Naunyn-Schmiedeberg's Arch Pharmacol (1997) 356 : 356–363

ORIGINAL ARTICLE

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Capsaicin-sensitive local sensory innervation is involved in pacing-induced preconditioning in rat hearts: role of nitric oxide and CGRP?

Received: 2 April 1997 / Accepted: 3 June 1997

Abstract Among several mediators, nitric oxide (NO) and calcitonin gene-related peptide (CGRP) were suggested to be involved in the mechanism of preconditioning. We examined the possible role of the cardiac capsaicin-sensitive sensory innervation in pacing-induced preconditioning, as well as in the cardiac NO and CGRP content. Wistar rats were treated subcutaneously with capsaicin or its solvent in the sequence of 10, 30, and 50 mg/kg increasing single daily doses for 3 days to deplete neurotransmitters of the sensory innervation. Isolated hearts from both groups were then subjected to either preconditioning induced by three consecutive periods of pacing at 600 beats per minute for 5 min with 5 min interpacing periods, or time-matched non-preconditioning perfusion, followed by a 10-min coronary occlusion. NO content of left ventricular tissue samples was assayed by electron-spin resonance, and CGRP release was determined by radioimmunoassay. CGRP immunohistochemistry was also performed. In the non-preconditioned, solvent-treated group, coronary occlusion decreased cardiac output (CO) from 68.1 to 32.1 mL/min, increased left ventricular end-diastolic pressure (LVEDP) from 0.58 to 1.90 kPa, and resulted in 200 mU/min/g LDH release. Preconditioning significantly increased ischaemic CO to 42.9 mL/min ($P < 0.05$), decreased ischaemic LVEDP to 1.26 kPa ($P < 0.05$) and decreased LDH release to 47 mU/min/g ($P < 0.05$) in the solvent-treated group. Preconditioning did not confer protection in the capsaicin-pretreated group (ischaemic CO: 35.6 mL/min; LVEDP: 1.76 kPa; LDH 156 mU/min/g). Capsaicin-treatment markedly decreased cardiac NO content, CGRP release, and CGRP-immunoreactivity. Conclusions: (i) The presence of an intact local sensory innervation is a prerequisite to elicit pacing-induced preconditioning in the rat heart. (ii) A significant portion of cardiac basal NO content may be of neural origin. (iii) Release of NO and CGRP from capsaicin-sensitive nerves may be involved in the mechanism of pacing-induced preconditioning.

Key words Preconditioning · Rapid pacing · Capsaicin · Nitric oxide · Electron-spin resonance · Calcitonin gene-related peptide · Immunohistochemistry · Rat heart

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Introduction

Since the original observation by Murry et al. (1986) more than a decade ago, the ability of the heart to adapt to ischaemic stress has been well established and termed ischaemic preconditioning. The cardioprotective effect of preconditioning shows two phases, an acute phase (classical preconditioning) and a 'second window' phase, and involves reduction of necrotic tissue mass, improvement of ischaemic/reperfused cardiac performance, and reduction of incidence/severity of arrhythmias (see for reviews: Parratt 1995; Baxter and Yellon 1994). In spite of intensive research in the past 10 years, there is considerable controversy in the literature regarding the mechanism of ischaemic preconditioning. A variety of substances and ion channels, i.e. adenosine, bradykinin, nitric oxide (NO), cyclic guanosine monophosphate (cGMP), prostacyclin, protein-kinases, noradrenaline, adenosine-triphosphate-sensitive K^+-channels (K_{ATP}) etc. were suggested and also refuted to be potential mediators of preconditioning. These discrepancies are generally attributed not only to species differences (see for reviews: Parratt 1995; Baxter et al.

1996; Walker and Yellon 1992), but also to the different triggers of preconditioning, like rapid pacing and no-flow ischaemia (Ferdinandy et al. 1995b). Moreover, the possible ischaemia sensor, if any exists, and the formation site of the potential mediators of preconditioning are still not known.

Concentrating on the well known adrenergic and cholinergic effector innervation of the heart, cardiovascular researchers generally leave out of consideration the rich sensory innervation of the myocardium and the coronary vascular system, which may have strong influence on cardiac function due to its calcitonin gene-related peptide (CGRP), NO, and substance P content (Franco-Cereceda 1988; Sosunov et al. 1995, 1996). The thin sensory nerve endings may act as potential sensor for ischaemia, since ischaemia, hypoxia, lactate, K^+, and low pH were shown to stimulate cardiac sensory nerves in association with the release of their transmitters (see for review: Franco-Cereceda 1988). The involvement of cardiac CGRP in preconditioning was recently assumed by Li et al. (1996). The role of NO in the mechanism of preconditioning is controversial. Vegh et al. (1992) found that the antiarrhythmic effect of preconditioning induced by short periods of coronary occlusion was abolished by administration of N^G-nitro-L-arginine methyl ester in anaesthetized dogs; Lu et al. (1995) and Weselcouch and associates (1995) could not confirm these results in anaesthetized rats and in isolated rat hearts. However, these studies focused on the antiarrhythmic effect of preconditioning induced by 'no-flow' ischaemia, and no direct measurement of myocardial NO was attempted. We have recently reported that a significant decrease in cardiac NO content assessed by electron spin resonance (ESR) abolished pacing-induced preconditioning in rat heart (Ferdinandy et al. 1996). We have also shown that the cardioprotective effect of pacing-induced preconditioning was proportional to the elevation of myocardial cGMP content (Szilvassy et al. 1994a), and that the reduced cardiac cGMP content due to experimental nitroglycerin tolerance led to the loss of preconditioning in rabbits (Szilvassy et al. 1994b). These data suggest that the NO-cGMP pathway may be involved in the mechanism of pacing-induced preconditioning in rabbits and rats, however, the major source of NO in the heart has not been identified.

Here we examine the hypothesis that cardiac sensory innervation is involved in the mechanism of pacing-induced preconditioning possibly via neural NO and CGRP release. Capsaicin is a highly selective sensory neurotoxin which, upon systemic administration, results in a selective depletion of neurotransmitters from a morphologically well defined population of primary sensory neurons (Jancso 1968; Jancso et al. 1977). Since these original observations, capsaicin has become the most important probe for sensory neural mechanisms (see for reviews: Franco-Cereceda 1988; Holzer 1991; Jancso 1992).

Therefore, the present study was devoted to examine whether capsaicin-pretreatment-induced abolition of local sensory neural control leads a decrease in cardiac NO and CGRP content and results in the loss of the preconditioning in the rat heart. The results presented here show that capsaicin-pretreatment significantly reduces cardiac NO and CGRP content and abolishes the cardioprotective effect of pacing-induced preconditioning.

Methods

The 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).

Animals and treatments

Male Wistar rats (300–360 g) housed in a room maintained at 12 h light-dark cycles and a constant temperature of 22 ± 2°C were treated with capsaicin/solvent subcutaneously in the sequence of 10, 30, and 50 mg/kg single daily doses for 3 days. Capsaicin (1% w/v) (Fluka, Buchs, Switzerland) was dissolved in physiological saline containing 3% v/v ethanol and 4% v/v Tween 80. Capsaicin/solvent-pretreated animals, 3 days after the last injections, were used for isolated heart studies (including measurements of cardiac performance, CGRP and lactate dehydrogenase [LDH] release), for NO measurements by means of ESR, and CGRP immunohistochemistry in separate experiments, respectively.

Isolated heart preparation

Hearts were excised after anaesthesia with diethylether, and perfused retrogradely according to Langendorff at 37°C with Krebs-Henseleit bicarbonate buffer containing (in mM) NaCl 118, KCl 4.3, $CaCl_2$ 2.4, $NaHC_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 11.1, gassed with 95% O_2 and 5% CO_2. The left atrium was then cannulated and the preparation was converted to a working preparation as described (Ferdinandy et al. 1993, 1995a). Briefly, the perfusion fluid enters the left atrium, then passes to the left ventricle from which it is spontaneously ejected through the cannulated aorta. Preload (1.7 kPa) and afterload (9.8 kPa) were kept constant throughout the experiments. A suture was placed around the left main coronary artery close to its origin, allowing induction of regional ischaemia (Ferdinandy et al. 1995a). The coronary occlusion results in a 37.4 ± 1.3% ischaemic zone of the left ventricle, which cannot be changed significantly even by strong coronary dilator/constrictor agents, as described previously in this model (Ferdinandy et al. 1995a), since the rat is a coronary collateral deficient species. Pacing at 10 Hz (600 beats per minute) with double threshold square impulses (impulse duration: 5 ms) was performed by an electric stimulator (Experimetria, Budapest, Hungary) through silver electrodes attached directly to the surface of the right ventricle. Heart rate [HR] derived from the left ventricular pressure curve, coronary flow [CF] measured by collecting effluent from the right atrium in a measuring cylinder for a timed period, aortic flow [AF] measured by a calibrated rotameter (KDG Flowmeters, Sussex, England), cardiac output [CO] calculated as the sum of AF and CF, left ventricular developed pressure [LVDP] counted as peak systolic pressure minus left ventricular end-diastolic pressure [LVEDP], positive and negative first derivatives of left ventricular pressure [$\pm$ dP/dt_{max}], and LVEDP were recorded. Ventricular pressure was measured by means of a pressure transducer (B. Braun, Melsungen, Germany) connected to a small polyethylene catheter inserted into the left ventricle through the left atrial cannula (Ferdinandy et al. 1995b). Ventricular pressure was on-line digitized with 200 Hz sampling frequency, recorded, and stored on an IBM computer.

Experimental groups. A pacing-induced preconditioning protocol was applied in hearts obtained from (i) solvent-treated [PS, Preconditioned Solvent], and (ii) capsaicin-treated [PC, Preconditioned Capsaicin] rats. A time-matched non-preconditioning proto-

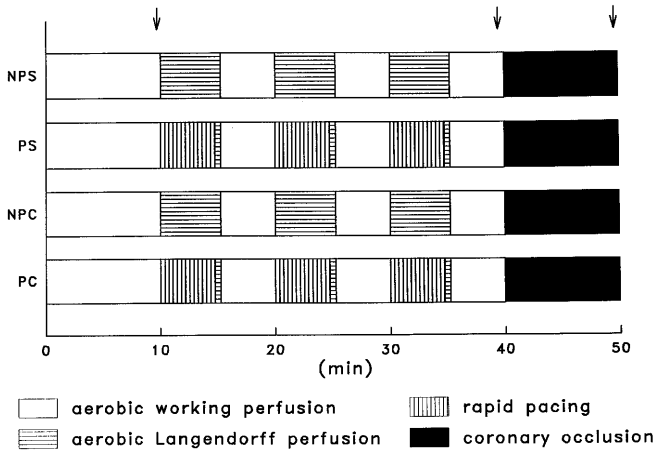

Fig. 1 Perfusion protocol of isolated rat hearts subjected to pacing-induced preconditioning followed by test ischaemia produced by coronary occlusion. Rapid ventricular pacing at 10 Hz (600 beats per minute) was carried out under Langendorff perfusion. *Arrows* indicate time points of measures of cardiac function. NPS and NPC, non-preconditioned hearts isolated from solvent-treated and capsaicin-pretreated rats, respectively; PS and PC, preconditioned hearts isolated from solvent and capsaicin-pretreated rats, respectively

col was also applied in hearts from (iii) solvent-treated [NPS, Non-Preconditioned Solvent], and (iv) capsaicin-treated [NPC, Non-Preconditioned Capsaicin] (n = 7 in each group, Fig. 1) animals.

Preconditioning with rapid ventricular pacing. After a 10-min aerobic working perfusion, hearts were subjected to 3 intermittent periods of pacing/non-pacing protocols for 5 min and 15 s separated by a 4-min and 45-s aerobic working perfusion performed after each period of preconditioning/non-preconditioning protocols as described (Ferdinandy et al. 1995b) (Fig. 1). In the non-preconditioned groups [NPS, NPC] 3 periods of aerobic Langendorff perfusion, 5 min and 15 s each, were applied without pacing. In groups preconditioned with pacing at 10 Hz under Langendorff perfusion [PS, PC], 3 periods of 4-min and 45-s pacing followed by a 30-s aerobic Langendorff perfusion was performed. The last 30 s allowed spontaneous restoration of sinus rhythm before switching to working perfusion. Langendorff perfusion was used during the periods of pacing and therefore during corresponding control periods as well, since in the working heart pacing at 10 Hz dramatically decreased AF, which could have deteriorated the preparation (Ferdinandy et al. 1995b). Cardiac functional parameters were recorded before and after preconditioning and at the 10th min of test ischaemia (Fig. 1). The specified duration and frequency of pacing was selected to induce preconditioning, since a single 4-min and 45-s pacing at 10 Hz increased significantly cardiac oxygen consumption, carbon dioxide production, and lactate efflux, however, LDH release was not increased. Cardiac functional parameters recovered within 3 min after termination of pacing, which indicates that a single pacing induced a completely reversible ischaemia, as described previously (Ferdinandy et al. 1995b). LDH was assayed from coronary effluents using an automatic analyzer (Hitachi-911) with Boehringer-Mannheim (Mannheim, Germany) kits. CGRP release was determined before preconditioning by means of a radioimmunoassay method as described (Varro et al. 1988).

Test ischaemia with coronary occlusion. After the preconditioning/non-preconditioning protocol, test ischaemia of 10-min duration was produced by occlusion of the left main coronary artery. Ten-minutes test ischaemia was chosen for assessing myocardial function, because this short-term regional ischaemia induced considerable deterioration of myocardial function, but did not result in ischaemia-induced arrhythmias that might have disturbed measures of myocardial function (Ferdinandy et al. 1995a). Reperfusion after coronary occlusion was not applied, since reperfusion after 10 min coronary occlusion results in a high incidence of ventricular fibrillation (VF) disabling measures of cardiac function, and pacing-induced preconditioning does not reduce VF due to activation of K_{ATP} (Ferdinandy et al. 1995b). Ischemia-induced LDH release was measured at the 10th min of coronary occlusion.

Design of electron-spin resonance studies

Solvent-treated and capsaicin-treated rats were used for ESR studies, respectively (n = 4 in each group). The spin-trap diethyl-dithiocarbamate (DETC, 200 mg/kg), 50 mg/kg $FeSO_4$ and 200 mg/kg sodium-citrate were slowly administered intravenously into the femoral vein under ether anesthesia. DETC dissolved in distilled water was injected separately from $FeSO_4$ and sodium-citrate in 0.5 ml volume to avoid precipitation of $Fe^{2+}(DETC)_3$. $FeSO_4$ and sodium-citrate were dissolved in distilled water, the pH being set to 7.4 with NaOH, and brought to 1 ml volume before injection. Five minutes after DETC, $FeSO_4$, and citrate treatment, hearts were isolated and perfused in the Langendorff mode for 1 min to eliminate blood, and 100 mg tissue samples of the left ventricles were placed into quartz tubes, and frozen in liquid nitrogen until assayed for ESR spectra of the NO-Fe^{2+}-$(DETC)_2$ complex. Fe^{2+}-$(DETC)_3$ has high affinity for NO while forming NO-Fe^{2+}-$(DETC)_2$. The specific triplet signal of the NO-Fe^{2+}-$(DETC)_2$ complex is superimposed on the dominant background spectra of Cu^{2+}-$(DETC)_2$. The detection limit of NO by this ESR method is 0.05 nM (Mülsch et al. 1992). ESR spectra were recorded with a Bruker ECS106 (Rheinstetten, Germany) spectrometer operating at X band with 100 kHz modulation frequency at a temperature of 160 K, using 10 mW microwave power to avoid saturation. Scans were traced with 2.85 G modulation amplitude, 340 G sweep width, and 3356 G central field as described (Mülsch et al. 1992; Ferdinandy et al. 1996). After subtraction of the background signal of Cu^{2+}-$(DETC)_2$, analysis of NO content was performed with double integration.

Immunohistochemistry

Control (n = 3) and capsaicin-treated animals (n = 3) were deeply anaesthetized with ether and perfused transcardially through the left ventricle with saline (50 ml) followed by Zamboni's fixative (400 ml). The heart was then cut out and stored in the same fixative for 4 h. Tissue samples were stored in 0.1 M phosphate buffer until frozen sections were prepared on a cryostat. An indirect immunofluorescence microscopic technique was used to demonstrate CGRP immunoreactivity. Briefly, sections were incubated with a rabbit polyclonal antibody raised against synthetic rat CGRP (1:500, Paesel + Lorei, Germany) for 12 h at room temperature. This was followed by an incubation with goat anti rabbit IgG labelled with carboxymethylindocyanine (Jackson, purchased from Dianova, Germany, 1:200) for 2 h at room temperature. Preparations were coverslipped with Citifluor AF1 (Citifluor, UK) and viewed under a Leitz DMLB fluorescence microscope. Omission of the first antibody resulted in an abolition of the specific immunofluorescence.

Statistics

Data were expressed as means ± SEM. All groups were analysed with one way analysis of variance (ANOVA). If a difference was established, each group was compared to the solvent-treated non-preconditioned group using a modified t-test corrected for simultaneous multiple comparisons according to the Bonferroni method (Wallenstein et al. 1980).

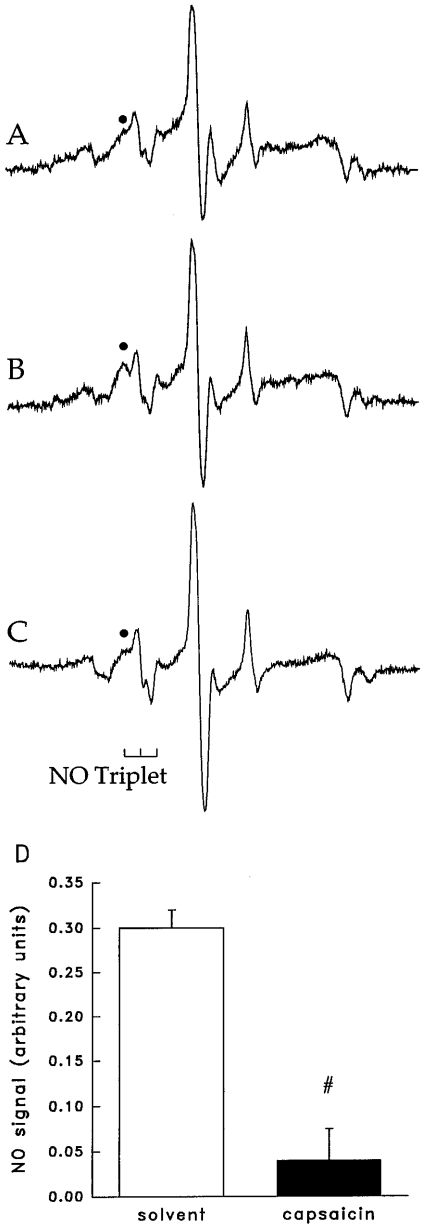

Fig. 2 Decrease of the basal cardiac nitric oxide (NO) content of the heart by capsaicin pretreatment. Curves **A–C**: representative electron spin resonance (ESR) spectra of the background Cu^{2+}-$(DETC)_2$ complex (**A**), and the NO-Fe^{2+}-$(DETC)_2$ complex in left ventricular tissue samples obtained from solvent-treated (**B**) and capsaicin-treated (**C**) rats. ESR parameters: X band, 100 kHz modulation frequency, 160 K temperature, 10 mW microwave power, 2.85 G modulation amplitude, 150 G sweep width, 3356 G central field, ● shows (g = 2.047) specific peak of NO-triplet (+1 0 –1). Panel **D**: NO content of left ventricular tissue samples in arbitrary units obtained from solvent and capsaicin treated groups. Values are means ± SEM ($n = 4$ in each group), # ($P < 0.05$) shows significant decrease

Results

Effects of capsaicin-pretreatment on cardiac NO and CGRP

In the solvent-treated group, basal cardiac NO content was detected by ESR spectroscopy. In the capsaicin-treated group, the specific signal for NO-Fe_2^+-$(DETC)_2$ complex was markedly reduced to near the detection limit (Fig. 2).

In hearts obtained from solvent-treated animals, numerous CGRP-immunoreactive fibres were observed both in the atria and the ventricles. In the ventricles, immunoreactive nerve fibres were more numerous in the subepicardial myocardium as compared to subendocardial and septal regions. In samples obtained from capsaicin-treated rats, immunoreactive fibres were scarce in the atria. In the ventricles, capsaicin-treatment resulted in a complete depletion of CGRP-immunoreactivity (Fig. 3). Similarly, CGRP release detected from coronary effluents of solvent-treated rats (11.27 ± 2.87 fmol/min/$g_{wet\ weight}$) was completely eliminated by capsaicin-pretreatment.

Effects of capsaicin-pretreatment on preconditioning

In the non-preconditioned solvent-treated [NPS group] group, test ischaemia produced by coronary occlusion resulted in a marked decrease in AF, CF, CO, LVDP, ± dP/dt_{max}, and a considerable increase in LVEDP and LDH release (Figs. 4 and 5, Table 1). When test ischaemia was preceded by preconditioning [PS group] elicited by three intermittent periods of rapid ventricular pacing, the AF, CO, LVDP, LVEDP, and LDH release responses were significantly attenuated showing the beneficial effect of preconditioning (Figs. 4 and 5, Table 1). HR and CF (Table 1) were not influenced by preconditioning. Preconditioning and non-preconditioning protocols did not significantly alter cardiac functional parameters before test ischaemia (Table 1, and Figs. 4 and 5).

When hearts were isolated from capsaicin-treated rats, the protective effect of pacing-induced preconditioning was not seen [PC group]. During test ischaemia, AF, CO, LVDP, ± dP/dt_{max}, LVEDP, and LDH release (Figs. 4 and 5, Table 1) were not improved by preconditioning [PC group] when compared to the non-preconditioned solvent-treated [NPS] group. LVEDP before coronary occlusion, was significantly increased, while other functional parameters were not affected in the capsaicin-treated groups (Figs. 4 and 5, Table 1).

Discussion

The present results show that pretreatment with capsaicin depletes the cardiac CGRP content, markedly reduces the cardiac NO content, and inhibits the cardioprotective effect of pacing-induced preconditioning in isolated working rat hearts with coronary occlusion. These findings indicate that an intact sensory innervation is required to

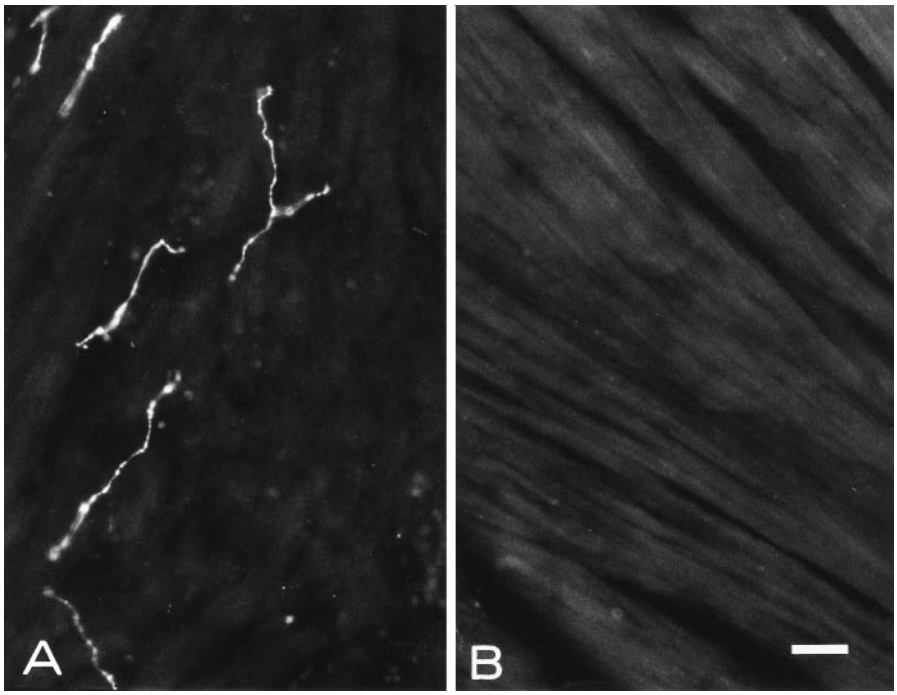

Fig. 3 Calcitonin gene-related peptide (CGRP) immunoreactivity in the myocardium of the left ventricle of solvent (**A**) and capsaicin-pretreated (**B**) rats. Note the complete absence of immunoreactive fibres in the preparation obtained from the capsaicin-pretreated rat. *Scale bar* represents 10 μm. × 400

elicit pacing-induced preconditioning in the rat, and may suggest that the loss of preconditioning after capsaicin-pretreatment is due to the lack of neural NO and CGRP. The study also shows that a significant amount of the basal NO content of the heart may be derived from the capsaicin-sensitive sensory innervation.

Possible role of sensory innervation in the mechanism of endogenous cardiac stress adaptation

Available experimental evidence shows that most cardiac sensory neurons associated with vagal and sympathetic afferents display polymodal behavior responding to mechanical and chemical stimuli (Franco-Cereceda 1988; Hunag et al. 1996), especially the capsaicin-sensitive subpopulation of the thin afferent fibres (see for review: Franco-Cereceda 1988; Bevan and Geppetti 1994). These afferent C-fibres show local efferent functions which are attributed to their CGRP, NO, and SP content (Holzer 1991; Jancso 1992). NO (Vegh et al. 1992; Ferdinandy et al. 1996) and CGRP (Li et al. 1996) were found as important mediators of preconditioning. Therefore, capsaicin-sensitive nerve endings are obvious candidates for the involvement in the mechanism of cardiac stress adaptation as sensors for metabolic (e.g. ischaemia) and mechanical stressors. In our present study, treatment with the sensory neurotoxin capsaicin was utilized to achieve a selective depletion of afferent fibres as described (Holzer 1991; Franco-Cereceda 1988). In hearts isolated from these rats, the protective effect of preceding rapid ventricular pacing against the deterioration of myocardial function and LDH release due to coronary occlusion was not observed, showing that an intact sensory innervation is a prerequisite to elicit pacing-induced preconditioning in the rat heart. Capsaicin-treatment decreased the NO signal in left ventricular tissue samples to approximately the detection limit of ESR, which suggests that a significant portion of total cardiac basal NO content derives from capsaicin-sensitive fibres. This is supported by recent observations showing that capsaicin-sensitive sensory ganglion cells express nitric oxide synthase (Ren and Ruda 1995). A very recent study of Pabla and Curtis (1996) suggesting a neural origin of cardiac NO release also strongly supports this assumption. Cardiac CGRP release and the rich CGRP innervation found in the ventricles were completely eliminated by capsaicin treatment in our present study. It shows the excellent effectiveness of the pretreatment with capsaicin to deplete sensory fibres, since it is well known that CGRP is located only in afferent axons in the heart (Franco-Cereceda 1988; Holzer 1991).

NO and preconditioning

The importance of NO in the conventional no-flow ischaemia-induced preconditioning is controversial (Vegh et al. 1992; Weselcouch et al. 1995; Lu et al. 1995), however, our previous studies support a role of the NO-cGMP system in pacing-induced preconditioning in rabbits and rats (Ferdinandy et al. 1996; Szilvassy et al. 1994a, b). The discrepancy may be explained by the different biochemical mechanisms in no-flow ischaemia-induced preconditioning and that induced by rapid pacing (Ferdinandy et al. 1995b). Rapid pacing may represent a stronger stimulus than no-flow ischaemia to the release of cardiac NO (Cooke et al. 1991; Kitakaze et al. 1995) and/or cGMP (Ohno et al. 1993) either through increased shear-stress in the coro-

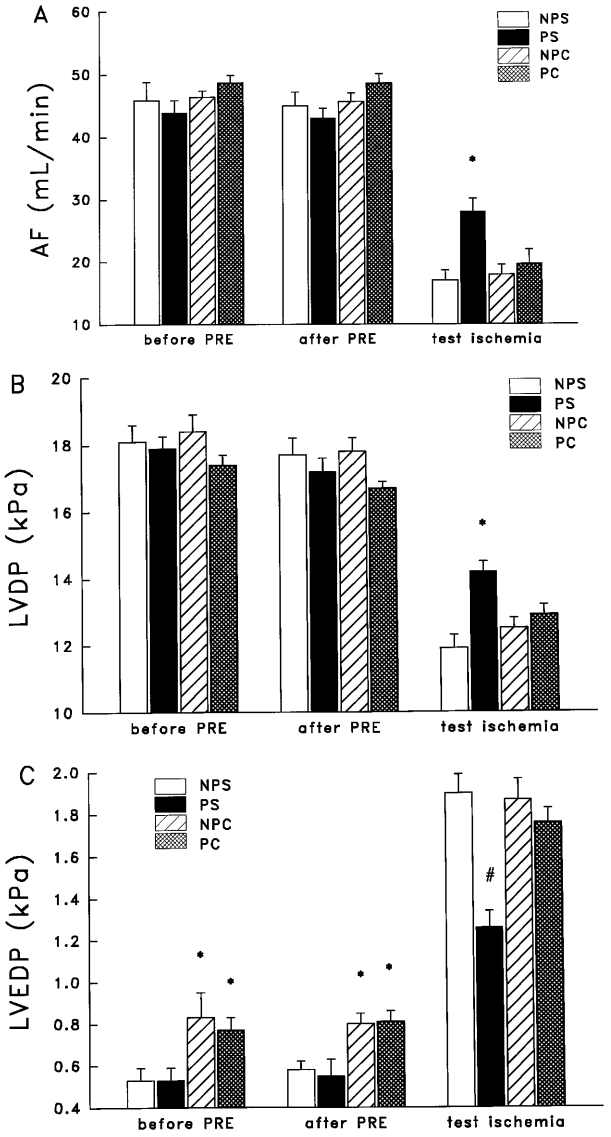

Fig. 4 A–C Abolition by capsaicin-pretreatment of the pacing-induced preconditioning (PRE) phenomenon in isolated working rat hearts: functional parameters. Aortic flow (AF, panel **A**), left ventricular developed pressure (LVDP, panel **B**), and left ventricular end-diastolic pressure (LVEDP, panel **C**) were measured before and after PRE and the corresponding control periods, and at the 10th min of test ischaemia produced by coronary occlusion. NPS and NPC, non-preconditioned hearts isolated from solvent and capsaicin-pretreated rats, respectively; PS and PC, preconditioned hearts isolated from solvent and capsaicin-pretreated rats, respectively. Values are means ± SEM (n = 7 in each group). * ($P < 0.05$) shows significant increase, # ($P < 0.05$) shows significant decrease compared to NPS group

Table 1 Cardiac functional parameters obtained during preconditioning/non-preconditioning protocols and after test ischemia

	HR (bpm)	CF (mL/min)	CO (mL/min)	+dP/dt$_{max}$ (kPa/s)	–dP/dt$_{max}$ (kPa/s)
Before preconditioning/corresponding control period					
NPS	276 ± 5	23.4 ± 1.7	69.3 ± 3.9	884 ± 57	462 ± 23
PS	270 ± 7	22.9 ± 0.5	66.7 ± 2.3	840 ± 50	459 ± 39
NPC	271 ± 9	22.7 ± 0.6	69.1 ± 1.4	907 ± 49	481 ± 35
PC	271 ± 8	22.7 ± 1.2	71.4 ± 2.0	854 ± 92	481 ± 38
After preconditioning/corresponding control period					
NPS	269 ± 6	23.1 ± 1.8	68.1 ± 2.8	820 ± 51	446 ± 29
PS	264 ± 4	22.1 ± 0.9	65.6 ± 1.4	814 ± 23	462 ± 37
NPC	273 ± 8	23.1 ± 0.9	68.9 ± 1.4	886 ± 23	497 ± 32
PC	261 ± 10	21.4 ± 1.0	70.0 ± 2.1	781 ± 62	497 ± 26
10th min of test coronary occlusion					
NPS	265 ± 5	15.1 ± 0.7	32.1 ± 2.1	467 ± 36	304 ± 23
PS	262 ± 7	14.9 ± 0.9	42.9 ± 2.2*	575 ± 25	381 ± 22*
NPC	261 ± 8	13.4 ± 0.6	31.3 ± 2.2	521 ± 38	313 ± 14
PC	271 ± 7	16.0 ± 0.9	35.6 ± 2.5	446 ± 28	352 ± 20

HR, heart rate; bpm, beats per minute; CF, coronary flow; CO, cardiac output; NPS and NPC, non-preconditioned hearts isolated from solvent-treated and capsaicin-treated rats; PS and PC, preconditioned hearts isolated from solvent-treated and capsaicin-treated rats; respectively. Values are means ± SEM (n = 7 in each group), * ($P < 0.05$) shows significant difference as compared to NPS group

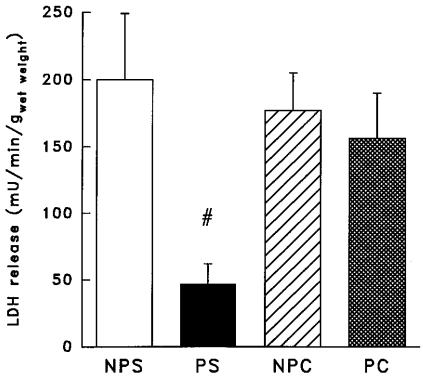

Fig. 5 Abolition by capsaicin-pretreatment of the pacing-induced preconditioning (PRE) phenomenon in isolated working rat hearts: cardiac lactate dehydrogenase (LDH) release. Ischemia-induced LDH release determined at the 10th min of test ischemia produced by coronary occlusion. NPS and NPC, non-preconditioned hearts isolated from solvent and capsaicin-pretreated rats, respectively; PS and PC, preconditioned hearts isolated from solvent-treated and capsaicin-treated rats, respectively. Values are means ± SEM (n = 7 in each group). # ($P < 0.05$) shows significant decrease compared to NPS group

nary vasculature, or through increased mechanical activation of mechanosensitive nerve fibres and mechanosensitive gating of K_{ATP}-channels in cardiac myocytes (Ten Eick et al. 1992; Van Wagoner 1993; Koning et al. 1996). In our earlier study (Ferdinandy et al. 1996), pretreatment with a blocker of NO synthesis (1 mg/kg N^G-nitro-L-arginine, LNNA) considerably decreased cardiac NO content, increased nonischaemic LVEDP, and abolished the effect of pacing-induced preconditioning on ischaemic myocardial function and LDH release in rat hearts. In our present study, capsaicin-pretreatment similarly decreased cardiac NO content, slightly increased nonischaemic LVEDP, and affected similarly ischaemic myocardial function and LDH release. Conclusively, our present finding not only suggests the sensory neural localization of a significant portion of cardiac NO, but also proposes that the loss of

the preconditioning effect in capsaicin-pretreated rats may be – at least in part – attributed to a decreased level of neural NO.

Mechanism of pacing-induced preconditioning: a hypothesis

In contrast to the effect of the traditional no-flow ischaemia-induced preconditioning, the effect of pacing-induced preconditioning was found to be sensitive to the K_{ATP} blocker glibenclamide (Ferdinandy et al. 1995b; Koning et al. 1996). Our previous studies demonstrated a role of the NO-cGMP system in pacing-induced preconditioning (Ferdinandy et al. 1996; Szilvassy et al. 1994a, b). Adenosine, the most extensively studied preconditioning mediator (see for review: Downey et al. 1993) may also act via NO, since it enhances NO production (Li et al. 1995). NO is known to activate K_{ATP} and other potassium channels (Murphy and Brayden 1995; Yao et al. 1995; Cooke et al. 1991). Our present results show the importance of sensory neurons containing NO and CGRP in the mechanism of preconditioning. CGRP was also shown to activate K_{ATP} (Kitazono et al. 1993). Conclusively, the following biochemical mechanism of pacing-induced preconditioning can be suggested as a working hypothesis for further experiments. Rapid pacing-induced slight ischaemia and mechanical stress result in a release of several ischaemic metabolites from the myocytes and/or coronary endothelium, which in turn activate the capsaicin-sensitive sensory nerve endings. The concomitant neural release of NO and/or CGRP leads to an activation of K_{ATP} and an increase in cGMP concentration, which may significantly contribute to the cardioprotective effect of preconditioning (Szilvassy et al. 1993, 1994a; Pabla et al. 1995; Gross and Auchampach 1992).

We conclude that the presence of an intact local sensory innervation is a prerequisite to elicit pacing-induced preconditioning; that a significant portion of basal cardiac NO content may be derived form capsaicin-sensitive neural sources; and that NO and CGRP released from these nerve fibres may be involved in the mechanism of pacing-induced preconditioning in the rat heart.

Acknowledgements This work was supported by grants from National Scientific Research Found (OTKA F19946, D23736, T20653), National Technical Development Committee (OMFB 05201950616), and the Hungarian Space Research Office, Budapest, Hungary.

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