ELSEVIER

European Journal of Pharmacology 376 (1999) 247–250

ejp

www.elsevier.nl/locate/ejphar

Short communication

Endothelin release by capsaicin in isolated working rat heart

János Szolcsányi [a,*], Gábor Oroszi [a], József Németh [a], Zoltán Szilvássy [a], Árpád Tósaki [b]

[a] *Department of Pharmacology and Pharmacotherapy, University Medical School of Pécs, H-7643, Szigeti ut 12, PO Box 99, Pecs, Hungary*
[b] *Department of Cardiology, First Department of Internal Medicine, University Medical School of Pécs, H-7643, Szigeti ut 12, Pecs, Hungary*

Received 8 March 1999; received in revised form 7 May 1999; accepted 18 May 1999

Abstract

Capsaicin (1 nM–1 μM) induced a concentration-dependent decrease in heart rate, coronary flow, aortic flow, left ventricular developed pressure and its first derivative, dP/dt_{max} in isolated working rat heart. The effect of 10 nM capsaicin was mimicked by 0.1 nM endothelin. PD142893 (200 nM), a non-selective endothelin receptor blocking agent antagonized the effect of either endothelin (0.1 nM) or capsaicin (10 nM). We conclude that the majority of the effects of capsaicin in the rat heart are mediated by neural endothelin release.

Keywords: Capsaicin; Heart, isolated, working; Endothelin

1. Introduction

Stimulation of primary sensory nerves of the heart by capsaicin results in an increase in heart rate and coronary flow in isolated guinea-pig hearts. Regarding the neurotransmitters involved, the first evidence favoured a role for calcitonin gene-related peptide (CGRP) (Franco-Cereceda, 1988). Beyond CGRP, nitric oxide (NO) has also been shown to contribute to sensory nerve-mediated regulation of coronary flow and cardiac function, moreover the integrity of both transmitter systems has been suggested to be a prerequisite for the ability of the heart to adapt to repetitive ischaemic challenges in isolated working rat hearts (Ferdinandy et al., 1997). Nevertheless, the pharmacological effects of capsaicin in isolated heart of the rat have not been investigated (Szolcsanyi, 1996). The aim of the present work was therefore to study the effects of capsaicin on heart rate and cardiac function in isolated working rat heart.

2. Materials and methods

2.1. Ethics

The experiments performed in the present work conformed to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied was approved by the Ethical Committee of the University Medical School of Pécs.

2.2. Study design

Hearts from male Wistar rats (320–350 g) were prepared for working heart preparations perfused with temperature- (37°C) and pH- (7.2) controlled oxygenized Krebs–Henseleit bicarbonate buffer as described (Tosaki and Hellegouarch, 1994). After 10-min aerobic working perfusion, the hearts underwent various experimental protocols as follows.

Six hearts were exposed to increasing concentrations of capsaicin at log increments (1 nM–1 μM). Heart rate, coronary flow, aortic flow were monitored. Left ventricular developed pressure and its first derivative, dP/dt_{max} were also determined. The selected concentration (10 nM: EC_{50}) was then used for further studies. Since capsaicin decreased heart rate, coronary flow and deteriorated cardiac function, opposite to that would have been expected from studies with guinea-pig, we hypothesized the involve-

* Corresponding author. Tel.: +36-72-324-122; fax: +36-72-211-761; E-mail: szolcs@apacs.pote.hu

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00374-X

ment of sensory neural endothelin (Giaid et al., 1989) immunolocalised in perivascular nerve fibres (Loesch et al., 1998). Therefore, the effect of 0.1 nM endothelin (separate group of 6 hearts) was also analyzed on these variables. In the last series of experiments, 10 nM capsaicin ($n = 6$) or 0.1 nM endothelin ($n = 6$) was co-perfused with 200 nM PD142893, a non-selective endothelin receptor antagonist (Brunner and Opie, 1998). The control for these series of experiments served those hearts exposed to 10 nM capsaicin or 0.1 nM endothelin alone ($n = 6$). Four hearts were given 200 nM PD142893 to study the per se cardiac effects of the endothelin antagonists. Five hearts were excluded from the study due to development of arrhythmias in response to high concentrations of capsaicin, so that altogether 34 hearts entered the whole study.

2.3. Drugs and chemicals

All drugs and chemicals were purchased from Sigma (St. Louis, Mo).

2.4. Statistical analysis

The data expressed as means ± standard error of means (S.E.M.) were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's *t*-test (Wallenstein et al., 1980). Changes were considered significant at $P < 0.05$.

3. Results

Capsaicin induced a concentration-dependent decrease in heart rate, coronary flow, aortic flow, left ventricular developed pressure and its first derivative, dP/dt_{max} (Table 1). The decrease in these parameters by the selected concentrations of capsaicin (10 nM) or endothelin (0.1 nM) was similar in magnitude. PD142893 (200 nM), a non-selective endothelin receptor blocking agent equally antagonized the effects of the selected concentrations of either endothelin or capsaicin (Fig. 1). It is worthy of note that the capsaicin-evoked responses were long-lasting after washout similarly as in the case of endothelin, i.e., the effects persisted 15 min subsequent to termination of the washout periods.

PD 142893 at the concentration applied was without effect on any of the baseline parameters studied.

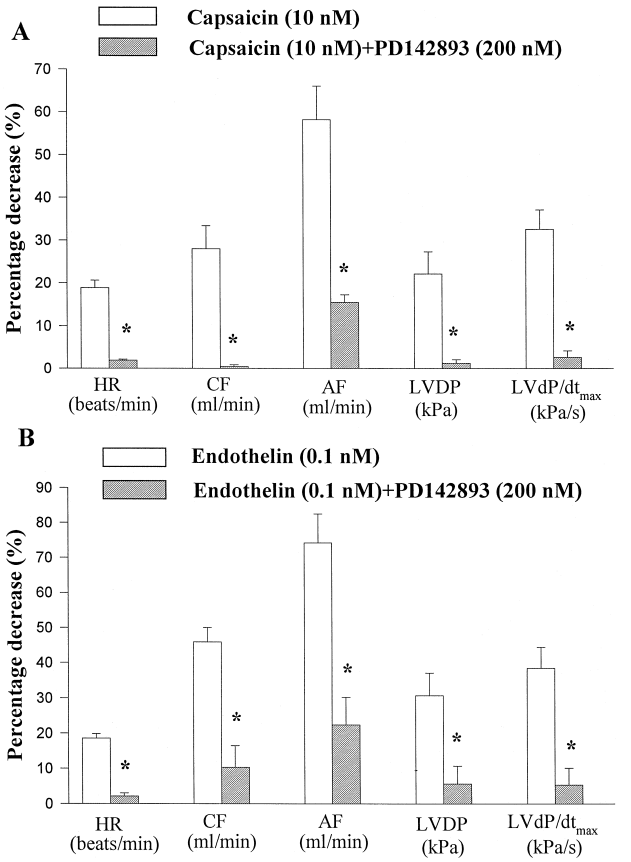

Fig. 1. Effect of capsaicin (A) or endothelin (B) on heart rate (HR), coronary flow (CF), aortic flow (AF), left ventricular developed pressure (LVDP), and its first derivative ($LVdP/dt_{max}$) in isolated working rat heart and an interaction with PD142893, an endothelin receptor antagonist. The data are means ± S.E.M. obtained with 6 preparations per group. * Indicates a significant difference between ''capsaicin'' vs. ''capsaicin + PD142893'' (A), and ''endothelin'' vs. ''endothelin + PD142893'' (B) values at $P < 0.05$.

Table 1
Effects of capsaicin on cardiac function
Data are expressed as means ± S.E.M. obtained with 6 hearts exposed to increasing concentrations of capsaicin at log increments in each group. Comparisons were made to the corresponding drug-free values. Abbreviations: HR (heart rate), CF (coronary flow), AF (aortic flow), LVDP (left ventricular developed pressure), $LVdP/dt_{max}$ (first derivative of LVDP).

Concentrations of capsaicin (nM)	HR (beats/min)	CF (ml/min)	AF (ml/min)	LVDP (kPa)	$LVdP/dt_{max}$ (kPa/s)
0	318 ± 7	28.2 ± 3	58.8 ± 2.9	18.1 ± 0.1	801 ± 8.3
1	315 ± 8	27.0 ± 0.8	48.0 ± 3.0[a]	17.6 ± 0.3	767 ± 23
10	262 ± 10[a]	17.6 ± 1.5[a]	23.3 ± 3.6[a]	14.1 ± 0.9[a]	539 ± 35[a]
100	181 ± 13[a]	14.5 ± 2.1[a]	12.4 ± 4.7[a]	12.1 ± 1.0[a]	469 ± 29[a]
1000	92 ± 9[a]	7.0 ± 3.2[a]	3.6 ± 2.0[a]	2.6 ± 1.0[a]	97 ± 28[a]

[a] $P < 0.05$.

4. Discussion

The results show that exposure of the isolated working rat heart to capsaicin results in a progressive deterioration of cardiac function. It is also suggested that this effect is underlain by a secondary endothelin release, since (i) endothelin mimics the effects of capsaicin and (ii) PD142893 a non-selective endothelin receptor antagonist almost abolishes the aforementioned capsaicin effects.

Capsaicin-sensitive nerve endings serve as powerful peptidergic effector sites in several tissues (Szolcsanyi, 1996) including the heart (Franco-Cereceda, 1988) but the involvement of endothelin in tissue responses attributed to influences coming from these nerve endings has not been identified (Maggi, 1995). Sensory nerve fibres operating with NO and CGRP mediators in the rat heart have been found essential for the ability of the heart to tolerate ischaemia (Ferdinandy et al., 1997). In the guinea-pig, Franco-Cereceda (1988) described a decrease in coronary resistance and positive inotropy attributed to the release of CGRP from C fibres in guinea-pig heart. Surprisingly, in the rat direct stimulation of the heart by capsaicin attained a marked and abrupt decline in cardiac function possibly deriving from a conspicuous decrease in coronary flow, a finding contrary to that would have been expected from the effects of either CGRP or NO. The controversy at least in part is explained by a difference in the species, and differences in the process of sensory neurotransmitter release in response to myocardial ischaemia and that pharmacologically provoked by capsaicin in the non-ischaemic/normoxic heart. Similarly, a contradiction exists between results obtained by Sigrits et al. (1986), who found an increased rate and force of contraction by capsaicin using spontaneously beating isolated rat atria and those presented in our work. In the intact heart, coronary vasoconstriction induced by capsaicin is of dominant influence on cardiac contractility, whereas in isolated atrial preparations any effect on coronary tone has minimum or no effect on inotropy.

PD 142893 at the concentration applied blocked but did not reverse the effect of capsaicin on either coronary flow or heart rate, similar to other indicators of cardiac function although the concomitant release of CGRP, NO or substance P would have been expected to be responsible for an opposite effect. This may be explained by either an incomplete blockade of endothelin receptors by the antagonist with a substantial *residual* endothelin effect that masked the weak positive cardiac effects of the other sensory mediators or the lack of sufficient amounts of these latter substances in response to the capsaicin concentration applied in the intact heart. Moreover, it is important that the blocking effect with lack of reversal was seen at selected concentrations of capsaicin and the endothelin receptor antagonist, leaving the possibility that the spectrum of sensory neuropeptides released in response to higher capsaicin concentrations might be different with a more significant proportion of CGRP or substance P that might eventually be able to overcome the negative effects of endothelin.

As far as the mechanism of action of capsaicin in the heart is concerned, capsaicin opens cation channels gated by vanilloid receptors in a subset of sensory nerve terminals (Szolcsanyi, 1996). According to the best of our knowledge, sensory neurons are exclusive for vanilloid receptor expression (Caterina et al., 1997), thus, endothelin is probably released by capsaicin from the perivascular nerve fibres (Loesch et al., 1998; Giaid et al., 1989; Franco-Cereceda et al., 1991) and produces a marked coronary vasoconstriction with ensuing deterioration of cardiac function. However, the present study does not exclude the possibility of implication of vascular endothelium or endocardium as sources of endothelin. Nevertheless, neither endothelial cells nor cardiac myocytes express vanilloid receptors (Caterina et al., 1997); therefore, the endothelin releasing effect of capsaicin at least in the heart is possibly confined to sensory nerve fibres.

In summary, the study provides the first pharmacological evidence for the involvement of endothelin as new sensory neuropeptide underlying the effector function of cardiac capsaicin-sensitive sensory nerves.

Acknowledgements

This work was supported by OTKA T-016945, T-029428, T-030766 and ETT 372 1996 and the Hungarian Academy of Sciences and the Volswagen Stifftung, Berlin, Germany.

References

Brunner, F., Opie, L.H., 1998. Role of endothelin-A receptors in ischemic contracture and reperfusion injury. Circulation 97, 391–398.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Ferdinandy, P., Csont, T., Csonka, Cs., Torok, L., Dux, M., Nemeth, J., Horvath, L.I., Szilvassy, Z., Jancso, G., 1997. Capsaicin-sensitive local sensory innervation is involved in pacing-induced preconditioning in rat hearts: role of nitric oxide and CGRP?. Naunyn-Schmiedeberg's Arch. Pharmacol. 356, 356–363.

Franco-Cereceda, A., 1988. Calcitonin gene-related peptide and tachykinins in relation to local sensory control of cardiac contractility and coronary vascular tone. Acta Physiol. Scand. 133, 1–63.

Franco-Cereceda, A., Rydh, M., Lou, Y.-P., Dalsgaard, C.-J., Lundberg, J.M., 1991. Endothelin as a putative sensory neuropeptide in the guinea pig: different properties in comparison with calcitonin gene-related peptide. Regul. Pept. 32, 253–265.

Giaid, A., Gibson, S.J., Ibrahim, B.N., Legon, S., Bloom, S.R., Yanagisawa, M., Masaki, T., Varndell, I.M., Polak, J.M., 1989. Endothelin 1, an endothelium-derived peptide, is expressed in neurons of human spinal cord and dorsal root ganglia. Proc. Natl. Acad. Sci. U.S.A. 86, 7634–7638.

Loesch, A., Milner, P., Burnstock, G., 1998. Endothelin in perivascular nerves. An electron-immunocytochemical study of rat basilar artery. Ann. Auton. Nerv. Syst. 17, 3093–3096.

Maggi, C.A., 1995. Tachykinins and calcitonine gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. Prog. Neurobiol. 45, 1–98.

Sigrits, S., Franco-Cereceda, A., Muff, R., Henke, H., Lundberg, J.M., Fischer, J.A., 1986. Specific receptor and cardiovascular effects of calcitonin gene-related peptide. Endocrinology 119, 381–389.

Szolcsanyi, J., 1996. Capsaicin-sensitive sensory nerve terminals with local and systemic efferent functions: facts and scopes of an unorthodox neuroregulatory mechanism. Prog. Brain Res. 113, 343–359.

Tosaki, A., Hellegouarch, A., 1994. Adenosine triphosphate-sensitive potassium channel blocking agent ameliorates, but the opening agent aggravates ischemia/reperfusion-induced injury: heart function studies in nonfibrillating isolated hearts. J. Am. Coll. Cardiol. 23, 487–496.

Wallenstein, S., Zucker, C.L., Fleiss, J.L., 1980. Some statistical methods useful in circulation research. Circ. Res. 47, 1–9.