Endocannabinoids are implicated in the infarct size-reducing effect conferred by heat stress preconditioning in isolated rat hearts

Marie Joyeuxa,*, Claire Arnauda, Diane Godin-Ribouta, Pierre Demengea, Daniel Lamontagneb, Christophe Ribuota

aLaboratoire Stress Cardiovasculaires et Pathologies Associées, Faculté de Pharmacie, Domaine de la Merci, 38706 La Tronche, France
bFaculté de Pharmacie, Université de Montréal, Montréal, Québec, Canada H3C 3J7

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Abstract

Objective: We have investigated the involvement of the endocannabinoid system in the delayed cardioprotection conferred by heat stress preconditioning in the isolated rat heart. Methods: Rats were divided into eight groups (n=7 in each group), subjected to either heat stress (42 °C for 15 min, HS groups) or sham anaesthesia (Sham groups). 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. Some hearts were perfused with either SR 141716 (a cannabinoid CB receptor antagonist, 1 µM), SR 144528 (a CB receptor antagonist, 1 µM) or l-NAME (a NOS inhibitor, 3 µM) 5 min before ischaemia and during the ischaemic period. Results: The infarct size-reducing effect conferred by heat stress (35.7±1.8% in Sham to 14.1±0.6% in HS groups) was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished by both SR 144528 (36.6±1.6%) and l-NAME (32.0±4.4%). In hearts from non-heat-stressed rats, perfusion with SR 141716 (32.8±1.6%), SR 144528 (33.4±2.2%) and l-NAME (31.6±2.9%) had no effect on infarct size. Conclusion: These results suggest an involvement of endocannabinoids, acting through CB receptors, and NO in the cardioprotection conferred by heat stress against myocardial ischaemia. The possible interaction between both mediators of the heat stress response remains to be determined.

Keywords: Shock; Preconditioning; Ischemia; Infarction; Nitric oxide

1. Introduction

Different preconditioning stimuli such as heat stress (HS) or lipopolysaccharide (LPS) or monophosphoryl lipid A (MLA) pre-treatment are known to trigger delayed endogenous protective mechanisms against myocardial ischaemia–reperfusion injury, appearing 24 h after exposure to stress and lasting for several days (for review see Ref. [1]). It is important to distinguish the molecular species that initiate the development of the protective response (trigger) from those which confer cardioprotection (mediator or end-effector).

Although the mechanisms implicated in heat stress preconditioning are not fully understood, different end-effectors, potentially responsible for cardiomyocyte protection, have been identified (for review see Ref. [2]). Amongst them, antioxidant enzyme activation [3], cardiac heat stress protein (HSP) synthesis [4,5] and ATP-sensitive potassium (KATP) channel opening [6–8] have been proposed. HS-induced cardioprotection resembles that triggered by LPS pre-treatment. Therefore, mediators under investigation for their role in LPS-induced ischaemic tolerance may provide a potential mechanism for HS-
induced cardioprotection [1]. Recently, we have reported an involvement of the endocannabinoid system in the cardioprotection triggered by LPS, in relation with a nitric oxide (NO) production [9].

Endogenous cannabinoids, first identified in the central nervous system, have recently attracted the attention of the scientific community. Two endocannabinoids, namely anandamide and 2-arachidonoyl glycerol, which are endogenous lipids have been isolated so far. They act through interaction with G-protein-coupled membrane receptors, namely CB₁ and CB₂ receptors, which are present throughout the body (for review see Ref. [10]). Selective antagonists of these receptors have also been developed. SR 141716, a CB₁ receptor antagonist, was found to block the central effect induced by an in vivo exposure to Δ⁹-tetrahydrocannabinol, the main psychoactive component of marijuana [11]. SR 144528, recently discovered, was shown to be a potent and selective CB₂ receptor antagonist [12].

Endocannabinoids have also been implicated in the regulation of the inflammatory response. Indeed, it has been reported that cannabinoid agonists can modulate inflammatory cytokine production in endotoxaemic mice through CB₁ receptor activation [13]. In macrophages, Δ⁹-tetrahydrocannabinol has been shown to inhibit the LPS-induced expression of the inducible form of NO synthase (NOS) [14] as well as NO production [15].

Therefore, the aim of the present study was to examine the involvement of the endocannabinoid system as well as the role of NO in the cardioprotection conferred by HS.

2. Methods

2.1. Experimental treatment groups

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 1996).

This study was conducted in two parts. In the first part, male Wistar (280–300 g) rats were submitted to either heat stress (HS groups) or anaesthesia without hyperthermia (Sham groups) and left to recover for 24 h. The animals were then assigned randomly to either one of the eight experimental groups (n=7 in each group). In the second part, an ischaemia (30 min)–reperfusion (120 min) protocol was performed in isolated hearts. Perfusion with either 1 μM SR 141716 [9], 1 μM SR 144528 [9] or 3 μM l-NAME (a non-selective NOS inhibitor [16]) was initiated 5 min before ischaemia and maintained during the entire ischaemic period. The experimental protocol is summarised in Fig. 1. The extent of heat stress performed and the particular time point for the observation of the cardioprotection induced have been chosen in order to compare our results with those of numerous previous studies [3–8].

2.2. Heat stress protocol

Heat stress was achieved by placing anaesthetised (with 25 mg kg⁻¹ i.p. sodium pentobarbitone) 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 group animals were anaesthetised only. All rats were allowed to recover for 24 h.

2.3. Ischaemia–reperfusion protocol

Rats were anaesthetised 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, CaCl₂ 1.8, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 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 (75 mmHg) with oxygenated Krebs–Henseleit buffer. A water-filled latex balloon (Hugo Sachs no. 4), coupled to a pressure transducer (Statham), was inserted into the left ventricular cavity via the left atrium for pressure recording. Left ventricular end-diastolic pressure (LVEDP) was adjusted between 5 and 10 mmHg. Myocardial temperature was measured by a thermoprobe inserted into the left ventricle and 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.

![Fig. 1. Experimental protocol.](https://academic.oup.com/cardiovascres/article-abstract/55/3/619/395642)
After 20 min of stabilisation, 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). Incidence of ventricular arrhythmias, assessed according to the Lambeth Conventions [17], was also measured throughout ischaemia and reperfusion.

3. Results

3.1. Haemodynamic data

Table 1 summarises CF, HR and LVDP data recorded in the eight experimental groups during the stabilisation 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 experimental groups.

3.2. Arrhythmias

The incidence of ventricular tachycardia and/or fibrillation (VT-VF) occurring during ischaemia and reperfusion is presented in Table 2. Twenty-four hours after heat stress or sham anaesthesia, there was no statistically significant difference in VT-VF incidence between the eight experimental groups.

3.3. Infarct data

Fig. 2 represents the infarct-to-risk ratio (I/R) of the eight experimental groups. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (32.8±1.6%), SR 144528 (33.4±2.2%) and L-NAME-perfused (31.6±2.9%) hearts. In hearts from non-heat-stressed rats, perfusion with SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts. Heat stress significantly reduced I/R from 35.7±1.8% in Sham to 14.1±0.6% in HS (P<0.05) groups. This infarct size-reducing effect of heat stress was not altered by the perfusion of SR 141716 (11.2±1.5%) but was abolished in SR 144528-perfused (36.6±1.6%) and L-NAME-perfused (32.0±4.4%) hearts.
Table 1

<table>
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<th>Group</th>
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<tr>
<td></td>
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<tr>
<td>CF (ml/min)</td>
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<td>13.9±0.5</td>
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<tr>
<td>HS</td>
<td>14.0±0.6</td>
<td>13.9±0.6</td>
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<tr>
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<td>14.8±0.2</td>
<td>14.8±0.3</td>
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<tr>
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<td>14.1±0.5</td>
<td>14.2±0.5</td>
</tr>
<tr>
<td>Sham+SR 144528</td>
<td>14.4±0.8</td>
<td>13.9±0.9</td>
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<tr>
<td>Sham+1-TNAME</td>
<td>13.9±0.4</td>
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<tr>
<td>HS+1-TNAME</td>
<td>12.9±0.7</td>
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<td>HR (bpm)</td>
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<td>HS</td>
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</tr>
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<td>LVDP (mmHg)</td>
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<tr>
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<td>90±7</td>
</tr>
<tr>
<td>Sham+1-TNAME</td>
<td>102±5</td>
<td>99±4</td>
</tr>
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</table>

CF, coronary flow; HR, heart rate; LVDP, left ventricular developed pressure. Data are mean±S.E.M. (n=7 in each group).

receptors in the preconditioning phenomenon is further strengthened by our recent work showing an implication of CB2 receptors in mediating LPS-induced myocardial protection [9].

The endocannabinoid system appears to be implicated in the regulation of many cardiovascular functions. Indeed, CB1 and CB2 transcripts have been found to be expressed in many peripheral tissues including the myocardium [18] and CB1 receptors have been located in vascular smooth muscle [19] and endothelial [20] cells. Furthermore, the two endocannabinoids identified so far, namely anandamide and 2-arachidonoyl glycerol, have recently been detected in the heart [21]. Finally, numerous studies report that these compounds appear to be cardiovascular

Table 2

Incidence (%) of ventricular arrhythmias occurring during ischaemia and reperfusion periods in the different experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>VT-VF ischaemia</th>
<th>VT-VF reperfusion</th>
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<tbody>
<tr>
<td>Sham</td>
<td>7</td>
<td>71</td>
<td>100</td>
</tr>
<tr>
<td>HS</td>
<td>7</td>
<td>71</td>
<td>100</td>
</tr>
<tr>
<td>Sham+SR 141716</td>
<td>7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>HS+SR 141716</td>
<td>7</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sham+SR 144528</td>
<td>7</td>
<td>71</td>
<td>100</td>
</tr>
<tr>
<td>HS+SR 144528</td>
<td>7</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>Sham+1-TNAME</td>
<td>7</td>
<td>71</td>
<td>86</td>
</tr>
<tr>
<td>Sham+1-TNAME</td>
<td>7</td>
<td>71</td>
<td>86</td>
</tr>
</tbody>
</table>

VT-VF, tachycardia and/or fibrillations. Data are mean.

Fig. 2. Infarct size (I) expressed as a percentage of the risk zone (R) assessed in isolated rat hearts following regional ischaemia (30 min)-reperfusion (120 min) sequence, in sham or heat-stressed hearts (42°C for 15 min 24 h earlier), perfused or not with either SR 141716 (1 µM), SR 144528 (1 µM) or 1-TNAME (3 µM). Drug perfusions are initiated 5 min before ischaemia and maintained during the entire ischaemic period. Open circles, individual values; closed circles, mean±S.E.M. *P<0.05 vs. the other groups.
modulators. They have important hypotensive and bradycardic effects via the inhibition of noradrenaline release by presynaptic CB₁ receptor activation (for review see Ref. [22]). This suggests that endocannabinoids could be implicated in pathological situations associated with extreme hypotension, such as LPS-induced endotoxic shock.

We also observed here that the HS-induced infarct size reduction was abolished by L-NAME perfusion before and during ischaemia, providing the first evidence that NO could be a mediator of the heat stress response. In accordance with a previous study showing that NO is involved in HSP synthesis induced by heat stress [23], we have recently demonstrated that NO formation is an important trigger of the heat stress response [24]. Indeed, we have observed that L-NAME administered before HS, completely abolished the delayed protection against myocardial infarction. Thus, it seems that NO can act both as a trigger and as a mediator of the cardioprotection conferred by HS. The same phenomenon has been described concerning the delayed protection conferred by other preconditioning stimuli, such as ischaemic preconditioning or LPS and MLA pre-treatments (for review see Ref. [1]).

We have previously shown that a functional relationship exists between NO and the endocannabinoid system. Indeed, we have observed in rat hearts that cardioprotection induced by a NO donor perfusion was abolished by a CB₂ receptor antagonist, indicating that NO-induced protection is mediated by CB₂ receptor activation [9]. Furthermore, it seems that cannabinoid receptor activation also interacts with NO production but a controversy still exists on the exact nature of this interaction (facilitating or inhibiting NO production), depending on the agonist used [15,25,26].

Although the mechanisms involved in HS preconditioning are not fully understood, different signalling pathways, potentially leading to the cardiomyocyte protection, have been identified (for review see Ref. [2]). We have previously shown that catecholamines (activating α₁ adrenoceptors [27]) and NO [24] act both as triggers to initiate the HS response, presumably resulting in protein kinase C (PKC) activation [28] and Kᵦᵦᵦᵦ channel opening [8], providing one hypothetical transduction pathway for the HS-induced cardioprotection. Furthermore, mitogen-activated protein kinase cascade (resulting in small HSP phosphorylation) seems to be involved in the HS response [29], potentially coupled to the PKC transduction pathway.

In this study, we have shown that 24 h after HS, NO and CB₂ receptor activation can also mediate the cardioprotection by separate or overlapping mechanisms. We could hypothesise that NO could also interact with other actors of the HS response such as PKC and/or mitochondrial Kᵦᵦᵦᵦ channel since it has been shown that NO is able to activate these proteins [30,31]. Finally, all these hypothetical transduction pathways (presented in Fig. 3), initiated by HS and leading to the delayed cytprotection, remain to be confirmed as well as their possible relationship.

Similarities seem exist between cardioprotection conferred by LPS pre-treatment and by prior HS. Thus, as observed in the HS response, PKC is also implicated in the LPS-induced cardiomyocyte protection [32]. Furthermore,
the late protection induced by these preconditionings appear to be mediated by common end-effectors such as myocardial HSP [4,33], antioxidant enzymes [3,34] and K_{ATP} channel [8,35]. Finally, as seen in the cardioprotection conferred by LPS pre-treatment [9], we demonstrated here that NO production and CB_{2} receptor activation could also mediate the HS-induced delayed cytoprotection.

It could be noticed in this work that HS conferred protection against myocardial infarction but not against the development of ventricular arrhythmias during ischaemia–reperfusion. Indeed, the experimental model of ischaemia–reperfusion used here has been specifically chosen to study the cellular necrosis and not the incidence and duration of the ventricular arrhythmias. It is why no difference of arrhythmias incidence was observed among the experimental groups in this work although we previously showed an antiarrhythmic effect induced by HS using another model of ischaemia–reperfusion (with a short duration of ischaemia) [36].

In summary, this study provides the first demonstration that CB_{2} receptors and NOS activation appear to mediate resistance to myocardial infarction induced by HS in the isolated rat heart, since both SR 144528 and l-NAME perfusion abolished the protection conferred by HS preconditioning. Further investigations are required to elucidate the precise signalling pathways by which CB_{2} receptors and NOS are activated following HS. The separate or overlapping mechanisms by which CB_{2} receptor, NO and the other stress-inducible proteins protect myocytes against ischaemic injury remain to be determined.

References

[17] Walker MA, Curtis MJ, Hearse DJ et al. The Lambeth Conver-


