ELSEVIER

European Journal of Pharmacology 352 (1998) 223–228

ejp

# Ion channels involved in the release of calcitonin gene-related peptide by low pH, prostacyclin and capsaicin in the isolated guinea-pig heart

Göran Källner *, Anders Franco-Cereceda

*Department of Thoracic Surgery, Karolinska Hospital, S-171 76 Stockholm, Sweden*

Received 23 March 1998; revised 24 April 1998; accepted 30 April 1998

**Abstract**

The aim of this study was to characterise the release of calcitonin gene-related peptide (CGRP) by capsaicin, low pH and prostacyclin in terms of $Ca^{2+}$ channel dependence, interactions with $K_{ATP}$ channels and the role of action potential propagation, in the isolated, perfused guinea-pig heart. The $Ca^{2+}$ channel blocker $\omega$-conotoxin reduced CGRP release evoked by $10^{-7}$ M capsaicin, as well as CGRP release evoked by pH 7. CGRP release caused by capsaicin at low ($10^{-7}$ M) but not high ($10^{-6}$ M) concentrations was also attenuated by tetrodotoxin, indicating partial dependence on action potential propagation. CGRP release caused by prostacyclin was not altered by any of the tested drugs. The $K_{ATP}$ channel activator cromakalim and the $K_{ATP}$ channel blocker glibenclamide had no effect on CGRP release. Previous findings that low pH and capsaicin stimulate capsaicin-sensitive afferents in the isolated heart at least partly through common mechanisms are thus supported. Attenuation of capsaicin-evoked release of CGRP by tetrodotoxin suggests recruitment of additional nerve terminals by a local axon reflex. 

*Keywords:* CGRP (calcitonin gene-related peptide); Capsaicin; Heart; Ion channel; Prostacyclin; (Guinea pig)

## 1. Introduction

Myocardial ischaemia causes responses in the autonomous nervous system which mediate haemodynamic changes and symptoms of ischaemia such as pain. A subgroup of cardiac afferents belongs to the C-fibre group and is characterised by sensitivity to capsaicin, the irritant agent in certain hot peppers (see Szallasi, 1994; Bevan and Szolcsanyi, 1990). Upon stimulation multiple peptides are released from these fibres, including calcitonin gene-related peptide (CGRP), which has potent haemodynamic effects (Franco-Cereceda, 1988). Capsaicin opens a receptor-operated non-selective cation channel, leading to $Ca^{2+}$-dependent peptide release (Bevan and Szolcsanyi, 1990). In ion flux and voltage clamp studies the effects of capsaicin were competitively antagonised by capsazepine, strongly indicating the existence of a specific capsaicin receptor (Bevan et al., 1992). The tritiated capsaicin analogue [$^3$H]resiniferatoxin binds specifically to this postulated receptor and has been used for its further characterisation (Szallasi and Blumberg, 1990). A homovanilloid moiety is the key structure shared by capsaicin and [$^3$H]resiniferatoxin, and the receptor is referred to as the vanilloid receptor (see Szallasi, 1994).

In addition to sensitivity to the exogenous compounds capsaicin and [$^3$H]resiniferatoxin, acidic pH (Franco-Cereceda et al., 1993) and prostaglandins (Franco-Cereceda et al., 1994) constitute endogenous stimuli to capsaicin-sensitive afferents. It is well known that myocardial pH decreases in response to ischaemia, and ischaemia-induced myocardial acidosis correlates well with ischaemia-induced metabolic disturbances (Ichihara et al., 1991). Endothelial synthesis of prostaglandins is stimulated by hypoxia/ischaemia (Busse et al., 1984; Berger et al., 1976). The release of CGRP as well as the formation of the stable prostacyclin (prostaglandin $I_2$) metabolite 6-keto-prostaglandin $F_{1\alpha}$ caused by moderate acidosis (pH 6–7) is dependent on extracellular $Ca^{2+}$. Furthermore, the capsaicin receptor antagonist capsazepine attenuates CGRP release caused by moderate acidosis (pH 6–7), as well as the release of CGRP by exogenous prostaglandin $I_2$ (Franco-Cereceda et al., 1994). The involvement of prostaglandin $I_2$ in the activation of the vanilloid-receptor was further substantiated by data showing that the release of

* Corresponding author. Tel.: +46-8-517-73434; fax: +46-8-322701.

0014-2999/98/$19.00 

CGRP by moderate acidosis is dependent on an intact endothelium, capable of producing prostaglandin $I_2$ (Källner and Franco-Cereceda, 1995). Thus, the release of CGRP caused by low pH and prostaglandin $I_2$ has several features in common with the release of CGRP caused by capsaicin.

The peptide release in the guinea-pig lung elicited by low ($10^{-8}$ M) but not high ($10^{-6}$ M) concentrations of capsaicin is attenuated by $\omega$-conotoxin, indicating the involvement of N-type $Ca^{2+}$ channels (Lou et al., 1992). The pulmonary effects of capsaicin at low concentrations are similarly blocked by the $Na^+$ channel blocker tetrodotoxin (Lou et al., 1992; Kröll et al., 1990). This implies that peptide release at this moderate degree of stimulation is at least partly dependent on action potential propagation (axon reflex).

C-fibre activation with peptide release is enhanced after blockade of adenosine triphosphate-sensitive potassium channels ($K_{ATP}$ channels) (Lou and Lundberg, 1993). Interestingly, $K_{ATP}$ channels are involved in the local response to myocardial ischaemia, and potassium channel openers, such as cromakalim, seem to have an anti-ischaemic, local cardioplegic effect (see Hearse, 1995). This anti-ischaemic effect can be counteracted by the potassium channel blocker glibenclamide (Grover et al., 1990).

The aim of the present study was to further characterise and compare the cardiac release of CGRP by capsaicin, low pH and prostaglandin $I_2$ in terms of $Ca^{2+}$ channel dependence, interactions with $K_{ATP}$ channels and the role of action potential propagation.

## 2. Materials and methods

The Ethics Committee of the Karolinska Institute approved the experiments in this study.

Adult guinea-pigs (body weight 250–350 g, $n = 81$) were killed by stunning. The beating heart was rapidly excised and the ascending aorta was cannulated. Perfusion according to the Langendorff technique with Tyrode's solution (for composition of the perfusion buffers, see Table 1) at a perfusion pressure of 70–75 cm $H_2O$ was started immediately. The solution was warmed to 37°C and aerated with 95% $O_2$ and 5% $CO_2$. A water-filled latex balloon attached to a plastic cannula was inserted into the left ventricle and connected to a pressure transducer. Pressure tracings were used only to record heart rate as an assessment of the experimental set-up. The hearts were allowed to stabilise for 15 min before drug treatment was started. The drug treatment was then maintained throughout the experiment. After 10 min of drug treatment the perfusate was collected for 5 min for determination of basal perfusion volume and basal outflow of CGRP. The hearts were then subjected to consecutive challenge with buffer solution at pH 7, 6 and 5, or challenge with prostaglandin $I_2$ ($10^{-5}$ M), followed after 15 min by capsaicin ($10^{-7}$ or $10^{-6}$ M). Each challenge lasted 5 min, during which the perfusate was collected in beakers placed on ice. Each 5-min fraction was desalted, using SEP-PAK C18 cartridges, and lyophilised. After addition of appropriate buffer, CGRP was determined by radioimmunoassay (RIA) (for details see Franco-Cereceda, 1988). The hearts were weighed after the experiments, and perfusion volume and outflow of CGRP are expressed per gram. Control experiments at pH 7, 6 and 5 ($n = 9$), with prostaglandin $I_2$ $10^{-5}$ M and capsaicin $10^{-7}$ M ($n = 6$), and with capsaicin $10^{-6}$ M ($n = 4$) were performed according to the same protocol, but without drug treatment.

Table 1
Perfusion buffer: composition (mM) of the Tyrode's solution (pH 7.4) and low pH solutions (pH 7, pH 6 and pH 5)

| | pH 7.4 | pH 7 | pH 6 | pH 5 |
|---|---|---|---|---|
| NaCl | 137 | 136 | 140 | 140 |
| KCl | 2.7 | 4.0 | 0.7 | – |
| $CaCl_2$ | 1.8 | 2.5 | 2.5 | 2.5 |
| $MgSO_4$ | – | 1.5 | 1.5 | 1.5 |
| $NaHCO_3$ | 11.9 | – | – | – |
| $Na_2HPO_4$ | 0.3 | 4.1 | 0.8 | 0.1 |
| $KH_2PO_4$ | – | 2.6 | 5.9 | 6.6 |
| $MgCl_2$ | 0.5 | – | – | – |
| Glucose | 5.6 | 11 | 11 | 11 |

### 2.1. Drugs

Tetrodotoxin ($Na^+$ channel blocker) was obtained from Latoxan (Rosans, France) and dissolved to form a 1 mM stock solution in 10 mM acetic acid. The stock solution was diluted in buffer to $10^{-5}$ M, which was then added to the perfusion buffer to give a final concentration of $10^{-6}$ M.

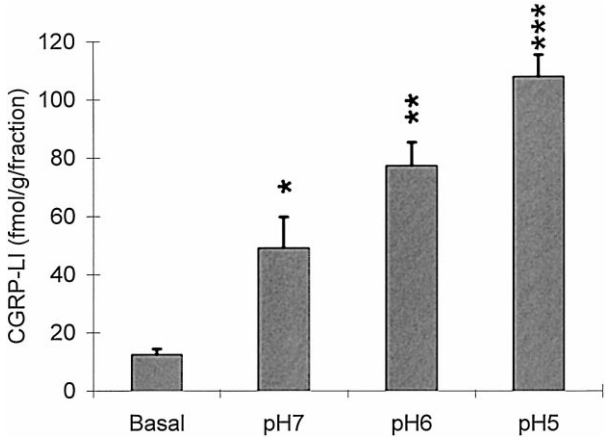


Fig. 1. Outflow of CGRP caused by acidic pH: control group. The perfusate was collected for 5 min under basal conditions (pH 7.4), and consecutively at pH 7, pH 6 and pH 5, and CGRP was measured as described in the Section 2. Values are presented as means ± S.E.M. $*\ P < 0.05$, $**\ P < 0.01$ and $***\ P < 0.001$, Friedman test, $n = 9$.

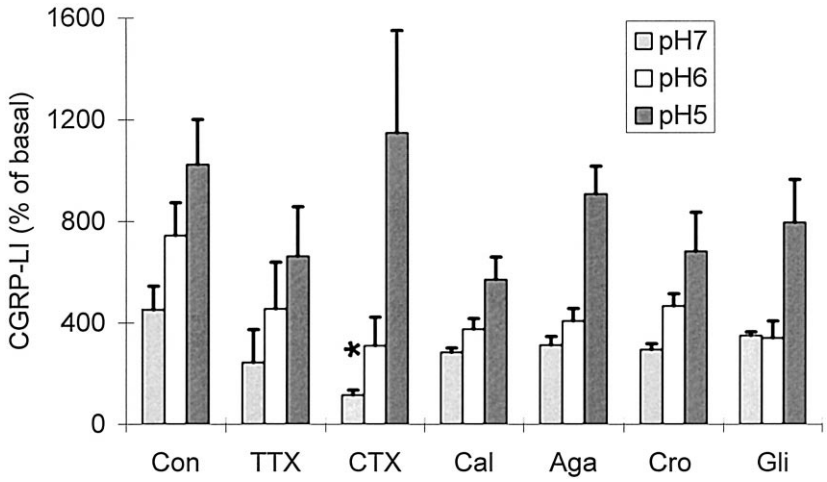


Fig. 2. Outflow of CGRP caused by acidic pH: effects of drug treatment. Effects of perfusion with tetrodotoxin (TTX), ω-conotoxin (CTX), Calciseptin (Cal), ω-agatoxin (Aga), Cromakalim (Cro) and Glibenclamide (Gli), $n = 4–5$ in each group, on release of CGRP evoked by consecutive perfusion at pH 7, pH 6 and pH 5. Release is expressed as percentage of the release under basal conditions with each drug, and presented as means ± S.E.M. $* P < 0.05$, Kruskall–Wallis test, each group vs. corresponding value in the control group (Con).

Cromakalim ($K_{ATP}$ channel activator) was obtained from Sigma (St. Louis, MO, USA), dissolved in 37°C polyethylenglycol and diluted in buffer solution to $10^{-4}$ M. This was added to the perfusion buffer to give a final concentration of $10^{-5}$ M.

Glibenclamide ($K_{ATP}$ channel blocker) was obtained from Hoechst (Frankfurt, Germany). It was diluted in perfusion buffer to $10^{-4}$ M and added to give a final concentration of $10^{-5}$ M.

ω-Conotoxin GVIA (blocker of N-type $Ca^{2+}$ channels) was obtained from Sigma and dissolved in buffer to $2.5 \times 10^{-6}$ M. This was added to the perfusion buffer to give a final concentration of $2.5 \times 10^{-7}$ M.

ω-Agatoxin IVA (blocker of P-type $Ca^{2+}$ channels) was obtained from Latoxan, and Calciseptin (blocker of L-type $Ca^{2+}$ channels) was from Alomone (Jerusalem, Israel). Both were dissolved in buffer to $10^{-6}$ M and added to the perfusion buffer to give a final concentration of $10^{-7}$ M.

Capsaicin was obtained from Fluka (Basel, Switzerland). It was dissolved as $10^{-3}$ M stock solution in 60% ethanol. It was further diluted in perfusion buffer to give a final concentration of $10^{-6}$ or $10^{-7}$ M.

Prostaglandin $I_2$ was obtained from Sigma and dissolved as stock solution (1 mg/ml) in 0.5% NaOH solution. This was diluted in buffer to a final concentration of $10^{-5}$ M.

All drugs were added to the perfusion buffer by a motor-driven syringe connected to a side-line in the aortic cannula.

### 2.2. Statistical evaluation

Values are expressed as means ± S.E.M. Differences within groups were evaluated by using Friedman's test, and differences between groups were evaluated by using Mann–Whitney *U*-test or Kruskall–Wallis' test. $P < 0.05$ was considered significant.

## 3. Results

### 3.1. Functional effects

In the control hearts subjected to low pH the basal heart rate was 200 ± 7 beats/min. Lowering of the pH was associated with a decrease in heart rate (at pH 7 150 ± 6, NS, at pH 6 26 ± 9, $P < 0.01$, and at pH 5 all hearts arrested, $P < 0.001$). In the control hearts treated with prostaglandin $I_2$ ($10^{-5}$ M) and capsaicin ($10^{-7}$ M and

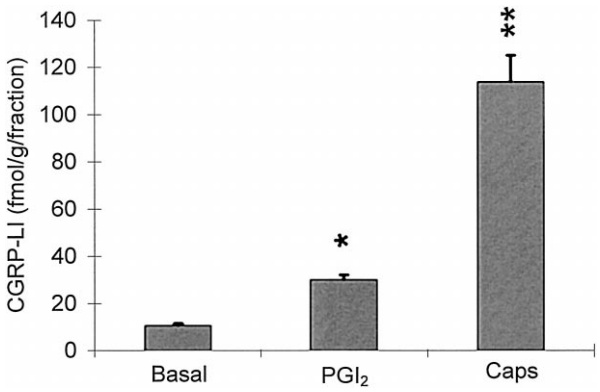


Fig. 3. Outflow of CGRP caused by prostaglandin $I_2$ and capsaicin $10^{-7}$ M: control group. The perfusate was collected for 5 min under basal conditions, followed by 5 min with addition of prostaglandin $I_2$. After 15 min of stabilisation, the same heart was exposed to capsaicin $10^{-7}$ M. Values are presented as means ± S.E.M. $* P < 0.05$, $** P < 0.01$, Friedman test, $n = 6$.

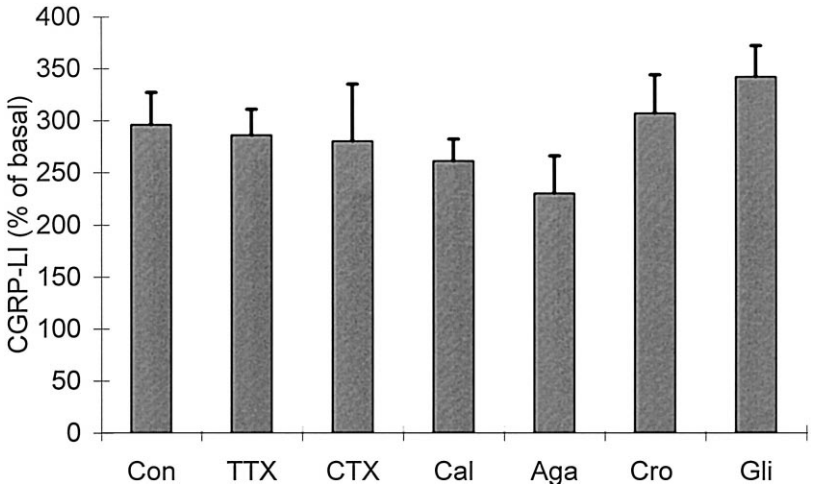


Fig. 4. Outflow of CGRP caused by prostaglandin $I_2$: effects of drug treatment. Effects of perfusion with tetrodotoxin (TTX), ω-conotoxin (CTX), Calciseptin (Cal), ω-agatoxin (Aga), Cromakalim (Cro) and Glibenclamide (Gli), $n = 4–7$ in each group, on release of CGRP evoked by perfusion with prostaglandin $I_2$. Release is expressed as percentage of the release under basal conditions with each drug, and presented as means ± S.E.M., Kruskall–Wallis test, each group vs. corresponding value in the control group (Con).

$10^{-6}$ M) there was no significant change in heart rate from the basal rate 198 ± 5 beats/min (212 ± 10, 212 ± 5 and 222 ± 3 beats/min, respectively). The tetrodotoxin-treated hearts had a significantly lower basal heart rate than control hearts (158 ± 5 vs. 199 ± 4 beats/min, $P < 0.01$), but otherwise there were no differences between drug-treated groups and controls in terms of heart rate.

The basal perfusion volume was 39 ± 7 ml/g in the control hearts subjected to low pH. Low pH-perfusion did not influence the perfusion volume. In the control hearts treated with prostaglandin $I_2$ and capsaicin, no effect on perfusion volume was observed. There was no significant difference in perfusion volume between drug-treated hearts and control hearts.

### 3.2. Outflow of CGRP

#### 3.2.1. Basal conditions

The basal outflow of CGRP was not significantly different in any of the drug-treated groups as compared to the control group.

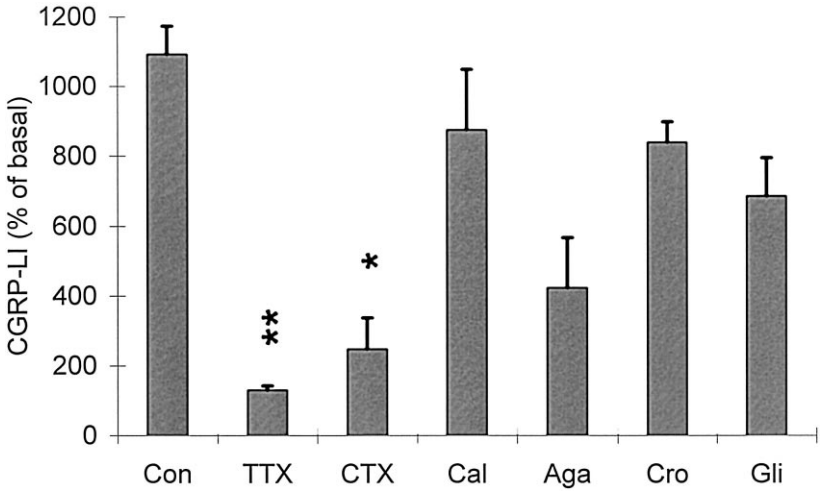


Fig. 5. Outflow of CGRP caused by capsaicin $10^{-7}$ M: effects of drug treatment. Effects of perfusion with tetrodotoxin (TTX), ω-conotoxin (CTX), Calciseptin (Cal), ω-agatoxin (Aga), Cromakalim (Cro) and Glibenclamide (Gli), $n = 4–7$ in each group, on release of CGRP evoked by perfusion with capsaicin $10^{-7}$ M. Release is expressed as percentage of the release under basal conditions with each drug, and presented as means ± S.E.M. $* P < 0.05$, $** P < 0.01$, Kruskall–Wallis test, each group vs. corresponding value in the control group (Con).

*3.2.2. Low pH perfusion*

In the control experiments, basal outflow of CGRP was $12 \pm 2$ fmol/g. The outflow at pH 7, 6 and 5 was significantly higher ($49 \pm 11$, $P < 0.05$; $77 \pm 8$, $P < 0.01$ and $108 \pm 7$, $P < 0.001$, fmol/g respectively) (Fig. 1). In all six drug-treated groups the general pattern of increased outflow of CGRP caused by low pH was maintained. In $\omega$-conotoxin-treated hearts, however, the increase in CGRP from basal level caused by pH 7 was almost completely abolished ($115 \pm 19\%$ of basal vs. $451 \pm 93\%$ of basal in the control group, $P < 0.05$). The increase in CGRP outflow at pH 6 or pH 5 was not significantly changed by any of the drug treatments (Fig. 2).

*3.2.3. Prostaglandin $I_2$ perfusion*

In the control experiments, basal outflow of CGRP was $11 \pm 1$ fmol/g. Prostaglandin $I_2$ was associated with an increase in outflow of CGRP to $30 \pm 2$ fmol/g, $P < 0.05$ (Fig. 3). This increased outflow was maintained in all the drug-treated groups with there being no difference compared to the control group (Fig. 4).

*3.2.4. Capsaicin perfusion*

In the control experiments, capsaicin perfusion ($10^{-7}$ and $10^{-6}$ M) increased the outflow of CGRP to $114 \pm 11$ vs. $11 \pm 1$ fmol/g at basal level, $P < 0.01$ (Fig. 3) and $206 \pm 6$ vs. $20 \pm 1$ fmol/g at basal level, $P < 0.05$, respectively. The response to capsaicin ($10^{-7}$ M) was markedly reduced in tetrodotoxin-treated hearts ($131 \pm 13\%$ of basal, $P < 0.01$) and in $\omega$-conotoxin-treated hearts ($247 \pm 90\%$ of basal, $P < 0.05$) as compared to control ($1092 \pm 81\%$ of basal) (Fig. 5). After the capsaicin concentration was increased to $10^{-6}$ M, tetrodotoxin did not influence the release of CGRP.

## 4. Discussion

Even though the heart has a substantial buffering capacity to counteract acidosis (Franco-Cereceda et al., 1994), a reduction in extracellular pH to 5.5 has been demonstrated after acute coronary artery occlusion (Hirche et al., 1980). Capsaicin-evoked CGRP release from capsaicin-sensitive C-fibre afferents is dependent on extracellular $Ca^{2+}$, and perfusion with $Ca^{2+}$-free medium totally abolishes the CGRP release observed at pH 7 and pH 6, while the marked release observed at pH 5 is only partially dependent on extracellular $Ca^{2+}$ (Franco-Cereceda et al., 1994). Thus, the present finding that the release of CGRP caused by both capsaicin and moderately low pH (pH 7) was attenuated by the same N-type $Ca^{2+}$ channel blocker ($\omega$-conotoxin) further supports the view that moderately low pH and capsaicin cause CGRP release by a common, $Ca^{2+}$-dependent mechanism. Previous studies suggest that as pH is brought down even further, other mechanisms, such as metabolic disturbances and tissue disruption, primarily account for the peptide release (Franco-Cereceda et al., 1994; Källner and Franco-Cereceda, 1995). The competitive capsaicin antagonist capsazepine (Bevan et al., 1992) inhibited not only capsaicin-evoked CGRP release, but also the release observed at low pH (Franco-Cereceda et al., 1993), further indicating a common release mechanism. It should be mentioned, however, that recent studies have shown that capsazepine, in addition to its competitive antagonism of vanilloid receptors, in high concentrations has a non-specific blocking action on voltage-activated $Ca^{2+}$ channels (Docherty et al., 1997). Prostaglandin $I_2$, the predominant cyclo-oxygenase product formed during myocardial ischaemia (Coker et al., 1981), also caused capsazepine-sensitive release of CGRP, and the release of CGRP observed at low pH was attenuated by cyclo-oxygenase inhibitors, indicating that cyclo-oxygenase products formed in response to low pH may be endogenous capsaicin receptor ligands (Franco-Cereceda et al., 1994). However, in spite of several common features in capsaicin- and prostaglandin $I_2$-evoked CGRP release, it can not be determined from the present results to what extent prostaglandin $I_2$ and capsaicin act through common ion channel mechanisms.

The release of CGRP caused by a low capsaicin concentration ($10^{-7}$ M) was also markedly attenuated by tetrodotoxin, indicating that propagation of an action potential along nerve fibres, i.e., an axon reflex, is involved in this response to capsaicin. After the capsaicin concentration was increased to $10^{-6}$ M, tetrodotoxin was ineffective in blocking the release of CGRP. In accord, findings in the guinea-pig isolated lung have demonstrated that low concentrations of capsaicin cause CGRP release through an axon reflex (Kröll et al., 1990). Other investigators have found the response to capsaicin to be tetrodotoxin-resistant (Szolcsanyi, 1983), indicating that capsaicin evokes a response only locally at the receptor site. Indeed, there are data in the literature to support the view that there are two distinct modes of neuropeptide release: one tetrodotoxin- and $\omega$-conotoxin-resistant, and one that is sensitive to these blockers (see Holzer, 1991; Maggi, 1995). The relative importance of these two modes of peptide release seems to vary with the stimulation mode (i.e., capsaicin concentration, electrical nerve stimulation, high $K^+$ concentration) as well as with the experimental model used.

Recently, interest has focused on myocardial protection by ischaemic preconditioning (see Parratt, 1994), and various endogenous substances that are released from the ischaemic myocardium have been proposed as mediators of this protective effect. In the isolated rat heart, pre-treatment with CGRP or capsaicin produced significant improvement of postischaemic cardiac function, and CGRP-(8-37), a selective CGRP receptor antagonist, abolished the cardioprotection of ischaemic preconditioning (Li et al., 1996). $K_{ATP}$ channel activators such as cromakalim also have cardioprotective properties and may be involved in ischaemic preconditioning (Rohmann et al., 1994; Gross

and Auchampach, 1992). The present study could not identify activation or blockade of $K_{ATP}$ channels as important factors in the release of CGRP.

## 5. Conclusion

In summary, this study indicates that N-type $Ca^{2+}$ channels are involved in the CGRP release caused by moderately low pH and capsaicin. This supports previous findings that low pH and capsaicin stimulate capsaicin-sensitive afferents in the isolated heart at least partly through common mechanisms. Furthermore, peptide release evoked by capsaicin was attenuated by tetrodotoxin, suggesting recruitment of additional nerve terminals by an axon reflex. The mechanisms of prostaglandin $I_2$ interaction with the proposed capsaicin receptor and subsequent CGRP release remain to be further evaluated. In this context, the recent cloning of the vanilloid receptor (VR1) (Caterina et al., 1997) represents a unique opportunity to identify endogenous capsaicin-receptor ligands.

## Acknowledgements

This study was supported by grants from the Swedish Medical Research Council, the Heart–Lung Foundation, the Wallenberg Foundation, and funds from the Karolinska Institute.

## References

Berger, H.J., Zaret, B.L., Speroff, L., Cohen, L.S., Wolfson, S., 1976. Regional cardiac prostaglandin release during myocardial ischaemia in anaesthetised dogs. Circ. Res. 38, 566–571.

Bevan, S., Szolcsanyi, J., 1990. Sensory neuron-specific actions of capsaicin: mechanisms and applications. Trends Pharmacol. Sci. 11, 330–333.

Bevan, S., Hothi, S., Hughes, G., James, I.F., Rang, H.P., Shah, K., Walpole, C.S.J., Yeats, J., 1992. Capsazepine: a competitive antagonist of the sensory neurone excitant capsaicin. Br. J. Pharmacol. 107, 544–552.

Busse, R., Förstermann, U., Matsuda, H., Pohl, U., 1984. The role of prostaglandins in the endothelium-mediated vasodilatory response to hypoxia. Pflüg. Arch. 401, 77–83.

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.

Coker, S.J., Parratt, J.R., Ledingham, I.Mc.A., Zeitlin, I.J., 1981. Thromboxane and prostacyclin release from ischaemic myocardium in relation to arrhythmias. Nature 291, 323–324.

Docherty, R.J., Yeats, J.C., Piper, A.S., 1997. Capsazepine block of voltage-activated calcium channels in adult rat dorsal root ganglion neurones in culture. Br. J. Pharmacol. 121, 1461–1467.

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 (Suppl. 569), 1–63.

Franco-Cereceda, A., Källner, G., Lundberg, J.M., 1993. Capsazepine-sensitive release of calcitonin gene-related peptide from C-fibre afferents in the guinea-pig heart by low pH and lactic acid. Eur. J. Pharmacol. 238, 311–316.

Franco-Cereceda, A., Källner, G., Lundberg, J.M., 1994. Cyclooxygenase products released by low pH have capsaicin-like actions on sensory nerves in the isolated guinea pig heart. Cardiovasc. Res. 28, 365–369.

Gross, G.J., Auchampach, J.A., 1992. Blockade of ATP-sensitive potassium channels prevents myocardial preconditioning in dogs. Circ. Res. 70, 223–233.

Grover, G.J., Sleph, P.G., Dzwonczyk, S., 1990. Pharmacologic profile of cromakalim in the treatment of myocardial ischaemia in isolated rat hearts and anaesthetised dogs. J. Cardiovasc. Pharmacol. 16, 853–864.

Hearse, D.J., 1995. Activation of ATP-sensitive potassium channels: a novel pharmacological approach to myocardial protection?. Cardiovasc. Res. 30, 1–17.

Hirche, H., Franz, C., Bös, L., Bissig, R., Lang, R., Schramm, M., 1980. Myocardial extracellular $K^+$ and $H^+$ increase and noradrenaline release as possible cause of early arrhythmias following acute coronary artery occlusion in pigs. J. Mol. Cell. Cardiol. 12, 579–593.

Holzer, P., 1991. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurones. Pharmacol. Rev. 43, 143–201.

Ichihara, K., Morimoto, T., Shiba, T., Tsujitani, M., Abiko, Y., 1991. Attenuation of ischaemia-induced regional myocardial acidosis by LP-805, a newly developed vasodilator, in dogs. Eur. J. Pharmacol. 204, 127–133.

Källner, G., Franco-Cereceda, A., 1995. Attenuation of low pH-, but not capsaicin- or prostaglandin $I_2$-evoked CGRP-release by endothelium removal using saponin. Acta Physiol. Scand. 155, 251–256.

Kröll, F., Karlsson, J.A., Lundberg, J.M., Persson, C.G.A., 1990. Capsaicin-induced bronchoconstriction and neuropeptide release in guinea pig perfused lungs. J. Appl. Physiol. 68, 1679–1687.

Li, Y.J., Xiao, Z.S., Peng, C.F., Deng, H.W., 1996. Calcitonin gene-related peptide-induced preconditioning protects against ischaemia-reperfusion injury in isolated rat hearts. Eur. J. Pharmacol. 311, 163–167.

Lou, Y.P., Lundberg, J.M., 1993. Different effects of the $K^+$ channel blockers 4-aminopyridine and charybdotoxin on sensory nerves in guinea-pig lung. Pharmacol. Toxicol. 72, 139–144.

Lou, Y.P., Franco-Cereceda, A., Lundberg, J.M., 1992. Different ion channel mechanisms between low concentrations of capsaicin and high concentrations of capsaicin and nicotine regarding peptide release from pulmonary afferents. Acta Physiol. Scand. 146, 119–127.

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

Parratt, J.R., 1994. Protection of the heart by ischaemic preconditioning: mechanisms and possibilities for pharmacological exploitation. Trends Pharmacol. Sci. 13, 19–25.

Rohmann, S., Weygandt, H., Schelling, P., Soei, L.K., Becker, K.H., Verdouw, P.D., Lues, I., Häusler, G., 1994. Effect of bimakalim (EMD 52692), an opener of ATP sensitive potassium channels, on infract size, coronary blood flow, regional wall function, and oxygen consumption in swine. Cardiovasc. Res. 28, 858–863.

Szallasi, A., 1994. The vanilloid (capsaicin) receptor: receptor types and species differences. Gen. Pharmacol. 25, 223–243.

Szallasi, A., Blumberg, P., 1990. Specific binding of resiniferatoxin, an ultrapotent capsaicin analogue, by dorsal root ganglion membranes. Brain Res. 524, 106–111.

Szolcsanyi, J., 1983. Tetrodotoxin-resistant non-cholinergic neurogenic contraction evoked by capsaicinoids and piperine on the guinea-pig trachea. Neurosci. Lett. 42, 83–88.