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Infarct size-reducing effect of heat stress and α_1 adrenoceptors in rats

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1 Noradrenaline (NA), which is abundantly released during heat stress (HS), is known to induce both delayed cardioprotection and heat stress protein (HSP) 72 expression by the mediation of α_1 adrenoceptors. Therefore, we have investigated the implication of α_1 adrenoceptors in HS-induced resistance to myocardial infarction, in the isolated rat heart model.

2 Rats were pretreated with prazosin (1 mg kg^{-1}, i.p., Praz) or 5-methylurapidil (3 mg kg^{-1}, i.v, 5MU) or chloroethylclonidine (3 mg kg^{-1}, i.v., CEC) or vehicle (V) in order to selectively antagonize α_1, α_{1A} and α_{1B} adrenoceptors. They were then either heat stressed (42°C for 15 min) or sham anaesthetized. Twenty-four hours later, their hearts were isolated, retrogradely perfused, and subjected to a 30 min occlusion of the left coronary artery followed by 120 min of reperfusion.

3 Infarct-to-risk ratio was significantly reduced in HS+V (15.4±1.8%) compared to Sham+V (35.7±1.3%) hearts. This effect was abolished in Praz-treated (29.1±1.6% in HS+Praz *vs* 34.1±4.0% in Sham+Praz), 5MU-treated (34.5±2.2% in HS+5MU *vs* 31.2±2.0% in Sham+5MU) and CEC-treated (33.4±3.0% in HS+CEC *vs* 32.4±1.3% in Sham+CEC) groups. Western blot analysis of myocardial HSP72 showed an HS-induced increase of this protein, which was not modified by Praz, 5MU and CEC pretreatments.

4 We conclude that both α_{1A} and α_{1B} adrenoceptor subtypes appear to play a role in the heat stress-induced cardioprotection, independently of the HSP72 level. Further investigations are required to elucidate the precise role of HSPs in this adaptative response.

Keywords: Heat stress; infarct size; α_1 adrenoceptors; heat stress protein

Introduction

It is well known that an increased synthesis of heat shock proteins (HSPs) is induced in most organisms in response to heat stress (HS) and other environmental stresses (Mestril & Dillmann, 1995). This phenomenon confers to the cell ability to survive noxious stresses such as a myocardial ischaemia-reperfusion sequence (Currie *et al*., 1988; Donnelly *et al*., 1992; Yellon *et al*., 1992). In particular, 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). However, the precise mechanisms underlying HSP synthesis and the signal transduction pathways associated with the development of this HS-induced cardioprotection remain to be determined.

In vivo treatment with noradrenaline (NA) has been shown to induce delayed cardioprotection against postischaemic dysfunction in the isolated rat heart with an associated expression of myocardial HSP72 (Meng *et al*., 1996b). These NA-induced effects appear to be mediated by α_1 adrenoceptors (Meng *et al*., 1996b). On the other hand, Kregel and co-workers (1991, 1993) have demonstrated in the conscious rat that whole body hyperthermia increases plasma catecholamine concentrations and myocardial NA turnover.

Moreover, the cardioprotection induced 24–48 h following heat shock resembles that observed during the second window of protection following ischaemic preconditioning (Marber *et al*., 1993; Yellon & Baxter, 1995). Mediators under investigation for their role in ischaemic preconditioning may therefore provide a potential mechanism for HS-induced protection (Parratt & Szekeres, 1995; Richard *et al*., 1996). Hence, in the rat (Banerjee *et al*., 1993; Hu & Nattel, 1995) and in the rabbit (Kariya *et al*., 1997), α_1 adrenoceptors have been shown to be implicated in the cardioprotection following acute ischaemic preconditioning.

Therefore, our working hypothesis was that α_1 adrenoceptor stimulation could mediate both the cardioprotection and the myocardial HSP72 expression induced by heat stress. In this study, we have investigated the implication of α_1 adrenoceptor subtypes in the heat stress response leading to the resistance to myocardial infarction in the isolated rat heart. The selective α_1 adrenoceptor antagonist prazosin, the α_{1A} adrenoceptor blocker 5-methylurapidil and the α_{1B} adrenoceptor blocker chloroethylclonidine were used in this study.

Methods

Experimental treatment groups

This study was conducted in two parts. In the first part, rats were submitted to either heat stress (HS groups) or anaesthesia without hyperthermia (Sham groups). Prior to this procedure, the animals received either prazosin (Praz) or chloroethylclonidine (CEC) or 5-methylurapidil (5MU) or vehicle (V). Subsequently, all animals were allowed to recover for 24 h. In the second part, ischaemia (30 min)-reperfusion (120 min) was performed in isolated hearts. Eight experimental groups were studied: Group Sham+V (n=8)-four rats received vehicle of Praz (0.4 ml saline containing 5% ethanol, i.p.) and four rats received vehicle of 5MU or CEC (0.4 ml saline only,

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i.v.) prior to sham HS; Group Sham + Praz ($n = 6$)-animals were treated with Praz (1 mg kg^{-1}, i.p., Meng *et al.*, 1996a) prior to sham HS; Group Sham + 5MU ($n = 6$)-animals were treated with 5MU (3 mg kg^{-1}, i.v., Kariya *et al.*, 1997) prior to sham HS; Group Sham + CEC ($n = 6$)-animals were treated with CEC (3 mg kg^{-1}, i.v., Kariya *et al.*, 1997) prior to sham HS. In Groups HS + V ($n = 8$), HS + Praz ($n = 6$), HS + 5MU ($n = 6$) and HS + CEC ($n = 6$), rats were similarly treated prior to undergoing heat stress. The experimental protocol is summarized in Figure 1.

Heat stress protocol

One hour before whole body hyperthermia, anaesthetized animals (with 30 mg kg^{-1}, i.p. sodium pentobarbitone) received either vehicle or Praz (1 mg kg^{-1}, i.p.) or 5MU (3 mg kg^{-1}, i.v.) or CEC (3 mg kg^{-1}, i.v.). The i.v. injections were performed *via* the penian vein. Heat stress was achieved by placing rats in an environmental chamber under an infrared light. Their body temperature, recorded with a rectal probe, was increased to 42 ± 0.2°C for 15 min. Sham control animals were anaesthetized only. All rats were allowed to recover for 24 h.

Ischaemia-reperfusion protocol

Twenty-four hours after heat stress, rats were anaesthetized with 60 mg kg^{-1}, i.p. sodium pentobarbitone and treated with heparin (1000 u kg^{-1}, i.p.). The heart was rapidly excised and immediately immersed in 4°C Krebs-Henseleit buffer solution (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 mM). The aortic stump was then cannulated and the heart perfused retrogradely using the Langendorff technique at a constant pressure (102 cm) with oxygenated Krebs-Henseleit buffer. A water-filled balloon, coupled to a pressure transducer (Statham), was inserted into the left ventricular cavity *via* the left atrium for pressure recordings. Left ventricular end-diastolic pressure (LVEDP) was adjusted between 8–12 mmHg. Myocardial temperature was measured by a thermoprobe inserted into the left ventricle and was maintained constant close to 37°C. For temporary occlusion of the left coronary artery (LCA), a 3/0 silk suture (Mersilk W546, Ethicon) was placed around the artery a few millimetres distal to the aortic root. After 20 min of stabilization, regional ischaemia was induced by tightening the snare around the LCA for 30 min. Thereafter the heart was reperfused for 120 min. Coronary flow (CF) was measured throughout the ischaemia-reperfusion procedure, by collecting the effluent. Heart rate (HR) and left ventricular developed pressure (LVDP = difference between left ventricular systolic pressure and LVEDP) were continuously recorded on a polygraph (Windograph, Gould Instrument). At the end of the reperfusion period, the coronary artery ligature was retied and unisperse blue dye was slowly infused through the aorta to delineate the myocardial risk zone. After removal of the right ventricle and connective tissues, the heart was frozen and then sectioned into 2 mm transverse sections from apex to base (6–7 slices/heart). Following defrosting, the slices were incubated at 37°C with 1% triphenyltetrazolium chloride (TTC) in phosphate buffer (pH 7.4) for 10–20 min and fixed in 10% formaldehyde solution to distinguish clearly stained viable tissue and unstained necrotic tissue. Left ventricular infarct zone (I) was determined using a computerized planimetric technique (Minichromax, Biolab) and expressed as the percentage of the risk zone (R) and of the left ventricle (LV).

Determination of myocardial HSP72 content

To determine myocardial HSP72 content, additional animals ($n = 2$ in each group) were treated with either vehicle or Praz or 5MU or CEC and submitted to HS or sham HS. Twenty-four hours later, animals were re-anaesthetized and treated with heparin as described above before the hearts were quickly excised. Approximately 50 mg ventricular tissue samples were rapidly powdered in liquid nitrogen and homogenized in 500 μl SDS–PAGE sample buffer (20% glycerol, 6% sodium dodecyl sulphate, 1.4% TrisHCl, pH 6.8). Ten per cent 2-mercaptoethanol was added and the samples heated at 100°C for 5 min. Samples were cooled and centrifuged at $11,000 \times g$ for 5 min. Eight per cent bromophenol blue was added to the supernatant and the samples were stored at −20°C. Proteins

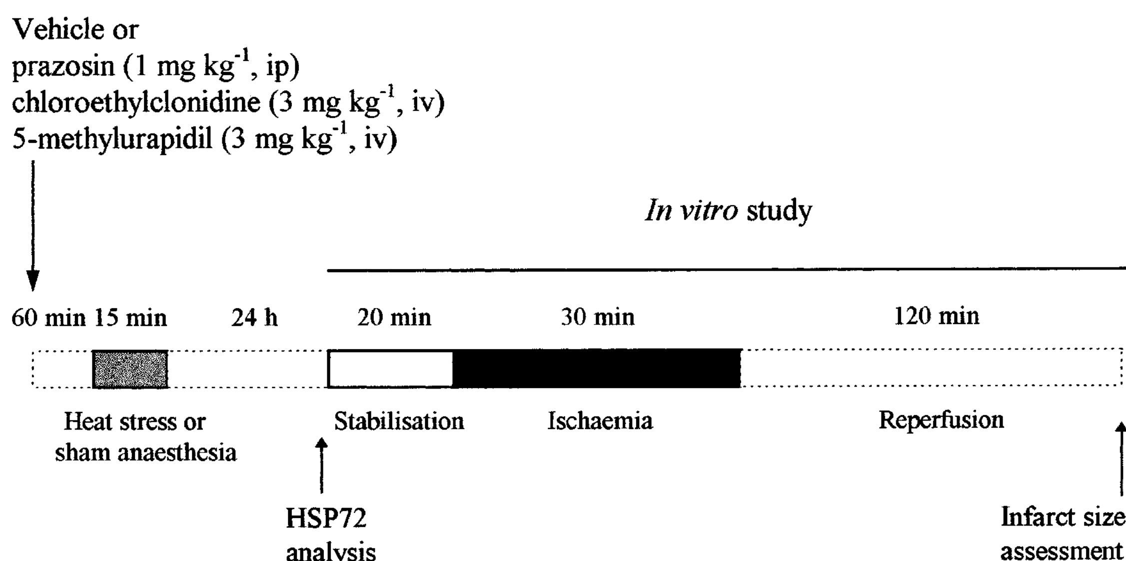

Figure 1 Experimental protocol.

were separated by electrophoresis on 12.5% polyacrylamide SDS–PAGE gels. Gels were stained in Coomassie blue R250 and subsequently destained to confirm equivalence of protein loading. For Western blot analysis of HSP72, proteins were transferred electrophoretically onto a nitrocellulose membrane (Hybond-C, Amersham, U.K.) overnight at 180 mA and 4°C. The membrane was placed in washing buffer for 30 min (phosphate buffered saline, pH 7.2, containing 0.05% Tween 20 and 0.1% dried milk powder) to block non-specific binding sites. The filter was then incubated for 60 min with a mouse monoclonal IgG cross-active to HSP72 (SPA-810, StressGen) at 1 : 1000 dilution and subsequently incubated for 60 min with horseradish peroxidase-conjugated rabbit anti-mouse IgG (P260, Dako, Denmark) at 1 : 2500 dilution. The filter was developed using an enhanced chemiluminescence detection system (Amersham).

Statistical analysis

The data are presented as means ± s.e.mean. Comparisons in CF, HR and LVDP were performed using two-way repeated measures ANOVA. Infarct size was analysed by a one-way ANOVA. *Post-hoc* comparisons were done using Tukey tests. P values $\leqslant 0.05$ were considered significant.

Exclusion criteria

Only hearts with CF within 8–15 ml min^{-1} and LVDP >70 mmHg at the end of the stabilization period were included in this study. The efficiency of coronary occlusion was indicated by a decrease in CF >30%. Hearts which developed ventricular fibrillation (VF) during ischaemia-reperfusion that could not be restored to normal sinus rhythm within 2 min were excluded. Moreover, the risk zone determined at the end of the ischaemia-reperfusion procedure had to represent 40–60% of the LV (Joyeux *et al*., 1998).

Materials

Male Wistar rats (280–340 g) were used for these studies. This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No 85–23, revised 1985).

Prazosin and 2,3,5-triphenyltetrazolium chloride were obtained from Sigma (France). 5-methylurapidil and chloroethylclonidine were obtained from Research Biochemical (France). Unisperse blue dye was obtained from Ciba-Geigy (France). All other reagents were of analytical reagent quality.

Results

Haemodynamic data

Table 1 summarizes CF, HR and LVDP data recorded in the eight experimental groups during the stabilization and ischaemia-reperfusion periods. Twenty-four hours after heat stress or sham anaesthesia, there was no statistically significant difference in haemodynamic performance between the eight groups.

Incidence of ventricular fibrillation

Twenty-four hearts developed VF during coronary occlusion or the first min of reperfusion: 4 Sham + V, 3 Sham + Praz, 2 Sham + 5MU, 3-Sham + CEC, 4 HS + V, 3 HS + Praz, 3 HS + 5MU, 2 HS + CEC. VF incidence was not different between groups. All converted back to sinus rhythm spontaneously.

Infarct data

Table 2 presents infarct size data expressed as the percentage of the risk zone (I/R) or of the left ventricle (I/LV) for the eight

Table 1 Haemodynamic data

			Ischaemia		*Reperfusion*			
	Group	*Stabilization*	*5 min*	*29 min*	*15 min*	*30 min*	*60 min*	*120 min*
CF (ml min^{-1})	Sham + V	11.3 ± 0.6	6.2 ± 0.4	5.6 ± 0.6	8.5 ± 0.8	7.9 ± 0.9	6.9 ± 0.7	5.2 ± 0.5
	HS + V	12.3 ± 0.8	6.6 ± 0.6	6.3 ± 0.6	10.2 ± 0.8	8.3 ± 1.3	8.8 ± 0.7	7.1 ± 0.6
	Sham + Praz	10.6 ± 0.4	6.1 ± 0.4	5.5 ± 0.5	9.9 ± 0.5	8.6 ± 0.6	7.6 ± 0.5	5.3 ± 0.4
	HS + Praz	10.0 ± 0.6	5.1 ± 0.5	4.9 ± 0.5	9.7 ± 0.7	8.6 ± 0.8	6.5 ± 0.5	5.5 ± 0.6
	Sham + CEC	11.1 ± 0.3	7.1 ± 0.5	6.3 ± 0.4	10.2 ± 0.9	9.5 ± 0.4	8.0 ± 0.3	6.6 ± 0.2
	HS + CEC	10.4 ± 0.2	5.1 ± 0.4	4.6 ± 0.6	10.0 ± 0.3	8.7 ± 6.9	6.8 ± 0.5	5.2 ± 0.4
	Sham + 5MU	10.9 ± 0.9	5.3 ± 0.5	5.0 ± 0.8	8.6 ± 1.2	8.2 ± 1.2	6.8 ± 1.3	5.4 ± 1.2
	HS + 5MU	11.9 ± 0.8	6.5 ± 0.7	6.2 ± 0.6	10.8 ± 0.8	9.6 ± 0.8	7.9 ± 0.9	6.4 ± 0.9
HR (beats min^{-1})	Sham + V	286 ± 11	296 ± 12	281 ± 13	273 ± 16	262 ± 16	268 ± 16	262 ± 14
	HS + V	307 ± 13	299 ± 17	273 ± 17	277 ± 13	271 ± 12	266 ± 12	264 ± 11
	Sham + Praz	280 ± 7	290 ± 9	268 ± 7	259 ± 5	256 ± 9	247 ± 11	232 ± 12
	HS + Praz	283 ± 6	271 ± 8	253 ± 9	254 ± 5	244 ± 8	241 ± 10	249 ± 10
	Sham + CEC	286 ± 7	245 ± 9	245 ± 11	247 ± 6	255 ± 6	245 ± 3	237 ± 2
	HS + CEC	274 ± 10	276 ± 16	256 ± 16	247 ± 8	246 ± 8	242 ± 12	250 ± 14
	Sham + 5MU	283 ± 7	274 ± 10	270 ± 7	246 ± 6	244 ± 9	250 ± 9	243 ± 7
	HS + 5MU	278 ± 7	272 ± 10	269 ± 15	260 ± 11	262 ± 12	253 ± 8	255 ± 9
LVDP (mmHg)	Sham + V	101 ± 16	43 ± 8	54 ± 6	84 ± 6	75 ± 5	68 ± 9	51 ± 5
	HS + V	106 ± 7	36 ± 4	62 ± 6	106 ± 12	95 ± 9	86 ± 10	63 ± 9
	Sham + Praz	108 ± 7	37 ± 5	61 ± 9	102 ± 9	97 ± 9	77 ± 8	59 ± 6
	HS + Praz	98 ± 6	27 ± 3	66 ± 5	90 ± 16	89 ± 9	84 ± 9	68 ± 6
	Sham + CEC	109 ± 4	45 ± 6	66 ± 2	96 ± 4	84 ± 5	78 ± 8	55 ± 6
	HS + CEC	108 ± 6	36 ± 5	57 ± 4	90 ± 12	79 ± 6	65 ± 8	57 ± 6
	Sham + 5MU	94 ± 9	35 ± 9	49 ± 11	90 ± 10	84 ± 8	66 ± 4	54 ± 6
	HS + 5MU	97 ± 4	31 ± 5	64 ± 4	102 ± 4	93 ± 4	70 ± 5	54 ± 6

CF – coronary flow, HR – heart rate, LVDP – left ventricular developed pressure, HS = heat-stressed, Sham = sham-anaesthetized, V = vehicle-treated, Praz = prazosin-treated, CEC = chloroethylclonidine-treated, 5MU = 5-methylurapidil-treated. Data are means ± s.e.mean.

Table 2 Risk (R) and infarct (I) sizes expressed as a percentage of the left ventricle (LV)

Group	n	*R/LV* (%)	*I/LV* (%)	*I/R* (%)
Sham + V	8	54.0 ± 1.1	19.4 ± 0.8	35.7 ± 1.3
HS + V	8	53.8 ± 1.5	8.5 ± 0.9*	15.4 ± 1.8*
Sham + Praz	6	49.0 ± 0.7	17.5 ± 1.9	34.1 ± 4.0
HS + Praz	6	53.7 ± 2.8	14.8 ± 1.1	29.1 ± 1.6
Sham + CEC	6	51.2 ± 1.5	15.9 ± 0.7	31.2 ± 2.0
HS + CEC	6	52.8 ± 1.4	19.1 ± 1.5	34.5 ± 2.2
Sham + 5MU	6	53.1 ± 1.2	17.3 ± 1.0	32.4 ± 1.3
HS + 5MU	6	53.3 ± 0.9	18.0 ± 1.6	33.4 ± 3.0

HS = heat-stressed, Sham = sham-anaesthetized, V = vehicle-treated, Praz = prazosin-treated, CEC = chloroethylclonidine-treated, 5MU = 5-methylurapidil-treated. Data are means ± s.e.mean. *$P \leqslant 0.001$ *vs* all the other groups.

experimental groups. In groups receiving vehicle, heat stress significantly reduced infarct size from 35.7 ± 1.3% in Sham + V to 15.4 ± 1.8% in HS + V ($P \leqslant 0.001$ by one-way ANOVA). This infarct size reducing effect of heat stress was abolished in Praz-treated (29.1 ± 1.6%), 5MU-treated (34.5 ± 2.2%) and CEC-treated (33.4 ± 3.0%) groups (Figure 2). In non-heat stressed rats, treatment with Praz (34.1 ± 4.0%), 5MU (31.2 ± 2.0%) and CEC (32.4 ± 1.3%) had no effect on infarct size (*vs* 35.7 ± 1.3% in Sham + V group). Similar results were observed concerning I/LV ratio between the eight groups (Table 2). Myocardial risk size expressed as the percentage of the left ventricle (R/LV) was similar for all groups (Table 2). Therefore, differences in infarct size did not result from variability in the risk zone.

HSP72 analysis

Western blot analysis of myocardial HSP72 content (Figure 3) showed a marked increase of this protein following heat stress in all HS groups compared to Sham groups. Praz- and 5MU-pretreatment did not modify the heat stress-induced increase of HSP72 (groups HS + Praz *vs* Sham + Praz and HS + 5MU *vs* Sham + 5MU). The heat stress-induced increase of HSP72 appeared to be attenuated by CEC-pretreatment (groups HS + CEC *vs* HS + V), but this effect was weak and rather inconclusive.

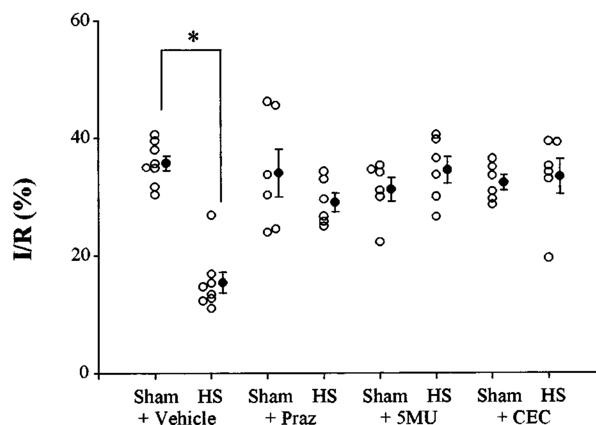

Figure 2 Infarct size expressed as a percentage of the risk zone in isolated rat hearts subjected to 30 min coronary occlusion followed by 120 min reperfusion. The day before, rats were treated with either vehicle (V), prazosin (Praz, 1 mg kg^{-1}, i.p.), 5-methylurapidil (5MU, 3 mg kg^{-1}, i.v.) or chloroethylclonidine (CEC, 3 mg kg^{-1}, i.v.) prior to undergoing heat stress (HS) or sham anaesthesia (Sham). *$P \leqslant 0.001$.

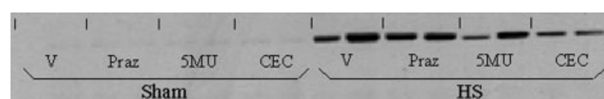

Figure 3 Western blot analysis of myocardial HSP72 content. HS = heat-stressed, Sham = sham-anaesthetized, V = vehicle-treated, Praz = prazosin-treated, 5MU = 5-methylurapidil-treated, CEC = chloroethylclonidine-treated. $n = 2$ in each group.

Discussion

This study provides the first demonstration of the implication of α_1 adrenoceptors in the heat stress-induced delayed cardioprotection. We observed that prior heat stress significantly reduced infarct size in the isolated rat heart subjected to an ischaemia-reperfusion sequence, in accordance with previous studies (Donnelly *et al.*, 1992; Marber *et al.*, 1993; Joyeux *et al.*, 1997). This myocardial ischaemic tolerance was abolished by the administration prior to heat stress of prazosin, 5-methylurapidil and chloroethylclonidine, which selectively inhibit α_1, α_{1A} and α_{1B} adrenoceptors, respectively. Western blot analysis of myocardial HSP72 content 24 h after heat stress, showed a heat stress-induced synthesis of this protein which was not modified by α_1 adrenoceptor blockade.

Alpha-1 adrenoceptors have been shown to be involved in acute ischaemic preconditioning in different species such as the rabbit (Bankwala *et al.*, 1994; Tsuchida *et al.*, 1994; Kariya *et al.*, 1997), the dog (Kitakaze *et al.*, 1994) and the rat (Banerjee *et al.*, 1993; Ravingerova *et al.*, 1995) where the α_{1B} subtype has been implicated (Hu & Nattel, 1995). To our knowledge, there is no study on the role of adrenoceptors in the delayed protection associated with ischaemic preconditioning.

Our results show that both subtypes of α_1 adrenoceptors appear to be involved in the cardioprotection induced by heat stress, which resembles that observed during the second window of protection following ischaemic preconditioning (Marber *et al.*, 1993; Yellon & Baxter, 1995). The three α_1 antagonists have been administrated 60 min before heat stress. Doses of prazosin and chloroethylclonidine used have been shown to be effective 60–120 min after their administration (Meng *et al.*, 1996a; Kariya *et al.*, 1997). 5-methylurapidil, at a lower dose than the one we used, has been shown to be effective up to 30 min after its administration (Buisson-Defferier *et al.*, 1993). We can presume that it was still active 60 min after its administration since it blocked the effects of heat stress.

Our results show that the heat stress-induced cardioprotection does not appear to be related to induction of HSP72 synthesis, since pretreatment with Praz, 5MU or CEC abolished myocardial ischaemic tolerance while it had little

or no effect on the increase in myocardial HSP72 levels. Several studies point to a relation between HSP72 induction and cardioprotection. Hence, Marber and co-workers (1993) have observed that prior hyperthermia induces a high level of myocardial HSP72 expression along with the enhanced myocardial tolerance to ischaemic injury. Moreover, the level of HSP72 has been directly correlated to the degree of heat stress-induced cardioprotection in the rat (Hutter *et al*., 1994) and in the rabbit (Marber *et al*., 1994). Furthermore, improved functional recovery has been observed in isolated perfused transgenic mouse and rat hearts overexpressing HSP72 and subjected to an ischaemia-reperfusion sequence (Marber *et al*., 1995; Suzuki *et al*., 1997).

Finally, *in vivo* treatment with noradrenaline can induce a delayed cardioprotection against postischaemic dysfunction which is associated with expression of myocardial HSP72 and appears to be mediated by α_1 adrenoceptors (Meng *et al*., 1996b). Our study shows that protection of myocardium can be blocked independently of the level of HSP72 induction. This finding is in agreement with a previous study showing that protein kinase C (PKC) inhibition abolished the cardioprotection brought about by heat stress without modifying myocardial HSP72 synthesis (Joyeux *et al*., 1997). One possible explanation is that α_1 adrenoceptor blockade or PKC inhibition could alter the functional state of HSP72 thus rendering it ineffective in protecting the myocardium. Also, although HSP72 is widely studied as a primary effector of heat stress-induced protection, other mechanisms can be involved. Hence, Calderwood and co-workers (1988) have demonstrated that heat shock induces an intracellular 1,4,5-inositol triphosphate release. This effect is antagonized by phospholipase C inhibition, suggesting that activation of phospholipase C is implicated in the heat stress response and induces a 1,4,5-inositol triphosphate and diacylglycerol release. We have demonstrated that PKC inhibition prior to whole body hyperthermia abolished the cardioprotection induced 24 h later in the isolated rat heart (Joyeux *et al*., 1997), a finding which is in accordance with an *in vivo* study (Yamashita *et al*., 1997). Moreover, ATP-sensitive potassium (K_{ATP}) channel opening appears to play a role in the heat stress-induced delayed cardioprotection in the rat (Joyeux *et al*., 1998) and in the rabbit (Pell *et al*., 1997). We can thus presume that activation of α_1 adrenoceptors, followed by phospholipase C activation and diacylglycerol release, could result in PKC activation and K_{ATP} channel opening, providing one hypothetical transduction pathway for the heat stress-induced cardioprotection.

Our results and those of previous studies show that during heat stress, different transductional signalling pathways are activated including α_1 adrenoceptor stimulation, resulting in a delayed myocardial protection and HSP72 expression. We can thus hypothesize that in a fashion similar to that seen with ischaemic preconditioning (Goto *et al*., 1995), the blockade of only one component or transduction pathway of this cytoprotective response can abolish the protection even if the others are functional.

In summary, this study provides the first demonstration of the implication of the α_1 adrenoceptors in resistance to myocardial infarction induced by heat stress in the isolated rat heart, since prazosin pretreatment abolished the heat stress-induced cardioprotection. Moreover, both α_{1A} and α_{1B} adrenoceptor subtypes appear to play a role in the cardioprotection associated with the heat stress response. The α_1 adrenoceptors appear to participate to this cardioprotection by a mechanism independent of HSP72 induction. Further investigations are required to clarify the signal transduction pathways which co-ordinate the heat stress response and the potential role of HSP72, and of other stress-inducible proteins, in mediating adaptative cytoprotection.

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