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# Functional and biochemical evidence for capsaicin-induced neural endothelin release in isolated working rat heart

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**Abstract**

In isolated working rat heart, capsaicin elicited a concentration-dependent constriction of coronary arteries accompanied by decline of all cardiac parameters recorded (heart rate, coronary and aortic flow, left ventricular developed pressure, and first derivative of left ventricular developed pressure). The following evidence suggests that capsaicin-induced changes are mediated by endothelin of neural origin: (1) the capsaicin (10 nM)-evoked decrease in coronary flow resulting in deterioration of cardiac functions was mimicked by endothelin (0.1 nM); (2) the selective endothelin $ET_A$ receptor antagonist, cyclo (D-α-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl) (1 μM), abolished the cardiac effects provoked by capsaicin (10 nM); (3) reduction of extracellular $Ca^{2+}$ concentration from 2.4 to 1.2 or 0.6 mM inhibited the cardiac effects of capsaicin (10 nM) but not those induced by endothelin (0.1 nM); (4) perfusion of the heart with 0.1% (v/v) Triton X-100 damaged the endothelium and reversed the enhancement of coronary flow evoked by bethanechol (1 μM), decreased the basal flow, but was without effect on capsaicin-induced coronary constriction; (5) in response to capsaicin challenge (10–100 nM), the endothelin concentration measured in coronary effluent by means of radioimmunoassay increased up to sevenfold but remained unchanged in the presence of 0.6 mM $Ca^{2+}$; (6) no reduction of coronary flow was induced by capsaicin (100 nM) applied to the heart of rats which were desensitised by capsaicin (150 mg/kg). It is concluded that, in the rat heart, capsaicin acting on VR1 capsaicin receptors elicits a release of endothelin from the sensory nerve terminals. 

*Keywords:* Capsaicin; Endothelin; BQ-123; Cardiac function; Heart, isolated, rat; Sensory neuropeptide

## 1. Introduction

Capsaicin, the pungent principle of red pepper due to its selective site of action on a subset of primary afferent neurones (Holzer, 1991; Szolcsányi, 1993; Maggi, 1995; Caterina et al., 1997), has become a valuable tool to reveal the function of the afferents. Activation of capsaicin-sensitive nociceptive, heat-sensitive and chemoceptive nerve endings (Szolcsányi, 1993) results in release of a variety of sensory neuropeptides, particularly tachykinins and calcitonin gene-related peptide (CGRP) which elicit various local tissue responses (Holzer, 1991; Maggi, 1995; Lundberg, 1996; Szolcsányi, 1996). The myocardium and coronary vessels are richly innervated by sensory neuropeptide-containing primary afferent neurones sensitive to capsaicin (Lundberg et al., 1985; Saito et al., 1986; Wharton et al., 1986; Maggi, 1995; Lundberg, 1996). Although the neuropeptide-releasing effects of capsaicin have been studied and well characterised in a wide variety of tissues, our knowledge concerning the capsaicin-elicited responses in the heart and coronary vessels is relatively sparse. The acute effects of capsaicin have been analysed in detail for the guinea-pig isolated perfused heart (Franco-Cereceda, 1988; Franco-Cereceda and Lundberg, 1985; Franco-Cereceda et al., 1991a; Oroszi et al., 1999) or the guinea-pig isolated atria (Fukuda and Fujiwara, 1969; Molnar et al., 1969; Franco-Cereceda, 1988; Maggi, 1995; Lundberg, 1996). Surprisingly, regarding rat heart, few data are available for the acute effects of capsaicin although studies on

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isolated perfused heart obtained from capsaicin-pretreated rats raised the issue of an important adaptive role in cardioprotection for CGRP and nitric oxide (NO) released from capsaicin-sensitive afferents (Ferdinandy et al., 1997; Song et al., 1999). In the rat, positive inotropic and chronotropic effects of capsaicin were described in the isolated atrial preparation similar to those seen with hearts from the guinea-pig and have been attributed to the release of sensory neuropeptide CGRP (Franco-Cereceda, 1988; Maggi, 1995; Lundberg, 1996). Recently, however, we observed that capsaicin applied to the isolated working rat heart induced a decrease in coronary and aortic flow with negative inotropic effects resembling the action of endothelin (Szolcsányi et al., 1999). The present aim was to shed light on the mechanism of capsaicin action on the isolated working rat heart. Particular emphasis was put on providing evidence for the hypothesis that capsaicin induced a release of endothelin from sensory nerve endings. It seemed to be important since, in spite of some histochemical data indicating the existence of endothelin in perivascular nerve fibres (Giaid et al., 1989; Franco-Cereceda et al., 1991b; Loesch et al., 1998), the presence of endothelin in capsaicin-sensitive sensory neurones or its release from sensory nerve endings in general has not yet been shown (Holzer, 1991; Maggi, 1995; Szolcsányi, 1996; Lundberg, 1996).

## 2. Methods

### 2.1. Animals

Male Sprague–Dawley rats (320–350 g body weight) were used for the experiments. The experimental protocols applied conformed to the European Community guiding principles for the care and use of laboratory animals and were approved by the local ethical committee of the University of Pecs, Hungary.

### 2.2. Isolated working heart preparation

Rats were anaesthetised with diethyl ether and then intravenous heparin (500 IU/kg) was given. After thoracotomy, the heart was excised and placed in ice-cold perfusion buffer. Immediately after preparation, the aorta and pulmonary vein were cannulated, and the heart was perfused (at 37°C) according to the Langendorff method for a 5-min washout period at a constant perfusion pressure equivalent to 100 cm of water (10 kPa). The perfusion medium consisted of a modified Krebs–Henseleit bicarbonate solution: sodium chloride 118, potassium chloride 4.7, calcium chloride 2.4, sodium bicarbonate 25, potassium biphosphate 0.36, magnesium sulphate 1.2 and glucose 10 (mM). The Langendorff preparation was switched to the working mode following the washout period as previously described in detail by Tosaki and Braquet (1990) and Tosaki and Hellegouarch (1994). Aortic flow was measured by a calibrated rotameter. Coronary flow rate was measured by a timed collection of the coronary effluent that dripped from the heart.

Throughout all experiments heart rate, coronary flow, and aortic flow rates were recorded. Left ventricular developed pressure, and the first derivative of left ventricular developed pressure was recorded by the insertion of a Millar catheter into the left ventricle via the left atrium and mitral valve.

### 2.3. Experimental protocols

In the first series of studies, extracellular $Ca^{2+}$ concentration was reduced from its control value of 2.4–1.2 or 0.6 mM. These extracellular $Ca^{2+}$ reductions were maintained during the perfusion of 10 nM of capsaicin (selected according to the capsaicin dose–response curve; Szolcsányi et al., 1999). Cardiac function was measured before (control), and after the perfusion of reduced $Ca^{2+}$ concentrations with capsaicin.

In the second series of experiments, 0.1 nM endothelin (Brunner and Opie, 1998) was perfused for 5 min and cardiac function was recorded.

In the third series of the study, 0.1 nM endothelin was perfused in the presence of 0.6 mM of extracellular $Ca^{2+}$ for 5 min, and cardiac function was monitored.

In the next series, 10 nM capsaicin was co-perfused with 1 μM of BQ-123 (Wang et al., 1998; Delpech et al., 1997), a selective endothelin $ET_A$ receptor antagonist, for 5 min and cardiac function was measured.

In the next protocol, 0.1 nM of endothelin was co-perfused with 1 μM of BQ-123.

In the sixth set of experiments, 0.1% (v/v) Triton X-100 (0.2 ml) was applied over 5 s into the coronary vasculature. This was succeeded by an immediate washout period according to aerobic Langendorff perfusion. Transient exposure to Triton X-100 served as a conventional method to destroy functional vascular endothelium in the intact heart (Kamata et al., 1996). The effect of capsaicin was then studied following the switch to the working mode.

In the last series of experiments on Langendorff preparations, the effect of capsaicin was tested on hearts obtained from rats pretreateded with capsaicin in vivo. Selective long-lasting impairment of the capsaicin-sensitive sensory nerve endings was produced (Holzer, 1991; Maggi, 1995; Szolcsányi, 1993) by subcutaneous injections of capsaicin (30 + 60 + 60 mg/kg) on three consecutive days under pentobarbitone anaesthesia (30 mg/kg i.p.). The hearts were removed for investigation 1 day after the last dose was given.

### 2.4. Measurement of endothelin in coronary effluents

The endothelin levels in response to capsaicin (10 and 100 nM) were determined in coronary effluents in the

presence of a normal (2.4 mM) or reduced (0.6 mM) extracellular $Ca^{2+}$ concentration.

Coronary effluents (100 ml) were collected in polystyrene containers spiked with ethylene diamine tetra-acetic acid disodium salt (EDTA) and Triton X-100 to give final concentrations of 5 mM and 0.5% (v/v), respectively, and were frozen to −70°C until they were processed. The perfusate was loaded onto 3 ml methanol and 5 ml water conditioned Sep-Pak $C_{18}$ cartridges, and endothelin was eluted with 2 ml 60% (v/v) acetonitrile in 0.1% (v/v) trifluoroacetic acid yielding a mean recovery of 60%. The eluates were freeze-dried and kept at −20°C until further radioimmunoassay determinations. Since the residue was re-dissolved in 0.5 ml of assay buffer, the coronary effluent samples were concentrated 200 times. Endothelin (i.e. endothelin-1, -2 and -3) immunoreactivity was then determined by radioimmunoassay using commercial endothelin radioimmunoassay kits with cross-reactivity for endothelin 2 (142%), endothelin 3 (98%), big endothelin 1–38, big endothelin fragments 22–38, (< 1%); with inter-assay and intra-assay variations of below 10% and 5%, respectively (see commercial kit descriptions for details).

### 2.5. Drugs and chemicals

Diethyl ether, Triton X-100 and Tween 80 were purchased from Reanal (Budapest, Hungary), bethanechol (acetyl-beta-methylcholine) from Schuchardt (Munchen, Germany), heparin from Richter (Budapest, Hungary), capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) and endothelin-1 from Sigma (St. Louis, USA), BQ-123 (cyclo [D-α-aspartyl-L-propyl-D-valyl-L-leucyl-D-tryptophyl]) from RBI (Natick, MA, USA), all constituents of the modified Krebs–Henseleit solution from Merck (Darmstadt, Germany). The endothelin radioimmunoassay kits, RIK 6910, were bought from Peninsula (Belmont, CA, USA) and Sep-Pak $C_{18}$ cartridges from Waters (Milford, MA, USA). Capsaicin was dissolved in a solution consisting of 10% (v/v) ethanol, 10% (v/v) Tween 80 and 80% (v/v) saline, then this stock solution was diluted with saline to the concentrations applied.

### 2.6. Exclusion criteria

Pre-selected exclusion criteria for the present studies demanded that hearts be excluded if: (1) ventricular arrhythmias occurred during the period prior to drug perfusions, (2) coronary flow and aortic flow were less than 19 and 35 ml/min, respectively, during drug-free aerobic perfusion. For the aforementioned reasons, eight hearts were excluded from the entire study (75 hearts at the start).

### 2.7. Statistical analysis

The data for myocardial function (heart rate, coronary flow, aortic flow, left ventricular developed pressure and first derivative of left ventricular developed pressure) were expressed as the means ± S.E.M. One-way analysis of variance was first carried out to test for any differences between the mean values of all groups. If differences were established, the values for the drug-treated groups were compared with those for the drug-free control group by a modified *t*-test. A change of $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Effects of reduced extracellular $Ca^{2+}$ concentration on cardiac responses evoked by capsaicin or endothelin

In these studies, the extracellular $Ca^{2+}$ concentration was reduced from its control value of 2.4–1.2 or 0.6 mM in the presence of 10 nM capsaicin. The capsaicin concentration was selected according to the dose–response curve from the previous experiments. These alterations in extracellular $Ca^{2+}$ concentrations did not produce any significant change in baseline heart rate, coronary flow, aortic flow, left ventricular developed pressure and first derivative of left ventricular developed pressure (data not shown). The effects of capsaicin (10 nM) were markedly inhibited in the presence of reduced $Ca^{2+}$ (Table 1). Thus, at 2.4 mM $Ca^{2+}$, coronary flow was reduced from its control value of 28.3 ± 0.3 to 18.2 ± 1.5 ml/min in the presence

Table 1
Effects of capsaicin and endothelin on cardiac function in the presence of normal or reduced extracellular calcium concentration $[Ca^{2+}]_o$. Data are expressed as means ± S.E.M. obtained with six hearts in each group. Comparisons were made to the corresponding drug-free values

| Concentration of substances | HR (beats/min) | CF (ml/min) | AF (ml/min) | LVDP (kPa) | $LVdP/dt_{max}$ (kPa/s) |
|---|---|---|---|---|---|
| 2.4 mmol/l $[Ca^{2+}]_o$ (control) | 313 ± 9 | 28.3 ± 0.3 | 55.2 ± 2.4 | 18. ± 0.1 | 794 ± 9 |
| 2.4 mmol/l $[Ca^{2+}]_o$ + Capsaicin $10^{-8}$ mol/l | 254 ± 12[a] | 18.2 ± 1.5[a] | 24.2 ± 3.1[a] | 13.8 ± 0.7[a] | 527 ± 31[a] |
| 1.2 mmol/l $[Ca^{2+}]_o$ + Capsaicin $10^{-8}$ mol/l | 278 ± 11[a] | 26.9 ± 1.5 | 38.6 ± 1.9[a] | 16.9 ± 0.2 | 701 ± 12[a] |
| 0.6 mmol/l $[Ca^{2+}]_o$ + Capsaicin $10^{-8}$ mol/l | 308 ± 7 | 26.7 ± 2.1 | 46.5 ± 1.2[a] | 17.6 ± 0.1 | 766 ± 10 |
| 2.4 mmol/l $[Ca^{2+}]_o$ + Endothelin $10^{-10}$ mol/l | 253 ± 8[a] | 16.4 ± 1.3[a] | 18.2 ± 3.9[a] | 11.8 ± 1.2[a] | 489 ± 35[a] |
| 0.6 mmol/l $[Ca^{2+}]_o$ + Endothelin $10^{-10}$ mol/l | 248 ± 11[a] | 16.7 ± 2.19[a] | 22.8 ± 4.3[a] | 13.8 ± 0.9[a] | 543 ± 40[a] |

Abbreviations: HR (heart rate), CF (coronary flow), AF (aortic flow), LVDP (left ventricular developed pressure), $LVdP/dt_{max}$ (first derivative of LVDP).
[a] $P < 0.05$.

of 10 nM capsaicin. When capsaicin was co-perfused with 1.2 mM of $Ca^{2+}$, the capsaicin-induced vasoconstriction was completely blocked, as indicated by the non-significant reduction in coronary flow ($26.9 \pm 1.5$ vs. $28.3 \pm 0.3$ ml/min). A similar non-significant reduction of the coronary flow was observed in the presence of 0.6 mM $Ca^{2+}$. The reduction in extracellular calcium prevented the capsaicin-induced diminution in heart rate, aortic flow, left ventricular developed pressure and first derivative of left ventricular developed pressure as well (Table 1).

Perfusion of the heart with 0.1 nM of endothelin-1 elicited an inhibition in heart rate, coronary flow, aortic flow, left ventricular developed pressure and first derivative of left ventricular developed pressure similar to that seen with capsaicin at a concentration of 10 nM (Table 1). At a low $Ca^{2+}$ concentration (0.6 mM), the endothelin-induced responses were not prevented, in contrast to the effects of capsaicin which were markedly inhibited at this $Ca^{2+}$ concentration (Table 1).

### *3.2. Impact of BQ-123, a selective endothelin $ET_A$ receptor antagonist on changes of cardiac function evoked by capsaicin or endothelin*

Table 2 shows that BQ-123, a selective endothelin $ET_A$ receptor antagonist, eliminated the effect of both capsaicin and endothelin, suggesting that the capsaicin-induced responses were due to a release of endogenous endothelin in isolated working rat hearts.

### *3.3. Interaction between capsaicin and removal of functional endothelium by Triton X-100*

Bethanechol at concentration of 1 μM induced an increase in coronary flow, a response reversed by a preceding 0.1% (v/v) Triton X-100 challenge over 5 s (Fig. 1A). Capsaicin (100 nM), however, elicited a decrease in coronary flow irrespective of the Triton X-100 pretreatment (Fig. 1B). Note that in both cases, destruction of the coronary endothelium with Triton X-100 markedly decreased the basal coronary flow.

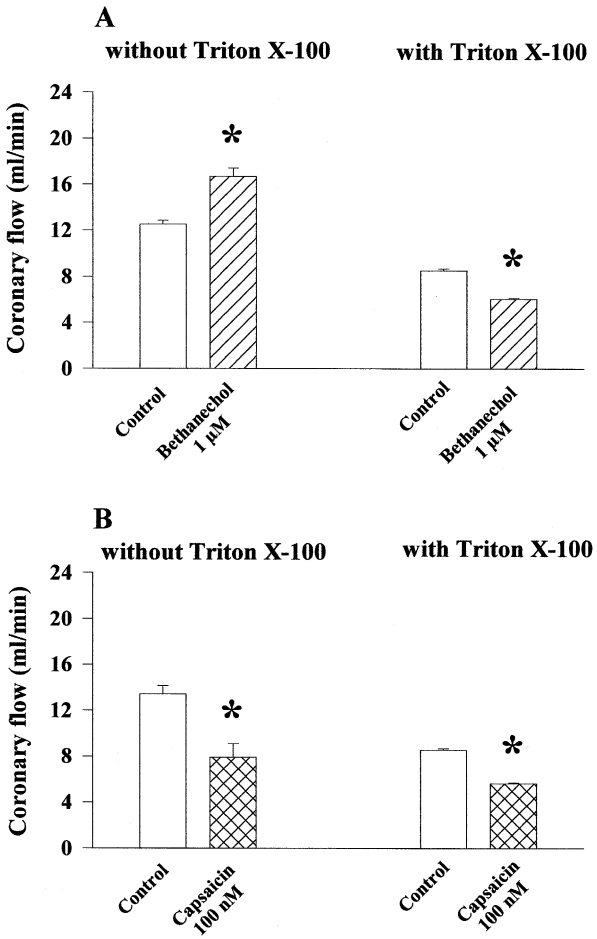


Fig. 1. (A) Endothelium-dependent vasorelaxation by betanechol in isolated Langendorff-perfused rat heart. (B) Endothelium-independent vasoconstriction by capsaicin. Removal of functional coronary endothelium resulted from a preceding brief (5 s) exposure to 0.1% (v/v) Triton X-100. Data are means ± S.E.M. obtained with six preparations. *: Significantly different from corresponding control at $P < 0.05$.

### *3.4. Effect of capsaicin on hearts obtained from capsaicin-pretreated rats*

Hearts from rats ($n = 6$) pretreated with a total dose of 150 mg/kg capsaicin were perfused according to the Langendorff method. Coronary flow before ($12.9 \pm 0.4$ ml/min) and after ($12.4 \pm 0.4$ ml/min) capsaicin (100

Table 2
Effects of capsaicin and endothelin on cardiac function in the presence or absence of BQ-123, a selective endothelin A receptor blocking agent. Data are expressed as means ± S.E.M. obtained with six hearts in each group. Comparisons were made to the corresponding drug-free values

| Concentration of substances | HR (beats/min) | CF (ml/min) | AF (ml/min) | LVDP (kPa) | $LVdP/dt_{max}$ (kPa/s) |
|---|---|---|---|---|---|
| Control | 316. ± 7 | 29.5 ± 1.68 | 60.7 ± 4.9 | 18.1 ± 0.1 | 808 ± 11.1 |
| Capsaicin $10^{-8}$ mol/l | 258 ± 11[a] | 16.9 ± 1.3[a] | 21.5 ± 2.8[a] | 14.2 ± 0.7[a] | 541 ± 39[a] |
| Capsaicin $10^{-8}$ mol/l + BQ-123 $10^{-6}$ mol/l | 327 ± 8 | 30.8 ± 1.47 | 58.6 ± 4.1 | 17.3 ± 0.2 | 796 ± 15 |
| Endothelin $10^{-10}$ mol/l | 257 ± 9[a] | 17.4 ± 1.7[a] | 17.4 ± 5.3[a] | 12.6 ± 1.1[a] | 498 ± 48[a] |
| Endothelin $10^{-10}$ mol/l + BQ-123 $10^{-6}$ mol/l | 311 ± 12 | 28.8 ± 1.2 | 56.1 ± 3.7 | 17.8 ± 0.1 | 785 ± 13 |

Abbreviations: HR (heart rate), CF (coronary flow), AF (aortic flow), LVDP (left ventricular developed pressure), $LVdP/dt_{max}$ (first derivative of LVDP).
[a] $P < 0.05$.

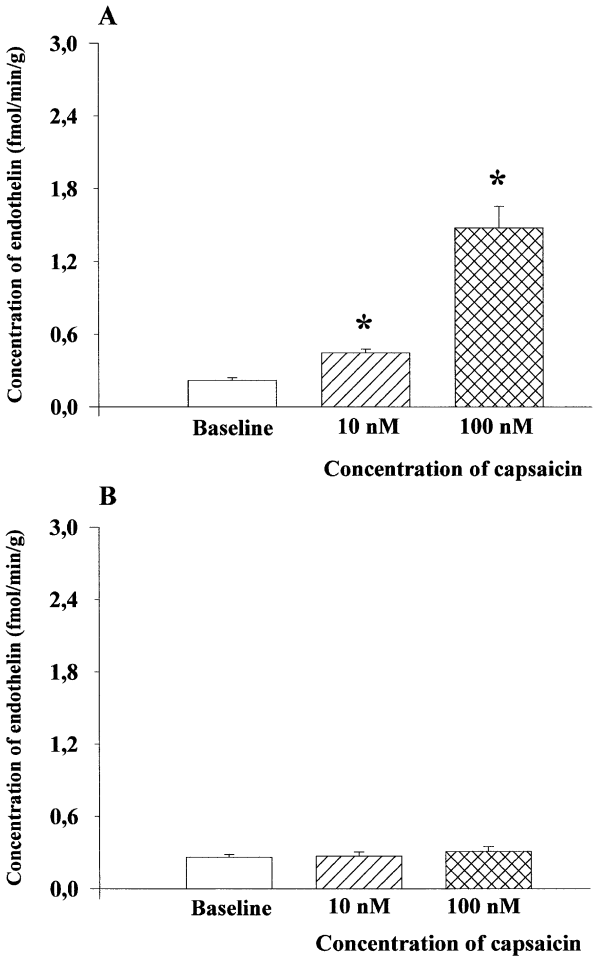


Fig. 2. Capsaicin-induced endothelin release in the presence of normal (A) and decreased (B) extracellular $Ca^{2+}$ concentration. Endothelin (endothelin 1, 2 and 3) concentration was determined in coronary effluent of the Langendorff-perfused rat hearts by radioimmunoassay. The results are means ± S.E.M. obtained from six determinations with six separate preparations. *: Significantly different from baseline at $P < 0.05$.

nM) challenge remained similar, indicating the absence of a capsaicin-induced decrease in coronary flow. Furthermore, the basal coronary flow values did not differ significantly from the values obtained with untreated control preparations (see Fig. 1).

### *3.5. Effect of capsaicin on endothelin release*

The endothelin concentration of the coronary effluent increased twofold and sevenfold compared to the baseline in response to 10 and 100 nM capsaicin, respectively, in the presence of a normal (2.4 mM) extracellular $Ca^{2+}$ concentration (Fig. 2A). In contrast, a reduced (0.6 mM) extracellular $Ca^{2+}$ concentration prevented the capsaicin-evoked endothelin release (Fig. 2B).

## 4. Discussion

The present findings confirm our previous results (Szolcsányi et al., 1999) that capsaicin at nanomolar concentrations markedly diminishes coronary flow, resulting in deterioration of all cardiac functions in the isolated working rat heart. The following experimental data support the concept that the effects observed are due to release of endothelin. (1) Direct radioimmunoassay determination revealed that capsaicin perfusion at low concentrations (10–100 nM) increased the endothelin level in the coronary effluent of the preparations up to sevenfold the baseline value. (2) Exogenous endothelin (0.1 nM) mimicked the effect of capsaicin (10 nM), inducing a similar inhibition with respect to heart rate, coronary and aortic flow, left ventricular developed pressure and its first derivative (Klemm et al., 1995; Brunner et al., 1997; Geller et al., 1998; Tanaka et al., 1997). In both cases, the changes were long lasting, practically irreversible. (3) Cardiac effects of both capsaicin and endothelin were antagonised by BQ-123, a selective endothelin $ET_A$ receptor blocking agent (Wang et al., 1998; Delpech et al., 1997). This finding is consistent with the results of our earlier experiments in which the non-selective endothelin receptor antagonist, PD142893 (*N*-[*N*-[*N*-[*N*-(*N*-Acetyl-β-phenyl-D-phenylalanyl)-L-leucyl]-L-α-aspartyl]-L-isoleucyl]-L-isoleucyl]-L-tryptophan), was applied (Szolcsányi et al., 1999), but provides new evidence for the mediation of the capsaicin effect on the rat heart through endothelin $ET_A$ receptors.

Genes encoding the endothelins are expressed in a wide array of cells, although the major source of endothelin is thought to be endothelial (Rubányi and Polokoff, 1994; Opgenorth, 1995; Miyauchi and Masaki, 1999). On the other hand, messenger RNA for endothelin-1 as well as the peptide itself have been localised in the dorsal root ganglia, where they co-exist with substance P and CGRP (Giaid et al., 1989). Endothelin-like immunoreactivity has also been shown in perivascular nerves of the rat basilar artery (Loesch et al., 1998). Furthermore, endothelin-like immunoreactivity of dorsal root ganglia was several-fold higher than that of the stellate ganglion, spinal cord, heart, pulmonary artery or aorta, being the highest among the large range of tissues investigated (Franco-Cereceda et al., 1991b).

A selective site of action of capsaicin on a subset of polymodal type nociceptors with C- and A-delta fibres was described by single unit studies, not only in cutaneous nerves, but also in nerve branches which innervate the heart and great vessels (Holzer, 1991; Szolcsányi, 1993, 1996; Maggi, 1995). Moreover, the recently cloned capsaicin receptor, termed as VR-1 receptor, was expressed in the small type sensory neurones but was undetectable in the heart tissue of rats (Caterina et al., 1997). Together, all these findings suggest that sensory nerve terminals, and not the endothelium of the coronary vessels or the heart, are the source of released endothelin.

In the present study, three lines of evidence support this conclusion. (1) Removal of functional endothelial cells with Triton X-100 (Kamata et al., 1996) was incapable of preventing the capsaicin-induced decrease of the coronary

flow and its deleterious effects on cardiac functions. On the other hand, the pretreatment reversed the vasodilator effect of the cholinergic agonist, bethanechol, and also decreased basal coronary flow, indicating that the Triton X-100 treatment used was appropriate to destroy functional endothelium. (2) The other approach exploited the fact that capsaicin elicits a $Ca^{2+}$-dependent release of sensory neuropeptides which has been analysed in detail for substance P and CGRP (Holzer, 1991; Maggi, 1995). We have now shown that the pronounced coronary and cardiac effects of capsaicin were inhibited and abolished by reducing the extracellular $Ca^{2+}$ concentration from its control value of 2.4–1.2 or 0.6 mM, respectively. The reduced extracellular $Ca^{2+}$ concentration prevented the release of endothelin evoked by capsaicin. Furthermore, the fact that the cardiac effects of exogenous endothelin remained practically unchanged under these conditions indicated that the reduced $Ca^{2+}$ concentration in the perfusate did not diminish the responsiveness of the coronary vessels or cardiac muscle to endothelin. (3) The capsaicin-induced reduction of the coronary flow was absent in hearts obtained from rats pretreated with capsaicin for 1–3 days before the experiment. Long-term selective damage of the capsaicin-sensitive nerve endings with depletion of neuropeptides such as tachykinins, CGRP and somatostatin is a cardinal feature of the actions of capsaicin (Holzer, 1991; Szolcsányi, 1993, 1996; Maggi, 1995). Similar lasting effects of capsaicin, unrelated to its action on capsaicin-sensitive, VR1-expressing sensory neural elements, were never found in various peripheral tissues. With respect to the present data, it is relevant that in isolated perfused rat heart subjected to 15-min ischaemia by coronary occlusion, subsequent perfusion with 1 μM capsaicin did not affect either coronary flow or heart rate, whereas in non-ischaemic rat hearts it reduced the left ventricular developed pressure and coronary flow (D'Alonzo et al., 1995). It is tempting to assume that the absence of the capsaicin response after ischaemia is an indicator of neural damage.

The deleterious effect of capsaicin on cardiac function of the rat working heart is probably mainly due to its pronounced inhibitory effect on coronary flow and oxygen supply, but its pronounced effect on aortic flow indicates an effect on the working muscle also. This conclusion is supported by the fact that capsaicin, as well as its putative sensory neuropeptide mediators of CGRP and endothelin, displayed positive inotropic and chronotropic effects in the isolated atrial preparations of the rat or guinea-pig (Fukuda and Fujiwara, 1969; Molnar et al., 1969; Franco-Cereceda, 1988; Lembeck et al., 1989). It is intriguing, however, that perfusion of the guinea-pig Langendorff heart preparation with 1 μM capsaicin produced no endothelin release in the outflow, while endothelin-like immunoreactivity in sensory neurones is high in this species (Franco-Cereceda et al., 1991b). The absence of evidence for the release of endothelin from guinea-pig hearts, and the relative insensitivity of the guinea-pig coronary arteries to endothelin (as compared to that of the rat; Lembeck et al., 1989), might explain why capsaicin enhances and does not diminish coronary flow in this species. It is remarkable that simultaneous detection of CGRP released and capsaicin-induced changes in frequency, tension and perfusion volume in the Langendorff-perfused guinea-pig heart preparation showed that 100 nM capsaicin induced a more than tenfold increase of the CGRP in the outflow without evoking any significant change in perfusion pressure or volume (Franco-Cereceda et al., 1991a). Consequently, in guinea-pig heart, the primary sensory neuropeptide of functional significance is CGRP, eliciting coronary vasodilatation and positive inotropic effects, while in the rat heart capsaicin releases endothelin which, probably owing to its potent and practically irreversible effects, prevents the manifestation of the increased coronary flow and positive inotropic effect mediated by the release of CGRP or NO (Franco-Cereceda, 1988; Franco-Cereceda et al., 1991a; Ferdinandy et al., 1997).

In rats, systemic pretreatment with high capsaicin doses several days before the experiment induces sensory blockade "desensitisation" with depletion of CGRP and NO from the cardiac sensory fibres (Ferdinandy et al., 1997). In these pretreated rats, an impairment of cardiac protection elicited by pacing-induced preconditioning or heat stress (Ferdinandy et al., 1997; Song et al., 1999) was observed which might indicate that several days after capsaicin treatment the impairment in the release of vasodilator mediators prevails over the now described endothelin-mediated responses.

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