart, using sodium nitroprusside (SNP) and 5-hydroxytryptamine (5-HT), respectively. Indeed, the vasodilatory response to 5-HT is indicative of the ability of endothelial cells to generate and release nitric oxide (Mankad *et al*., 1991) and could be used as an index of endothelial function, whereas coronary smooth muscle function could be evaluated by the vasodilatation produced by SNP (Bouchard & Lamontagne, 1996).

Methods

This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1985). The experiments were performed on male Wistar rats weighing 280–340 g.

*Author for correspondence;
E-mail: Christophe.Ribuot@ujf-grenoble.fr

Heat stress protocol

The animals were first submitted to either heat stress (H groups) or sham anaesthesia (S groups). Heat stress was achieved by placing rats, lightly anaesthetized with pentobarbitone sodium (25 mg kg^{-1} i.p.), in an environmental chamber under an infrared light. The body temperature, recorded with a rectal probe, was increased to $42.0 \pm 0.2^{\circ}C$ for 15 min. All animals were allowed to recover for 24 h before being submitted to the ischaemia-reperfusion procedure.

Ischaemia-reperfusion procedure

For this second part of the experimental protocol, the rats were re-anaesthetized with pentobarbitone sodium (50 mg kg^{-1} i.p.) and treated with heparin (1000 U kg^{-1} i.p.). Hearts were excised and perfused retrogradely using the Langendorff technique at a constant flow (pump Minipuls3, Gilson) with oxygenated Krebs-Henseleit buffer (in mM: NaCl 118.0, KCl 4.7, $CaCl_2$ 1.8, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.2 and glucose 11.0). The flow rate was adjusted during the stabilization period (15 min) to obtain a coronary perfusion pressure of approximately 75 mmHg. According to the protocol previously described (Bouchard & Lamontagne, 1996), isolated perfused hearts were subjected to an infusion of either 0.3 μM glibenclamide (Gli) or its vehicle (V, 0.02% dimethyl sulphoxide). As previously described, 30 min after the beginning of Gli or V infusion, hearts were then subjected to a low-flow ischaemia (30 min, 1 ml min^{-1})-reperfusion (20 min) sequence (I/R) or normally perfused (50 min). Coronary perfusion pressure (CPP) was measured with a pressure transducer connected to a side arm of the aortic perfusion cannula. Left ventricular developed pressure (LVDP) was assessed with a fluid-filled latex balloon inserted into the left ventricle and connected to a pressure transducer. The volume of the balloon was adjusted to obtain a diastolic pressure between 5 and 10 mmHg. CPP, LVDP and heart rate (HR) were recorded continuously on a polygraph (Windograph, Gould Instrument).

Endothelial function exploration

Following the I/R sequence or the time-matched normal perfusion, coronaries were precontracted with 0.1 μM U-46619 administered until the end of the experiment. Fifteen minutes after the beginning of U-46619 infusion, the endothelial function was evaluated by the vasodilatation produced by 10 μM 5-HT, whereas coronary smooth muscle function was evaluated with 3 μM SNP. These infusions were maintained for 10 min, which was long enough to reach a steady-state. A washout period of 10 min was allowed between each infusion. All drugs were administered through a Y connector in the aortic cannula at 20% of the coronary flow rate. Adequate mixing of the drugs was ensured by the turbulent flow created in the reverse drop-shaped aortic cannula. All concentrations mentioned refer to the final concentration after mixing. Vasodilatation was evaluated by computing per cent changes in coronary resistance (CPP divided by coronary flow), measured immediately before each drug infusion, and after a new steady-state. The concentrations of 5-HT and SNP used here have been described previously by our group (Bouchard & Lamontagne, 1996; Bouchard *et al*., 1998) and were determined in preliminary dose-response experiments to produce near-maximal vasodilatation.

Experimental groups

In S and SI groups ($n = 13$ per group), sham hearts were normally perfused or exposed to I/R, respectively. In H and HI groups ($n = 13$ per group), hearts from heat stressed rats were normally perfused or exposed to I/R, respectively. In addition, hearts from these groups were perfused with either glibenclamide ($n = 6$ per group) or its vehicle ($n = 7$ per group), resulting in eight different groups. The experimental protocol is summarized in Figure 1.

Drugs

Glibenclamide (from Sigma-Aldrich, France), dissolved in dimethyl sulphoxide, was added directly to the Krebs-Henseleit buffer. U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$, 28.5 mM) was dissolved in 100% ethanol and diluted in Krebs-Henseleit buffer to obtain the desired final concentration. This drug, as well as 5-HT and SNP were from Sigma-Aldrich (France).

Statistical analysis

Values are mean $\pm$ s.e.mean. Statistical significance of differences between means was evaluated by a 2-way ANOVA followed by *post-hoc* Tukey comparison tests. In the presence of an interaction between the different groups, 1-way ANOVA were used for each single group. $P < 0.05$ was considered to be statistically significant.

Table 1 Effect of 0.1 μM U-46619 infusion on perfusion pressure and coronary resistance

	n	*CF* (ml min^{-1} g^{-1})	*Perfusion pressure* (mmHg) *Before U-46619*	*After U-46619*	*Coronary resistance* (mmHg min ml^{-1}) *Before U-46619*	*After U-46619*
Vehicle-treated						
S	7	9.83 ± 0.42	72.1 ± 1.6	146.1 ± 7.8*	5.98 ± 0.39	12.17 ± 1.11*
SI	7	9.11 ± 0.62	77.7 ± 1.3	154.7 ± 5.2*	7.11 ± 0.40	14.13 ± 0.82*
H	7	9.34 ± 0.59	73.0 ± 1.6	148.4 ± 8.3*	6.20 ± 0.39	12.61 ± 1.01*
HI	7	8.73 ± 0.40	78.3 ± 1.7	151.9 ± 4.4*	7.67 ± 0.32	15.28 ± 0.71*
Glibenclamide-treated						
S	6	9.74 ± 0.85	137.8 ± 3.7†	158.2 ± 3.2*	11.19 ± 0.30†	13.89 ± 0.53*
SI	6	9.44 ± 0.76	140.8 ± 7.0†	167.6 ± 3.8*	12.13 ± 0.87†	14.38 ± 0.63*
H	6	9.17 ± 0.70	144.8 ± 6.4†	170.0 ± 3.1*	13.19 ± 0.72†	15.54 ± 0.80*
HI	6	9.23 ± 0.51	145.2 ± 8.7†	169.0 ± 5.4*	13.20 ± 1.20†	15.34 ± 1.05*

Coronary resistance was calculated as coronary perfusion pressure devided by coronary flow (CF). S = normally perfused hearts from sham-anaesthetized rats, H = normally perfused hearts from heat-stressed rats, SI = hearts from sham-anaesthetized rats subjected to an ischaemia-reperfusion, HI = hearts from heat-stressed rats subjected to an ischaemia-reperfusion. Values are mean ± s.e.mean. *$P < 0.005$ compared with corresponding 'before U-46619', value, †$P < 0.05$ compared with corresponding vehicle-treated group value.

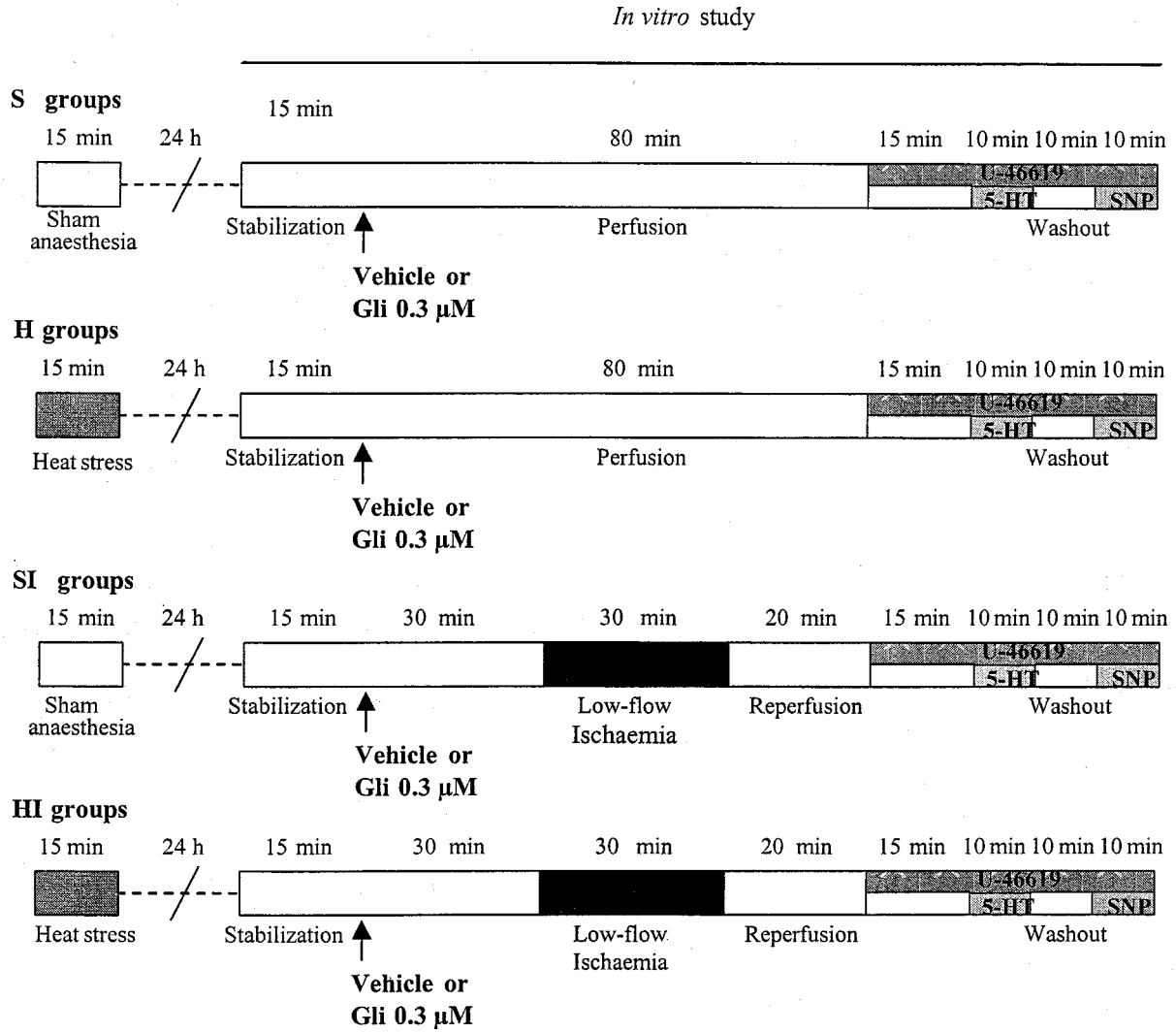

Figure 1 Graphic representation of the different experimental protocols. Each experiment started with a 15-min stabilization period followed by infusion of either glibenclamide (Gli, 0.3 μM) or its vehicle (0.02% DMSO). In sham (S) and heat-stressed (H) groups, a time-matched perfusion was applied. In SI and HI groups, hearts from sham and heat-stressed rats were subjected to a low-flow ischaemia (30 min, 1 ml min^{-1})-reperfusion (20 min) sequence after an additional (30 min) stabilization period. For each group, endothelial and smooth muscle function was tested at the end of these protocols. Coronary arteries were then precontracted by continuous infusion of 0.1 μM U-46619. After 15 min, a 10-min infusion of 5-hydroxytryptamine (5-HT; 10 μM) was started. A washout period of 10 min was allowed between 5-HT and a 10-min infusion of sodium nitroprusside (SNP; 3 μM).

Results

Coronary function

Vehicle-treated groups In S, H, SI and HI groups treated with vehicle ($n=28$), coronary resistance measured just before 0.1 μM U-46619 perfusion was 6.74 ± 0.22 mmHg min ml^{-1}, for a coronary flow rate of 9.24 ± 0.25 ml $min^{-1}g^{-1}$ (mean heart weight, 1.28 ± 0.02 g). Infusion of 0.1 μM U-46619 induced a significant vasoconstriction in all vehicle-treated groups, as shown in Table 1. Vasodilatation produced by 10 μM 5-HT in sham hearts was $-33.6\pm2.3\%$. Ischaemia-reperfusion significantly diminished this vasodilatation by more than one-half ($-14.1\pm2.4\%$ in SI group, Figure 2). Prior heat stress prevented the deleterious effect of ischaemia-reperfusion on endothelium-dependent vasodilatation since the vasodilatation produced by 5-HT in HI group ($-28.4\pm1.9\%$) was comparable to that of H group ($-30.4\pm1.6\%$), (Table 2 and Figure 2). Endothelium-independent vasodilatation to 3 μM SNP was comparable in the four vehicle-treated groups as shown in Figure 3.

Glibenclamide-treated groups Blockade of K_{ATP} channels with glibenclamide (0.3 μM) was accompanied by significant increases in coronary resistance when measured just before U-46619 perfusion (Table 1). In S, H, SI and HI groups treated with glibenclamide ($n=24$), the perfusion rate was 9.39 ± 0.34 ml min^{-1} g^{-1} (mean heart weight, 1.23 ± 0.02 g). Infusion of 0.1 μM U-46619 induced a significant vasoconstriction in all glibenclamide-treated groups, as shown in Table 1. Vasodilatation produced by 10 μM 5-HT in sham hearts ($-16.0\pm1.1\%$) was practically abolished by the ischaemia-reperfusion ($-4.0\pm1.0\%$ in SI group, Figure 2). In glibenclamide-treated hearts, prior heat stress failed to prevent the deleterious effect of ischaemia-reperfusion on 5-HT-induced vasodilatation (Table 2 and Figure 2). Vasodilatation

Table 2 Effects of 10 μM 5-HT and 3 μM SNP infusions on perfusion pressure and coronary resistance

	n	*Perfusion pressure* (mmHg)				*Coronary resistance* (mmHg min ml^{-1})			
		Before 5-HT	*After 5-HT*	*Before SNP*	*After SNP*	*Before 5-HT*	*After 5-HT*	*Before 5-HT*	*After SNP*
Vehicle-treated									
S	7	146.1±7.8	99.0±5.1*	146.4±7.0	66.0±2.6*	12.17±1.11	7.75±0.71*	12.18±1.04	5.44±0.28*
SI	7	154.7±5.2	136.7±5.5	152.1±5.6	78.9±3.9*	14.13±0.82	11.96±0.64	13.95±0.96	7.50±0.83*
H	7	148.4±8.3	103.4±7.0*	150.0±8.0	68.4±4.3*	12.61±1.01	8.75±0.70*	12.77±1.08	5.70±0.20*
HI	7	151.9±4.4	110.0±4.7*	160.1±4.1	70.7±7.9*	15.28±0.71	10.96±0.64*	15.08±0.56	6.55±0.54*
Glibenclamide-treated									
S	6	158.2±3.2	132.7±1.4*†	149.8±1.9	98.2±2.9*†	13.89±0.53	10.68±0.57*†	13.22±0.73	8.64±0.47*†
SI	6	167.7±3.8	161.2±5.0†	165.4±4.6	92.4±6.0*†	14.38±0.63	13.82±0.63†	14.23±0.84	8.06±1.11*†
H	6	170.0±3.1	144.2±3.0*†	162.8±2.8	102.7±5.0*†	15.54±0.80	12.18±0.68*†	14.91±0.84	9.32±0.45*†
HI	6	169.0±5.4	162.0±5.0†	160.8±3.9	101.0±1.1*†	15.34±1.05	14.70±0.98†	14.57±0.87	9.12±0.38*†

S = normally perfused hearts from sham-anaesthetized rats, H = normally perfused hearts from heat-stressed rats, SI = hearts from sham-anaesthetized rats subjected to an ischaemia-reperfusion, HI = hearts from heat-stressed rats subjected to an ischaemia-reperfusion. 5-HT = 5-hydroxytryptamine, SNP = sodium nitroprusside. Data are mean±s.e.mean. *$P<0.05$ compared with corresponding 'before' value, †$P<0.05$ compared with corresponding vehicle-treated group value.

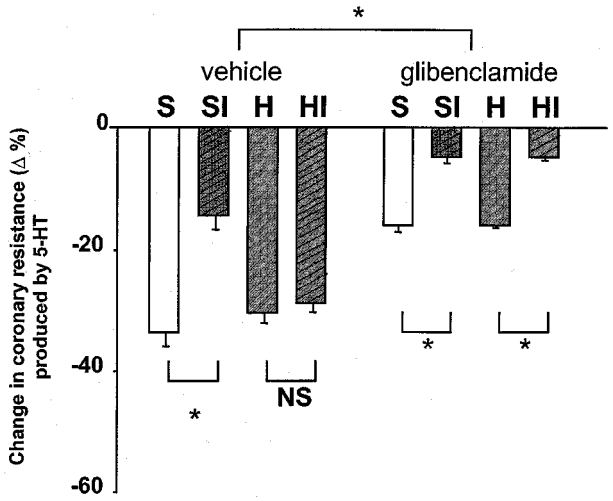

Figure 2 Change in coronary resistance (Δ%) induced by 10 μM 5-hydroxytryptamine (5-HT) in vehicle-treated hearts (left, $n=7$ per group) and in hearts treated with 0.3 μM glibenclamide (right, $n=6$ per group). S = normally perfused hearts from sham-anaesthetized rats, H = normally perfused hearts from heat-stressed rats, SI = hearts from sham-anaesthetized rats subjected to an ischaemia-reperfusion, HI = hearts from heat-stressed rats subjected to an ischaemia-reperfusion. Columns are mean±s.e.mean. *$P<0.05$ (2-way ANOVA). NS = not significant.

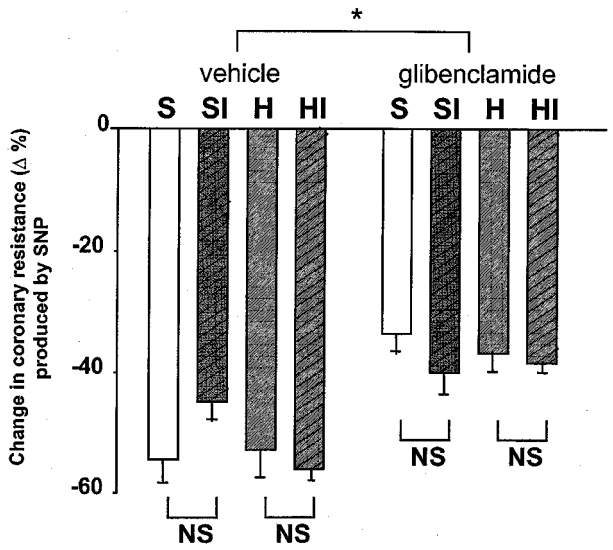

Figure 3 Change in coronary resistance (Δ%) induced by 3 μM sodium nitroprusside (SNP) in vehicle-treated hearts (left, $n=7$ per group) and in hearts treated with 0.3 μM glibenclamide (right, $n=6$ per group). S = normally perfused hearts from sham-anaesthetized rats, H = normally perfused hearts from heat-stressed rats, SI = hearts from sham-anaesthetized rats subjected to an ischaemia-reperfusion, HI = hearts from heat-stressed rats subjected to an ischaemia-reperfusion. Columns are mean±s.e.mean. *$P<0.05$ (2-way ANOVA). NS = not significant.

to 3 μM SNP was comparable in the four glibenclamide-treated groups, as shown in Figure 3. The vasodilatation produced by SNP (Figure 3) and 5-HT (Figure 2) in glibenclamide-treated groups was significantly less than in corresponding vehicle-treated group.

Haemodynamic data

HR, LVDP (data not shown) and CF (Table 2) did not differ neither between the four groups of normally perfused hearts (S and H treated with either vehicle or glibenclamide), nor between the four groups subjected to the ischaemia-reperfusion (SI and HI treated with either vehicle or glibenclamide) at any time point.

Discussion

Our results demonstrate that the endothelial function of rat coronary arteries is protected by heat stress against the consequences of a normothermic ischaemia-reperfusion sequence and that K_{ATP} channel activation seems to mediate this protective effect.

Prior hyperthermia is known to induce cardiac HSPs synthesis, in particular HSP70 (Currie *et al*., 1988, Joyeux *et al*., 1997b) which possess molecular chaperoning properties. This stress is able to protect the myocardium against ischaemia-reperfusion injury by improving mechanical function (Currie *et al*., 1988) or limiting infarct size (Donnelly *et al*., 1992; Marber *et al*., 1993; Joyeux *et al*., 1997a). In accordance with a previous study (Amrani *et al*., 1994), we observed that heat stress can also prevent the alteration in endothelial function of coronary circulation produced by ischaemia-reperfusion.

Mechanisms implicated in the heat stress-induced resistance to infarction and preservation of post-ischaemic mechanical function have been explored. Different potential end-effectors of the heat stress response, such as HSPs, catalase or K_{ATP}

channels, have been identified (Joyeux *et al*., 1999). However, little is known about the mechanisms underlying the coronary endothelial function preservation conferred by heat stress against ischaemic insult. In human endothelial cells, it has been observed that heat stress induces HSP27 phosphorylation and tolerance to ischaemic stress (in terms of preservation of cell morphology), (Loktionova *et al*., 1998). Moreover, overexpression of HSP70 attenuates hypoxic injury in coronary endothelial cells (Suzuki *et al*., 1998). Amrani *et al*. (1998) have noticed that following whole body hyperthermia, coronary endothelial cells are the main site of induction of HSP70 in the heart and appear to contribute to the coronary endothelial function preservation conferred by heat stress against a cardioplegic arrest. Thus, it seems that HSPs could represent one potential end-effector implicated in this heat stress response. Our study provides the first observation that K_{ATP} channel activation seems to contribute to the coronary vascular protection conferred by heat stress against ischaemic injury. Thus, these channels could represent another potential end-effector of the heat stress response and HSPs and K_{ATP} channels could act by separate or overlapping mechanisms to protect endothelial function (Joyeux *et al*., 1999).

Several hypotheses have been proposed to explain the mechanisms by which K_{ATP} channel activation protects the myocardium or the coronary endothelium against ischaemic insult (Bouchard & Lamontagne, 1996). In the myocardium, activation of these channels may inhibit ischaemic depolarization, which could reduce calcium entry *via* voltage-gated channels, resulting in a reduction in intracellular calcium levels (Grover, 1994). Furthermore, recent studies have shown the presence of K_{ATP} channel in mitochondrial membranes, which could maintain membrane polarity or even control mitochondrial calcium concentration (Grover, 1994). This effect can prevent mitochondrial calcium overload, a key factor in myocardial ischaemic damage. In endothelial cells, activation of K_{ATP} channels also produces hyperpolarisation (Janigro *et al*., 1993). Because of the absence of voltage-gated calcium channels in endothelial cells, hyperpolarisation will have an unusual effect on calcium influx in these cells and will increase the electrochemical gradient, facilitating calcium entry (Janigro *et al*., 1993) which will enhance nitric oxide release from endothelial cells (Lückhoff & Busse, 1990). The contribution of nitric oxide to the heat stress-induced endothelial protection remains to be determined.

Finally, protection afforded by heat stress to the coronary endothelial function may be analogous to that seen with ischaemic preconditioning (IP). Indeed, it has been shown in the rat that acute IP protect the coronary arterial bed against ischaemic injury (Richard *et al*., 1994; Bouchard *et al*., 1998) and that K_{ATP} channel activation is involved in this cardioprotective effect (Bouchard & Lamontagne, 1996). On the other hand, Kaeffer *et al*. (1997) have observed that IP also induces late protection against reperfusion-induced coronary endothelial injury, respecting the same window of protection than the heat stress response. Since there is increasing evidence that mitochondrial, rather than sarcolemmal K_{ATP} channels are involved in the cardioprotection conferred by IP (Garlid *et al*., 1997; Gross & Fryer, 1999), it would be of interest to determine, using a more selective mitochondrial K_{ATP} channel blocker, which type of channels are implicated in the HS-induced preservation of endothelial function. A better characterization of mechanisms underlying these cardioprotective responses, induced by both heat stress and IP, could lead to the development of new pharmacological interventions inducing delayed cardiac protection.

In summary, our results show that K_{ATP} channel opening appears to play a role in the preservation of coronary endothelial function against ischaemic insult induced by heat stress in the isolated rat heart. Indeed, K_{ATP} channel blocker glibenclamide abolished this cardioprotection. Further investigations are required to elucidate the mechanisms by which K_{ATP} channels contribute to the heat stress-induced protection as well as their possible interaction with HSPs.

References

AMRANI, M., CORBETT, J., ALLEN, N.J., O'SHEA, J., BOATENG, S.Y., MAY, A.J., DUNN, M.J. & YACOUB, M.H. (1994). Induction of heat-shock proteins enhances myocardial and endothelial functional recovery after prolonged cardioplegic arrest. *Ann. Thorac. Surg.,* **57,** 157–160.

AMRANI, M., LATIF, N., MORRISON, K., GRAY, C.C., JAYAKUMAR, J., CORBETT, J., GOODWIN, A.T., DUNN, M.J. & YACOUB, M.H. (1998). Relative induction of heat shock protein in coronary endothelial cells and cardiomyocytes: implication for myocardial protection. *J. Thorac. Cardiovasc. Surg.,* **115,** 200–209.

BOUCHARD, J-F., CHOUINARD, J. & LAMONTAGNE, D. (1998). Role of kinins in the endothelial protective effect of ischaemic preconditioning. *Br. J. Pharmacol.,* **123,** 413–420.

BOUCHARD, J-F. & LAMONTAGNE, D. (1996). Mechanisms of protection afforded by preconditioning to endothelial function against ischemic injury. *Am. J. Physiol.,* **271,** H1801–H1806.

CURRIE, R.W., KARMAZYN, M., KLOC, M. & MAILER, K. (1988). Heat-shock response is associated with enhanced postischemic ventricular recovery. *Circ. Res.,* **63,** 543–549.

DEFILY, D.V. & CHILIAN, W.M. (1993). Preconditioning protects coronary arteriolar endothelium from ischemia-reperfusion injury. *Am. J. Physiol.,* **265,** H700–H706.

DONNELLY, T.J., SIEVERS, R.E., VISSERN, F.L.J., WELCH, W.J. & WOLFE, C.L. (1992). Heat shock protein induction in rat hearts. A role for improved myocardial salvage after ischemia and reperfusion? *Circulation,* **85,** 769–778.

GARLID, K.D., PAUCEK, P., YAROV-YAROVOY, V., MURRAY, H.N., DARBENZIO, R.B., D'ALONZO, A.J., LODGE, N.J., SMITH, M.A. & GROVER, G.J. (1997). Cardioprotective effect of diazoxide and its interaction with mitochondrial ATP-sensitive K+ channels. Possible mechanism of cardioprotection. *Circ. Res.,* **81,** 1072–1082.

GROSS, G.J. & FRYER, R.M. (1999). Sarcolemmal versus mitochondrial ATP-sensitive K+ channels and myocardial preconditioning. *Circ. Res.,* **84,** 973–979.

GROVER, G.J. (1994). Protective effects of ATP sensitive potassium channel openers in models of myocardial ischemia. *Cardiovasc. Res.,* **28,** 778–782.

HOAG, J.B., QIAN, Y.Z., NAYEEM, M.A., D'ANGELO, M. & KUKREJA, R.C. (1997). ATP-sensitive potassium channel mediates delayed ischemic protection by heat stress in the rabbit heart. *Am. J. Physiol.,* **273,** 2458–2464.

HUTTER, M.M., SIEVERS, R.E., BARBOSA, V. & WOLFE, C.L. (1994). Heat-shock protein induction in rat hearts. A direct correlation between the amount of heat-shock protein induced and the degree of myocardial protection. *Circulation,* **89,** 355–360.

JANIGRO, D., WEST, G.A., GORDON, E.L. & WINN, H.R. (1993). ATP-sensitive K+ channels in rat aorta and brain microvascular endothelial cells. *Am. J. Physiol,* **265,** C812–C821.

JOYEUX, M., BAXTER, G.F., THOMAS, D.L., RIBUOT, C. & YELLON, D.M. (1997a). Protein kinase C is involved in resistance to myocardial infarction induced by heat stress. *J. Mol. Cell. Cardiol.,* **29,** 3311 – 3319.

JOYEUX, M., GODIN-RIBUOT, D. & RIBUOT, C. (1998). Resistance to myocardial infarction induced by heat stress and the effect of ATP-sensitive potassium channel blockade in the rat isolated heart. *Br. J. Pharmacol.,* **123,** 1085 – 1088.

JOYEUX, M., GODIN-RIBUOT, D., YELLON, D.M., DEMENGE, P. & RIBUOT, C. (1999). Heat stress response and myocardial protection. *Fund. Clin. Pharmacol.,* **13,** 1 – 10.

JOYEUX, M., RIBUOT, C., BOURLIER, V., VERDETTI, J., DURAND, A., RICHARD, M.-J., GODIN-RIBUOT, D. & DEMENGE, P. (1997b). *In vivo* antiarrhythmic effect of prior whole body hyperthermia: implication of catalase. *J. Mol. Cell. Cardiol.,* **29,** 3285 – 3292.

KAEFFER, N., RICHARD, V. & THUILLEZ, C. (1997). Delayed coronary endothelial protection 24 hours after preconditioning: role of free radicals. *Circulation,* **96,** 2311 – 2316.

LOKTIONOVA, S.A., ILYINSKAYA, O.P. & KABAKOV, A.E. (1998). Early and delayed tolerance to simulated ischemia in heat-preconditioned endothelial cells: a role for HSP27. *Am. J. Physiol.,* **275,** H2147 – H2158.

LÜCKHOFF, A. & BUSSE, R. (1990). Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. *Pflugers Arch.,* **416,** 305 – 311.

MANKAD, P.S., CHESTER, A.H. & YACOUB, M.H. (1991). 5-hydroxytryptamine mediates endothelium dependent coronary vasodilatation in the isolated rat heart by the release of nitric oxide. *Cardiovasc. Res.,* **25,** 244 – 248.

MARBER, M.S., LATCHMAN, D.S., WALKER, J.M. & YELLON, D.M. (1993). Cardiac stress protein elevation 24 hours after brief ischemia or heat stress is associated with resistance to myocardial infarction. *Circulation,* **88,** 1264 – 1272.

MARBER, M.S., WALKER, J.M., LATCHMAN, D.S. & YELLON, D.M. (1994). Myocardial protection after whole body heat stress in the rabbit is dependent on metabolic substrate and is related to the amount of the inducible 70-kD heat stress protein. *J. Clin. Invest.,* **93,** 1087 – 1094.

PELL, T.J., YELLON, D.M., GOODWIN, R.W. & BAXTER, G.F. (1997). Myocardial ischemic tolerance following heat stress is abolished by ATP-sensitive potassium channel blockade. *Cardiovasc. Drugs Ther.,* **11,** 679 – 686.

RICHARD, V., KAEFFER, N., TRON, C. & THUILLEZ, C. (1994). Ischemic preconditioning protects against coronary endothelial dysfunction induced by ischemia and reperfusion. *Circulation,* **89,** 1254 – 1261.

SUZUKI, K., SAWA, Y., KANEDA, Y., ICHIKAWA, H., SHIRAKURA, R. & MATSUDA, H. (1998). Overexpressed heat shock protein 70 attenuates hypoxic injury in coronary endothelial cells. *J. Mol. Cell. Cardiol.,* **30,** 1129 – 1136.

VAN BENTHUYSEN, K.M., MCMURTRY, I.F. & HORWITZ, L.D. (1987). Reperfusion after acute coronary occlusion in dogs impairs endothelium-dependent relaxation to acetylcholine and augments contractile reactivity *in vitro*. *J. Clin. Invest.,* **79,** 265 – 274.

(Received December 22, 1999
Revised February 18, 2000
Accepted February 18, 2000)