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Capsaicin-sensitive sensory neurons regulate myocardial nitric oxide and cGMP signaling

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Abstract

We studied whether tissue levels of nitric oxide (NO) and cGMP are regulated by sensory nerves in normoxic and ischemic hearts. Wistar rats were treated with capsaicin to deplete neurotransmitters from capsaicin-sensitive sensory nerves. In separate experiments, capsaicin was applied perineurally to both vagus nerves for selective chemodenervation of vagal cardiac afferent nerves. Systemic capsaicin administration significantly decreased basal myocardial NO content assessed by electron spin resonance (ESR) spectroscopy, whereas, local treatment of vagus nerves did not change it. Both systemic and local capsaicin treatment decreased cardiac cGMP content measured by radioimmunoassay. In separate experiments, isolated hearts from control and systemic capsaicin-treated rats were subjected to 30-min global ischemia. NO signal intensity increased 10-fold after ischemia, whereas, cardiac cGMP decreased. Capsaicin pretreatment did not influence ischemic NO or cGMP content. These results suggest a major role for capsaicin-sensitive sensory neurons in the maintenance of basal but not ischemic myocardial NO and cGMP content. Vagal sensory nerves may be involved in the regulation of basal myocardial cGMP but not basal NO level. Consequently, basal NO content in the heart is regulated primarily by spinal afferent nerves.

Keywords: Capsaicin; Vagal sensory nerve; Nitric oxide (NO); cGMP; Ischemia; Heart, rat

1. Introduction

In contrast to the adrenergic and cholinergic innervation of the heart, less attention has been paid to the functional significance of the rich sensory innervation of the myocardium and the coronary vascular system. Sensory nerves may have strong influence on cardiac function and adaptive responses due to their nitric oxide (NO) and vasoactive peptide content, such as calcitonin gene-related peptide (CGRP) and substance P (Ren and Ruda, 1995; Franco-Cereceda, 1988; Sosunov et al., 1995, 1996). The thin sensory nerve endings may act as potential sensor machinery for ischemia, since ischemia, hypoxia, lactate, K^+, and low pH were shown to stimulate cardiac sensory nerves in association with a release of their transmitters (see Franco-Cereceda, 1988 for review). Capsaicin is a highly selective sensory neurotoxin which leads to a selective functional blockade and/or ablation of a morphologically well defined population of primary sensory neurons (Jancso, 1968; Jancso et al., 1977). Hence, capsaicin has become one of the most important probes for investigations of sensory neural pathology and pharmacology (see Franco-Cereceda, 1988; Holzer, 1991; Jancso, 1992 for reviews).

Recently, the involvement of cardiac sensory nerves has been revealed in cardiac adaptation to ischemic stress, i.e. ischemic preconditioning (Ferdinandy et al., 1997a; Li et al., 1996). We have shown that systemic capsaicin treatment leading to depletion of sensory nerves resulted in a decrease in basal cardiac NO content. This suggests that cardiac sensory nerves play a role in basal cardiac NO synthesis in rats (Ferdinandy et al., 1997a). However, the origin of these cardiac sensory nerves, i.e. spinal or vagal, is still not known.

It has been shown that myocardial ischemia leads to a marked accumulation of myocardial NO in rat hearts (Csonka et al., 1999; Zweier et al., 1995). We have also shown that the

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cardioprotective effect of ischemic preconditioning was proportional to the elevation of myocardial cGMP content in rabbits (Szilvássy et al., 1994a), and that a reduction of cardiac NO and cGMP content due to either experimental hyperlipidemia (Ferdinandy et al., 1997b) or vascular nitroglycerin tolerance (Szilvássy et al., 1994b, 1997) led to the loss of the preconditioning effect in rats and rabbits (see Ferdinandy et al., 1998b for review). These data suggest that both NO and cGMP are involved in myocardial ischemia/reperfusion-induced pathophysiological changes and in the development of ischemic stress adaptation of the heart. However, it is not known whether cardiac sensory nerves play a role in the regulation of NO and cGMP in the ischemic myocardium.

Therefore, here we studied if capsaicin-sensitive sensory nerves of spinal and vagal origin contribute to NO and cGMP signaling in normoxic and ischemic rat hearts.

2. 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) and was approved by the Animal Research Ethics Committee of University of Szeged.

2.1. Experimental design

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 used throughout the experiments. Systemic capsaicin treatment was applied for chemodenervation of both spinal and vagal sensory nerves. Local application of capsaicin on both vagal nerves was used to deplete vagal sensory nerves. Hearts were then isolated from capsaicin-pretreated rats and subjected to normoxic perfusion followed by 30-min global normothermic ischemia. Cardiac NO and cGMP were measured from ventricular tissue.

2.2. Systemic and local capsaicin treatment

For selective chemodenervation of both spinal and vagal sensory nerves, rats were treated with capsaicin subcutaneously in the sequence of 10, 30, and 50 mg/kg single daily doses for 3 days as described (Ferdinandy et al., 1997a). Capsaicin (1% w/v, Fluka, Buchs, Switzerland) was dissolved in physiological saline containing 6% v/v ethanol and 8% v/v Tween 80. Animals treated with equivalent amounts of the solvent served as controls. Capsaicin- and solvent-treated animals were used for isolated heart studies 7 days after the last injection, when depletion of peptide-containing myocardial sensory nerves is already complete (Ferdinandy et al., 1997a).

For selective chemodenervation of vagal sensory nerves, local treatment of the vagus nerves with capsaicin was applied as described (Jancso et al., 1980; Jancso and Such, 1983). Briefly, rats were anaesthetized with chloral hydrate and the cervical vagus nerves were exposed through a midline incision on the neck. Approximately 1-cm-long segments of both vagus nerves were isolated with Parafilm® and small pieces of gelfoam moistened with a capsaicin solution (1%, 100 μl) were wrapped around the nerves for 30 min. The capsaicin-exposed area was then flushed with saline and the wound was closed. In some control animals, the vagus nerves were similarly treated with the solvent for capsaicin. Animals were used for isolated heart studies after a 2-week reconvalescence period.

2.3. Isolated heart perfusion

Hearts were excised after anesthesia with diethylether and prepared for working heart perfused at 37 °C with Krebs-Henseleit bicarbonate buffer containing (in mM) NaCl 118, KCl 4.3, $CaCl_2$ 2.4, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 11.1, gassed with 95% O_2 and 5% CO_2 as described (Csonka et al., 1999; Csont et al., 1999). After a 10-min normoxic, normothermic perfusion, hearts were subjected to 30-min global, normothermic ischemia by clamping the perfusion lines. Cardiac mechanical functional parameters, such as heart rate, coronary flow, aortic flow, cardiac output, left ventricular developed pressure and its first derivatives ($\pm dP/dt_{max}$), and left ventricular end-diastolic pressure (LVEDP) were monitored during normoxic perfusion as described (Csonka et al., 1999; Csont et al., 1999). Preload (1.7 kPa) and afterload (9.8 kPa) were kept constant throughout the experiments. To estimate severity of ischemia-induced deterioration of myocardial function after capsaicin pretreatment, 15-min reperfusion was applied to let us measure cardiac contractile function as described (Csonka et al., 1999).

2.4. Measurement of cardiac NO by electron spin resonance (ESR)

To study the role of spinal and vagal sensory neurons in cardiac NO signaling, cardiac NO was measured by ESR after in vivo spin trapping from rats subjected to systemic and vagal capsaicin treatment. 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+}–$(DETC)_2$. $FeSO_4$ and sodium-citrate were dissolved in distilled water, pH was 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 Langendorff mode for 1 min to eliminate blood, and 150 mg tissue samples of the left ventricles were placed into quartz ESR tubes, and frozen in liquid nitrogen until assayed for ESR spectra of NO–

Fe^{2+}–$(DETC)_2$ complex as described (Ferdinandy et al., 1997a,b).

To study the role of sensory neurons in ischemic NO signaling, cardiac NO was measured by ESR after ex vivo spin trapping before and after ischemia in hearts isolated from control and capsaicin-treated (systemic treatments) rats. The spin trap for NO, the complex of *N*-methyl-glucamine-dithio-carbamate (MGD, synthesized as described, Shinobu et al., 1984) with ferrous ion [$Fe^{2+}(MGD)_2$], was prepared freshly before each experiment. MGD (175 mg) and 50 mg $FeSO_4$ dissolved in distilled water (pH 7.4, volume 6 ml) was infused into the aortic cannula under Langendorff perfusion for 5 min at a rate of 1 ml/min before ischemia in order to measure basal myocardial NO content. Tissue samples from the apex of the heart (approximately 150 mg) were collected at the end of the infusion of $Fe^{2+}(MGD)_2$ and placed into quartz ESR tubes and frozen in liquid nitrogen. To measure the accumulation of NO during ischemia, in separate studies, $Fe^{2+}(MGD)_2$ infusion was started 5 min before the induction of ischemia to load the heart with the spin trap before clamping the perfusion line, and tissue samples were collected at the end of ischemia as described (Csonka et al., 1999; Csont et al., 1999).

The detection limit of NO by in vivo spin trapping with the lipophilic Fe^{2+}–$(DETC)_2$ is approximately 0.05 nM in a sample (Mülsch et al., 1992), whereas ex vivo spin trapping with the water soluble Fe^{2+}–$(MGD)_2$ is somewhat less sensitive. However, in buffer perfused isolated heart studies, only the water soluble spin trap can be applied. ESR spectra of NO–Fe^{2+}–$(DETC)_2$ and NO–Fe^{2+}–$(MGD)_2$ adducts ($g\| = 2.018$ and $g\perp = 2.039$ in the frozen state, with a triplet hyperfine structure at $g\perp$) were recorded with a Bruker ECS106 spectrometer (Rheinstetten, Germany; ESR parameters: X band, 100-kHz modulation frequency, 160-K temperature, 10-mW microwave power, 2.85-G modulation amplitude, 3356-G central field). After subtracting the background signal of Cu^{2+}-dithiocarbamate, spectra were analyzed for NO signal intensity with double integration as described (Csont et al., 1998; Ferdinandy et al., 2000). Cardiac NO content was expressed as arbitrary units/g tissue. Cu-dithiocarbamate signal intensity was not changed significantly in the different groups.

2.5. Measurement of cardiac cGMP by radioimmunoassay

To examine the role of sensory neurons in cardiac cGMP signaling, in separate studies, cardiac cGMP concentration was measured in solvent and capsaicin-treated animals. Before ischemia and at the end of ischemia, left ventricular tissue mass was frozen by means of a Wollenberger clamp prechilled in liquid nitrogen. Samples were then homogenized and centrifuged and the supernatants were extracted six times in water-saturated diethylether, evaporated, and assayed for cGMP by radioimmunoassay using Amersham kits as described (Csont et al., 1999; Szilvássy et al., 1994a).

2.6. Statistics

Data were expressed as means ± standard error of the mean (S.E.M.) and analyzed with one way analysis of variance (ANOVA). If a difference was established, each group was compared to the solvent-treated control group using the Bonferroni post-hoc test for simultaneous multiple comparisons (Wallenstein et al., 1980).

3. Results

3.1. Effects of systemic and local capsaicin treatments on cardiac NO

First we measured basal cardiac NO content in the solvent-treated control group, then we assessed cardiac NO after systemic capsaicin treatment to study if sensory neurons contribute to the maintenance of basal myocardial NO content. The specific ESR signal for NO after in vivo spin trapping was markedly reduced near to the detection limit after systemic capsaicin treatment (Fig. 1A,B). To assess the relative significance of spinal and vagal sensory neurons in this effect, we also measured cardiac NO after selective sensory chemodenervation of the vagus nerves by perivagal application of capsaicin. Vagal capsaicin treatment did not significantly change basal NO content when compared to controls (Fig. 1A,B).

We also measured accumulation of NO after 30 min of global no-flow ischemia (Fig. 2A) in separate experiments using ex vivo spin trapping in isolated rat hearts. In control hearts, cardiac NO signal intensity was markedly increased when compared to preischemic basal NO content (Fig. 2B). To study the role of sensory neurons in ischemic NO signaling, ischemic NO was measured after systemic capsaicin pretreatment. Systemic capsaicin pretreatment decreased preischemic NO below the detection limit by ex vivo spin trapping, however, it did not significantly affect ischemic accumulation of NO in the heart (Fig. 2B). Therefore, ischemic changes in NO content were not studied further after selective capsaicin treatment of the vagus nerves.

3.2. Effects of systemic and local capsaicin treatments on cardiac cGMP

In separate experiments, we measured basal cardiac cGMP content in the solvent-treated control group, then we assessed cardiac cGMP after systemic capsaicin treatment to study if sensory neurons regulate basal myocardial cGMP content (Fig. 1C). Cyclic GMP content was moderately reduced after systemic capsaicin treatment. To assess the relative significance of spinal and vagal sensory neurons, we also measured cardiac cGMP after selective chemodenervation of the vagus nerves by capsaicin. Vagal capsaicin treatment, similarly to systemic treatment, de-

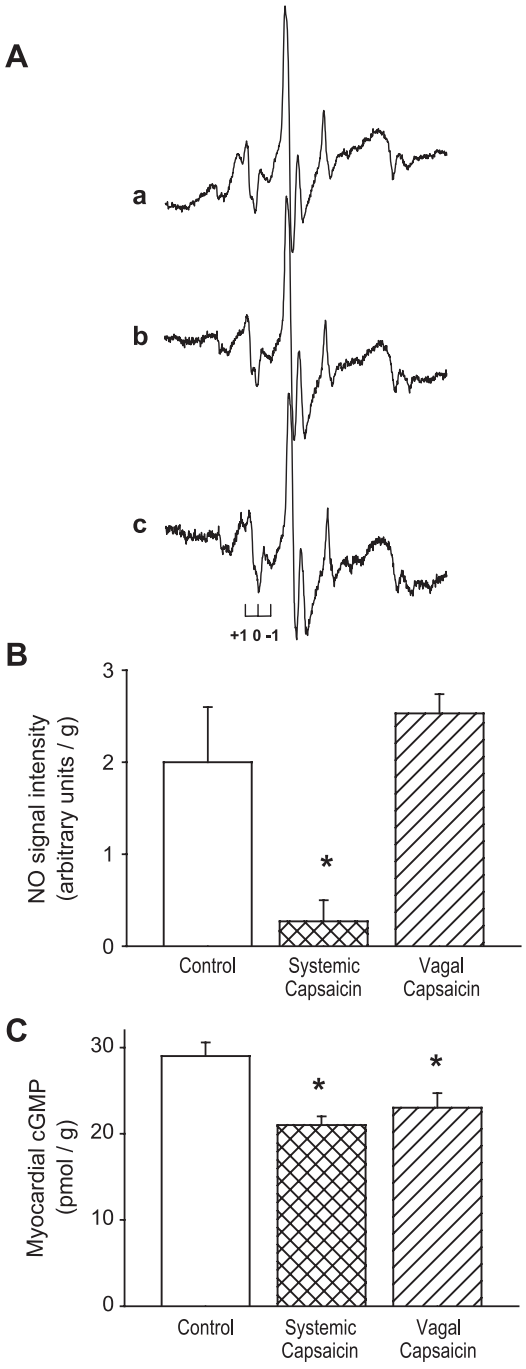

Fig. 1. Effect of systemic and vagal capsaicin pretreatment on basal cardiac nitric oxide measured by electron spin resonance after in vivo spin trapping (NO, panels A and B) and cyclic guanosine monophosphate measured by radioimmunoassay (cGMP, panel C) in rat hearts. Curves a–c: representative electron spin resonance spectra of the NO–Fe^{2+}–$(DETC)_2$ complex in left ventricular tissue samples obtained from solvent-treated control (a), and systemic (b) or vagal capsaicin-pretreated (c) rats. +1 0 −1 indicates the specific peaks of NO-triplet ($g = 2.039$, hyperfine splitting: 1.30 mT). Electron spin resonance spectroscopy parameters: X band, 100-kHz modulation frequency, 160 K, 10-mW microwave power, 2.85-G modulation amplitude, 340-G sweep width, and 3350-G central field. Values on panels B and C are means ± S.E.M. ($n = 5$ in each group). *($P < 0.05$) shows a significant decrease as compared to Control.

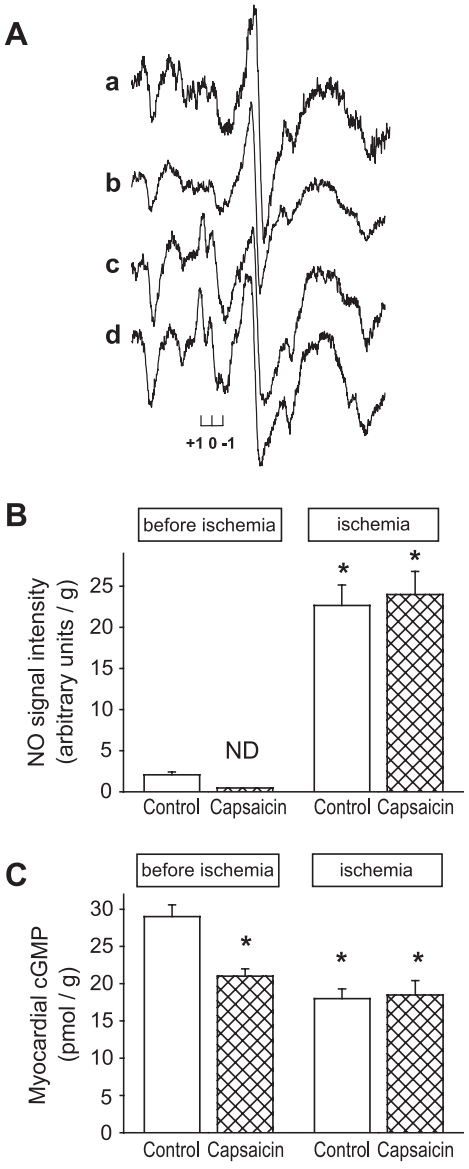

Fig. 2. Effect of systemic capsaicin pretreatment on cardiac nitric oxide measured by electron spin resonance after ex vivo spin trapping (NO, panels A and B) and cyclic guanosine monophosphate measured by radioimmunoassay (cGMP, panel C) in isolated rat hearts before and after 30 min of global no-flow ischemia. Curves a–d: representative electron spin resonance spectra of the NO–Fe^{2+}–$(MGD)_2$ complex in left ventricular tissue samples obtained from solvent-treated control (a), and systemic (b) or vagal capsaicin-pretreated (c) rats. +1 0 −1 indicates the specific peaks of NO-triplet ($g = 2.039$, hyperfine splitting: 1.30 mT). Electron spin resonance spectroscopy parameters: X band, 100-kHz modulation frequency, 160 K, 10-mW microwave power, 2.85-G modulation amplitude, 340-G sweep width, and 3350-G central field. Values on panels B and C are means ± S.E.M. ($n = 5$ in each group). *($P < 0.05$) shows significant difference as compared to Control before ischemia. ND, non-detectable.

creased basal cGMP content when compared to controls (Fig. 1C).

In separate experiments, we also studied the effect of 30-min global no-flow ischemia on cardiac cGMP content (Fig. 2C). In control hearts, in contrast to NO, cardiac cGMP was

Table 1
Effect of systemic and vagal capsaicin pretreatment on cardiac functional parameters before and after ischemia in isolated rat hearts

	HR[a] (bpm)	CF[b] (ml/min)	AF[c] (ml/min)	LVDP[d] (kPa)	$+dP/dt_{max}$ (kPa/s)	$-dP/dt_{max}$ (kPa/s)	LVEDP[e] (kPa)
Before ischemia							
Control ($n=8$)	278 ± 5	23.4 ± 1.7	57.4 ± 3.9	18.7 ± 1.2	873 ± 54	443 ± 26	0.44 ± 0.05
Systemic capsaicin ($n=7$)	272 ± 7	22.9 ± 0.5	60.7 ± 2.3	18.1 ± 1.4	837 ± 39	460 ± 37	0.87 ± 0.04*
Vagal capsaicin ($n=5$)	284 ± 9	24.2 ± 1.3	62.1 ± 3.4	19.1 ± 2.1	917 ± 63	479 ± 40	0.52 ± 0.06
Following 30-min global no-flow ischemia							
Control ($n=7$)	270 ± 5	20.2 ± 2.3	17.3 ± 2.1	13.1 ± 0.9	546 ± 34	306 ± 14	1.54 ± 0.08
Systemic capsaicin ($n=7$)	269 ± 7	21.4 ± 2.2	19.8 ± 2.7	12.6 ± 0.8	564 ± 29	328 ± 19	1.59 ± 0.10

Values are means ± S.E.M.
[a] Heart rate.
[b] Coronary flow.
[c] Aortic flow.
[d] Left ventricular developed pressure.
[e] Left ventricular end-diastolic pressure.
* ($P<0.05$) shows significant difference as compared to corresponding controls.

significantly decreased when compared to preischemic basal cGMP content. To study the role of sensory neurons on ischemia-induced changes in myocardial cGMP, ischemic cGMP was measured after systemic capsaicin treatment. Systemic capsaicin treatment did not significantly affect ischemia-induced decrease in cardiac cGMP. Therefore, ischemic cGMP was not studied further after perivagal capsaicin pretreatment.

3.3. Effects of systemic and local capsaicin treatments on myocardial function

We measured parameters of myocardial hemodynamics to assess the effect of capsaicin pretreatments on basal cardiac function. LVEDP was significantly increased when hearts were isolated after systemic capsaicin treatment, while other functional parameters were not affected as compared to solvent-treated controls (Table 1). After local treatment of vagal nerves with capsaicin, basal myocardial functional parameters were not changed significantly (Table 1).

In the control group, ischemia induced a marked deterioration of myocardial function when measured upon reperfusion. Systemic capsaicin treatment did not significantly change postischemic cardiac function (Table 1). Therefore, postischemic cardiac performance was not tested further after perivagal capsaicin treatment.

4. Discussion

The present results show that systemic capsaicin treatment decreased basal cardiac NO and cGMP, whereas selective chemodenervation solely of vagal capsaicin-sensitive afferent nerves decreased basal cardiac cGMP but not NO. We have also demonstrated that myocardial ischemia markedly increased cardiac NO and moderately decreased cGMP. However, ischemia-induced changes in cardiac NO and cGMP were not influenced by systemic capsaicin treatment. These results suggest that capsaicin-sensitive cardiac sensory nerves play a major role in maintaining basal cardiac NO and cGMP content, but these nerves do not influence ischemia-induced changes in NO and cGMP level. Our findings further indicate that spinal afferent nerves rather than vagal afferents are involved in the regulation of basal cardiac NO.

The present study confirms our previous findings that systemic capsaicin treatment leading to selective destruction of C-type thin sensory neurons decreases myocardial NO content, which indicates a fundamental role of the capsaicin-sensitive sensory innervation in the maintenance of basal cardiac NO (Ferdinandy et al., 1997a). Nevertheless, available experimental evidence shows that most cardiac sensory neurons associated with either vagal parasympathetic or spinal (thoracic) sympathetic afferentations display polymodal behavior responding to mechanical and chemical stimuli (Franco-Cereceda, 1988; Hunag et al., 1996). In our present study, systemic capsaicin treatment decreased cardiac NO content, but vagal capsaicin treatment did not affect cardiac NO. This is the first demonstration that capsaicin-sensitive nerves of spinal but not vagal origin are involved in the regulation of basal cardiac NO content. This may suggest that a significant portion of total cardiac basal NO content may derive from spinal capsaicin-sensitive afferent nerves. A previous study by Pabla and Curtis (1996) also suggested that basal cardiac NO release is of neural origin in rat hearts. It should be noted, however, that our present results does not show directly whether the sources of basal NO are the capsaicin-sensitive sensory nerves or other adjacent cells including cardiomyocytes, smooth muscle cells, and endothelial cells. In addition to NO, capsaicin-sensitive nerve endings contain different neurotransmitters such as CGRP and substance P (Ren and Ruda, 1995; Jancso et al., 1977). These nerve endings may control basal NO directly by releasing NO, or indirectly via release of their other neurotransmitters which in turn activates the synthesis of NO in adjacent cells.

NO is believed to exert its biological actions via activation of guanylate cyclase. However, cGMP-independent NO actions, as well as NO-independent cGMP level modifications have been described in many cell types (see, for reviews, Mayer and Hemmens, 1997; Lucas et al., 2000). We have previously described that myocardial cGMP does not necessarily reflect changes in NO in rat hearts (Csont et al., 1998). This could explain that local administration of capsaicin on vagal nerves did not influence cardiac NO but decreased cGMP level in the present study. These findings suggest that vagal nerves may regulate basal cardiac cGMP independently from NO.

Systemic capsaicin treatment resulted in an elevation of LVEDP, however, other parameters of cardiac mechanical function were not affected. LVEDP elevation due to systemic capsaicin treatment is most likely the consequence of decreased myocardial NO content, as different experimental conditions associated with decreased cardiac NO level lead to an elevation of LVEDP (Prendergast et al., 1997; Ferdinandy et al., 1997b). Although there is no dramatic change in cardiac function after systemic capsaicin treatment, our previous results show that the ability of these hearts to adapt to ischemic challenges was markedly decreased (Ferdinandy et al., 1997a). Vagal capsaicin treatment did not influence LVEDP or other functional parameters. This is a further indirect evidence showing that vagal sensory nerves do not influence basal cardiac NO metabolism.

We have shown here that 30-min global ischemia markedly increased cardiac NO content and slightly decreased cGMP level. As to the ischemia-induced NO accumulation, the present results confirm previous studies from our (Csonka et al., 1999; Ferdinandy et al., 1998a) and other research groups (see, for review, Shah and MacCarthy, 2000). The effect of ischemia on cardiac cGMP level is somewhat controversial in the literature, and it seems to depend on the duration of ischemia and the method used to provoke ischemia (Depre and Hue, 1994; Cheung et al., 2000; Du Toit et al., 1998; Dobson, 1981; Krause and Wollenberger, 1981). Severe ischemia may decrease cardiac cGMP possibly due to a depletion of high energy phosphates including GTP, the substrate for cGMP. Ischemia-induced alterations in the activity of either guanilate cyclase or phosphodiesterase enzymes may be also responsible for the decreased cGMP level in the ischemic heart. In our present study, systemic treatment with capsaicin did not influence ischemic changes in cardiac NO and cGMP and it did not affect postischemic myocardial function. Therefore, the present results suggest that although capsaicin-sensitive sensory neurons play a major role in the regulation of basal cardiac NO and cGMP signaling, they do not regulate ischemia-induced changes in cardiac NO and cGMP.

In summary, this is the first demonstration that (i) capsaicin-sensitive sensory neurons significantly contribute to the regulation of basal tissue levels of NO and cGMP under normoxic but not ischemic conditions; (ii) spinal afferents play a major role in the maintenance of basal cardiac NO; and that (iii) both vagal and spinal afferents regulate basal cGMP in the heart. These observations encourage further studies on pharmacological manipulation of cardiac sensory neurons to modulate basal cardiac NO-cGMP signaling and to study their functional significance in cardiac function under physiological and pathological conditions.

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