Br. J. Pharmacol. (1991), **103**, 1764–1768

Heterogeneous involvement of endothelium in calcitonin gene-related peptide-induced relaxation in coronary arteries from rat

[1]Dolores Prieto, [1]Sara Benedito & [2]Niels C. Berg Nyborg

Department of Pharmacology, University of Aarhus, DK-8000 Aarhus C, Denmark

1 The effects of rat- and human-CGRP and capsaicin were studied in isolated rings of rat proximal epicardial (PC) and distal intramyocardial (DC) coronary arteries.

2 The relaxing effect of rat-CGRP was dependent on the level of vessel tone induced by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in PC but not in DC arteries. Submaximally contracted DC and PC arteries were more sensitive to rat- than human-CGRP. There was no difference in sensitivity to rat- and human-CGRP between PC and DC arteries.

3 Substance P elicited a small relaxation only in 4 of the 6 PC arteries tested. PC and DC arteries were concentration-dependently relaxed by capsaicin. The relaxation was partly inhibited by ruthenium red, thus suggesting that capsaicin causes specific release of CGRP from sensory nerve endings in rat coronary arteries.

4 The relaxant effect of rat-CGRP was antagonized by endothelium removal and indomethacin but not methylene blue in endothelium-intact PC arteries. The relaxation in DC arteries was not affected by any of these treatments, indicating a heterogeneous involvement of the endothelium in CGRP-mediated coronary vasodilatation and the release of a cyclo-oxygenase product in PC arteries in rats.

5 Glibenclamide had no inhibitory effect on the CGRP-induced relaxation of PC and DC arteries, thus excluding the involvement of glibenclamide-sensitive K^+-channels in the mechanism of action of CGRP in rat coronary arteries.

Keywords: Calcitonin gene-related peptide; endothelium; indomethacin; methylene blue; capsaicin; glibenclamide; coronary artery of rat

Introduction

The vasodilator effect of calcitonin gene-related peptide (CGRP) (Amara *et al.*, 1982) has been extensively studied in the coronary circulation of several mammals (Holman *et al.*, 1986; Franco-Cereceda *et al.*, 1987; Greenberg *et al.*, 1987; Shoji *et al.*, 1987; Franco-Cereceda, 1988; Bény *et al.*, 1989) including man (McEwan *et al.*, 1986; Franco-Cereceda & Lundberg, 1987; Thom *et al.*, 1987). CGRP-immunoreactivity is present in high concentration in cardiac tissue and in nerve fibres associated with coronary blood vessels (Mulderry *et al.*, 1985). CGRP may be released upon activation of cardiac sensory nerves by capsaicin and during ischaemia (Franco-Cereceda *et al.*, 1989).

The relaxation induced by CGRP has been reported to be endothelium-dependent in rat aorta (Brain *et al.*, 1985; Grace *et al.*, 1987) and some human vessels (Thom *et al.*, 1987) and also endothelium-independent in cat cerebral arteries (Edvinsson *et al.*, 1985), human skeletal muscle and pig splenic arteries (Pernow, 1989), rabbit mesenteric resistance arteries (Nelson *et al.*, 1990) and bovine (Greenberg *et al.*, 1987) and porcine coronary arteries (Franco-Cereceda *et al.*, 1987; Shoji *et al.*, 1987). The CGRP-induced relaxation of rabbit mesenteric resistance arteries may possibly be mediated through opening of K^+-channels which are blocked by glibenclamide (Nelson *et al.*, 1990).

The present study examines the relaxant effects of rat- and human-CGRP in rat proximal epicardial (PC) and distal intramyocardial (DC) coronary arteries in relation to the level of vascular tone, the presence of endothelium and involvement of secondary mediators.

Methods

Segments (1–2 mm long) of the proximal (PC) and distal (DC) part of the left anterior descending coronary artery in hearts obtained from 3 month old male Wistar rats were dissected as previously described (Nyborg, 1990) and mounted as ring preparations on two 40 μm diameter stainless steel wires on a double myograph (Mulvany & Nyborg, 1980) which allowed direct determination of the isometric wall tension while the internal circumference of the vessels was controlled.

After mounting, the arteries were equilibrated in physiological saline solution (PSS) at 37°C, pH 7.4 for 30 min. The vessels were then stretched to their optimal lumen diameter $l_0 = 0.9 \times l_{100}$, where l_{100} is an estimate of the diameter the vessels would have if subjected a passive transmural pressure of 13.3 kPa (100 mmHg), for active tension development (Nyborg *et al.*, 1987). The arteries were then repetitively stimulated with K-PSS (equivalent to PSS except that NaCl is exchanged with KCl on an equimolar basis) until reproducible responses were recorded. The maximal contractile capacity of the vessels (E_{max}) was estimated by activating the vessels with K-PSS to which prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and 5-hydroxytryptamine, both 10^{-5} M, were added.

In the first series of experiments, cumulative concentration-response curves for rat-CGRP and human-CGRP, were constructed by using vessels submaximally (50–60% of E_{max}) or maximally (>70% of E_{max}) contracted with $PGF_{2\alpha}$ obtained by a final concentration of $PGF_{2\alpha}$ in the tissue bath between 10^{-7} M and 10^{-5} M. Because of the development of tachyphylaxis to rat- and human-CGRP, arterial segments were used only once when challenged with these agonists.

In a second series of experiments the role of the endothelium was determined by using two consecutive arterial segments; one was used as a control and the other segment had its endothelium removed as previously described (Nyborg, 1990). Successful removal of the endothelium was tested by the absence of relaxation to acetylcholine (10^{-5} M).

[1] Present address: Department of Physiology, Faculty of Veterinary Sciences, Universidad Complutense, 28040-Madrid, Spain.
[2] Author for correspondence.

Finally, the effect of rat-CGRP was tested after incubating the arteries with either indomethacin or methylene blue, both at 3×10^{-6} M, or 10^{-6} M glibenclamide, a blocker of the ATP-sensitive K^+-channels in arterial smooth muscle (Standen *et al.*, 1989). In this series of experiments, and also in those where the endothelium was removed, proximal coronary arteries were submaximally contracted with $PGF_{2\alpha}$ (10^{-7}–10^{-6} M).

PSS had the following composition (in mM): NaCl 119, NaH_2CO_3 25, KCl 4.7, $CaCl_2$ 1.5, KH_2PO_4 1.18, $MgSO_4$ 1.17, ethylene diamine tetraacetic acid (EDTA) 0.026 and glucose 11. K-PSS was similar to PSS escept that NaCl was exchanged for KCl on an equimolar basis.

Drugs used were rat- and human calcitonin gene-related peptide (CGRP), capsaicin, ruthenium red, 5-hydroxytryptamine creatinine sulphate complex, acetylcholine HCl (all Sigma, U.S.A.), prostaglandin $F_{2\alpha}$ (Dinoprost, UpJohn, Belgium), methylene blue (DAK, Denmark) and indomethacin (Merck, F.R.G.). Capsaicin was dissolved in 96% ethanol while other drugs were dissolved in distilled water. Stock solutions were stored at −20°C and dilutions were made just before experimentation.

Vessel responses are expressed as either active tension (Newton per meter of vessel wall, $N\,m^{-1}$) or as a percentage of the response induced by $PGF_{2\alpha}$. Sensitivities to the agonists are expressed as pD_2-values, where $pD_2 = -\log(EC_{50}[M])$, and $EC_{50}[M]$ is the agonist-concentration required to produce half-maximal relaxation. $EC_{50}[M]$s were estimated by fitting plots of responses (R) vs. concentration (A[M]) on a log-scale to the equation $R = R_{max} \times A[M]^n/(A[M]^n + EC_{50}[M]^n)$, where R_{max} is the maximal tissue response to agonist, using the commercial available GraphPAD programme. Results are given as mean ± s.e.mean, (number of vessels).

Differences between mean values were analyzed by Student's *t* test for paired or unpaired observation where appropriate. The level of significance was for both tests set at *P* values less than 0.05.

Results

Effect of initial vessel contraction on relaxation of PC and DC arteries

Concentration-response relations for rat- and human-CGRP in sub- and maximally $PGF_{2\alpha}$-contracted segments of PC and DC arteries with intact endothelium are shown in Figure 1. The maximal relaxation induced by rat-CGRP (3×10^{-8} M) was significantly ($P < 0.001$) greater in PC arteries contracted submaximally (50–60% of E_{max}) than in arteries contracted to a higher level (>70% of E_{max}) by $PGF_{2\alpha}$. Submaximally contracted PC arteries were more sensitive ($P < 0.05$) to rat- than human-CGRP, pD_2 values being 8.48 ± 0.03 ($n = 7$) and 8.12 ± 0.08 ($n = 9$).

The relaxation induced by rat-CGRP in DC arteries was in contrast to PC arteries independent of the magnitude of the preconstricting tone. Maximal relaxations were 85 ± 6% ($n = 4$) and 83 ± 4% ($n = 7$) and pD_2 values were 8.49 ± 0.09 ($n = 4$) and 8.53 ± 0.11 ($n = 7$) in DC arteries contracted with $PGF_{2\alpha}$ to 51 ± 5% ($n = 4$) and 81 ± 2% ($n = 7$) of E_{max}, respectively. DC arteries were also more sensitive ($P < 0.05$) to rat- than human-CGRP, pD_2 values being 8.52 ± 0.07 ($n = 11$) and 8.19 ± 0.10 ($n = 8$), respectively. PD_2 values in PC and DC arteries were not significantly different.

Effect of substance P and capsaicin

Substance P (SP) (10^{-10}–10^{-7} M) did not elicit a significant relaxation in rings of DC arteries with intact endothelium. However, SP induced a small relaxation, 10 ± 3% ($n = 4$) in 4 of 6 PC arteries contracted with 10^{-6} M $PGF_{2\alpha}$.

Capsaicin (10^{-6}–3×10^{-5} M) induced a concentration-dependent relaxation of PC and DC arteries (Figure 2). The maximal responses to capsiacin, 3×10^{-5} M, were 63 ± 7% ($n = 6$) and 66 ± 7% ($n = 6$) in PC and DC arteries, respectively. The capsaicin-induced relaxations were antagonized by 10^{-5} M ruthenium red (Figure 2). Higher concentration than 10^{-5} M of ruthenium red was not tested systematically because it caused a decrease in the $PGF_{2\alpha}$-induced contraction.

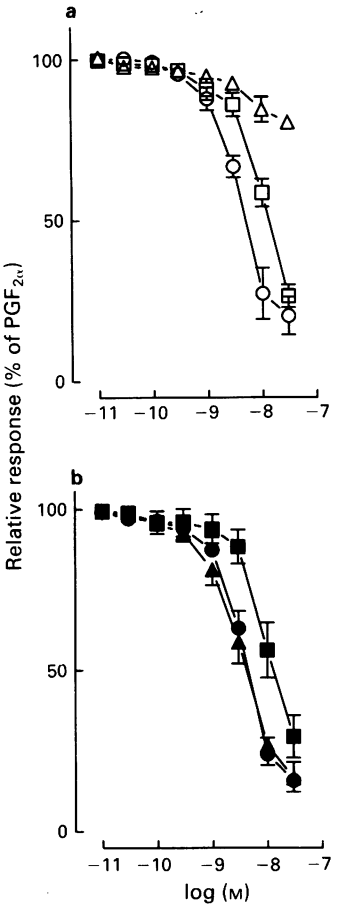

Figure 1 Relaxant effects of human-calcitonin gene-related peptide (CGRP) (squares) and rat-CGRP in proximal epicardial (a) and distal intra myocardial, (b) arteries submaximally (50–60% of E_{max}) (circles) and maximally (>70% of E_{max}) (triangles) contracted with prostaglandin $F_{2\alpha}$. Points represent mean values of 4–11 vessels. Vertical bars indicate s.e.mean where this value exceeds the size of symbol.

Effect of endothelium-denudation

Acetylcholine (ACh) (10^{-5} M) relaxed endothelium-intact and -denuded PC arteries 82 ± 5% ($n = 7$) and 8 ± 4% ($n = 6$) respectively. Removal of endothelium was accompanied by an increase in the spontaneous myogenic tone but the total active tension (spontaneous tone plus that induced by $PGF_{2\alpha}$) was always kept in a range between 50–60% of E_{max}, mean active tension being 52 ± 3% ($n = 6$) of E_{max}, in order to avoid bias comparing the relaxing effect of rat-CGRP in endothelium-intact and denuded arteries. The maximal relaxation elicited by rat-CGRP was significantly ($P < 0.001$) reduced in endothelium-denuded PC arteries compared to control vessels: 18 ± 5% ($n = 6$) vs. 80 ± 6% ($n = 7$), respectively (Figure 3a).

The 10^{-5} M ACh-induced relaxation of endothelium-intact and -denuded DC arteries was 83 ± 7% ($n = 6$) and 5 ± 3% ($n = 6$), respectively. The concentration-response relations for rat-CGRP were unaffected by the absence of endothelium in DC arteries (Figure 3b), pD_2 values being 8.42 ± 0.12 ($n = 6$) and 8.47 ± 0.09 ($n = 6$) and maximal relaxations 77 ± 4% ($n = 6$) and 74 ± 9% ($n = 6$) in endothelium-intact and -denuded arteries, respectively.

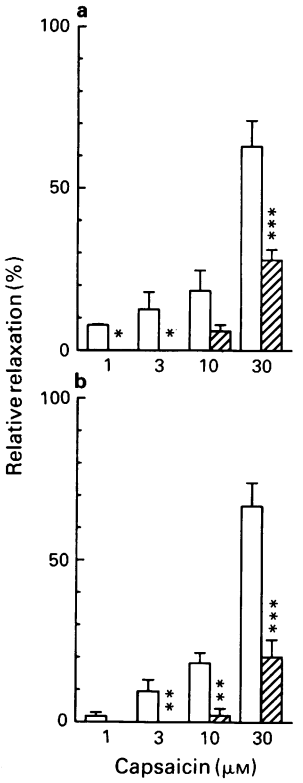

Figure 2 Columns showing the inhibitory action of 10^{-5} M ruthenium red (hatched columns) on the relaxant effect of capsaicin (open columns) in six rat proximal epicardial (a) and distal intramyocardial (b) arteries. Responses are expressed as relative relaxation of arteries contracted with 10^{-6} M prostaglandin $F_{2\alpha}$. Vertical lines show s.e.mean. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

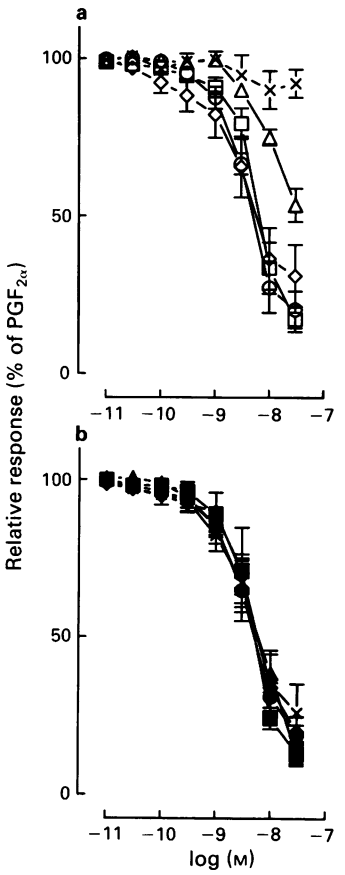

Figure 3 Rat-calcitonin gene-related peptide (CGRP) concentration-response relations of proximal epicardial (a) and distal intramyocardial (b) arteries in control condition (circles), and in presence of 3×10^{-6} M indomethacin (triangles), 3×10^{-6} M methylene blue (squares), 10^{-6} M glibenclamide (diamonds) and after removal of endothelium (crosses). Points show mean values of 3–15 observations. Vertical bars indicate s.e.mean where this value exceeds the size of symbol.

Effect of indomethacin, methylene blue and glibenclamide

Incubation of endothelium-intact PC arteries with 3×10^{-6} M indomethacin partially blocked the relaxation induced by rat-CGRP; maximal relaxations were $80 \pm 6\%$ ($n = 7$) and $52 \pm 3\%$ ($n = 7$) ($P < 0.001$) in the absence and presence of indomethacin, respectively (Figure 3a). However, the concentration-response curve for rat-CGRP in DC arteries was not affected by indomethacin (Figure 3b).

Methylene blue (3×10^{-6} M) and glibenclamide (10^{-6} M) had no effect on the relaxation induced by rat-CGRP in either PC or DC arteries (Figure 3).

Discussion

The present study demonstrates that CGRP induces potent relaxation of rat coronary arteries via endothelium-dependent and -independent mechanisms in the proximal and distal part of the left coronary artery, respectively, in rats. The present results are thus in agreement with earlier studies reporting that both rat- and human-CGRP evoke dose-dependent decreases in perfusion pressure in rat hearts (Al-Kazwini *et al.*, 1985; Holman *et al.*, 1986) and in agreement with *in vitro* studies on bovine (Greenberg *et al.*, 1987), porcine (Franco-Cereceda *et al.*, 1987; Shoji *et al.*, 1987; Bény *et al.*, 1989) and human (Franco-Cereceda & Lundberg, 1987) coronary arteries.

The magnitude of the CGRP-induced relaxation in PC arteries was, in contrast to DC arteries, highly dependent on the level of tone induced by $PGF_{2\alpha}$. Previous studies on mesenteric (Al-Kazwini *et al.*, 1986) and coronary (Shoji *et al.*, 1987) vascular smooth muscle have shown that the extent of relaxation elicited by CGRP is dependent on the type of vasoconstrictor used; thus CGRP was less effective when the arteries were contracted by high K^+-concentrations than by $PGF_{2\alpha}$. This suggests that the smooth muscle membrane potential may play an important role in determining the magnitude of relaxation induced by CGRP. However, the relaxation induced by CGRP was independent of the level of contraction in DC arteries suggesting either that the membrane potential is independent of the level of $PGF_{2\alpha}$-induced vessel tone or that the mechanism involved in the CGRP-mediated relaxation is independent of smooth muscle membrane potential as in pig distal coronary arteries (Bény *et al.*, 1989).

CGRP may be released from sensory nerve endings by myocardial ischaemia as well as by capsaicin (Franco-Cereceda *et al.*, 1989). Capsaicin may also induce release of other neuropeptides (Buck & Burks, 1986). Substance P had no effect in DC arteries at all and induced only a small and sporadic relaxation in PC arteries. Neuropeptide Y has only a constrictor effect in rat coronary arteries (Prieto *et al.*, 1990) and bradykinin is without effect (results not shown). Ruthenium red, which probably inhibits capsaicin-induced release of CGRP by a calcium-antagonistic action on sensory nerve endings (Maggi *et al.*, 1988), antagonized the relaxant effect of capsaicin in PC and DC arteries suggesting that at least a large part of the relaxation of these arteries is mediated primarily by release of CGRP. The density of CGRP-containing nerves or at least the amount of CGRP released in PC and DC arteries appears to be equal since the concentration-response relations to exogenous CGRP and capsaicin, respectively, were similar in these arteries. The concentration of capsaicin used to elicit mechanical responses in rat coronary

arteries is rather high compared to those necessary for responses in guinea-pig urinary bladder (Maggi *et al.*, 1989), bronchus and left atria (Maggi *et al.*, 1988).

Although the CGRP-receptor discriminated between human- and rat-CGRP there was no difference in effect of CGRP between PC (submaximally contracted) and DC arteries, as previously shown in the proximal and distal part of the guinea-pig mesenteric (Edvinsson *et al.*, 1989) arteries. However, there are important differences concerning the mechanisms underlying the CGRP-induced relaxation between large and small arteries in the rat coronary vascular bed. CGRP induced an endothelium-independent direct relaxation of DC vascular smooth muscle, which is consistent with that reported for pig (Franco-Cereda *et al.*, 1987; Shoji *et al.*, 1987) and bovine coronary arteries (Greenberg et al., 1987), and also for cerebral (Edvinsson *et al.*, 1985), splenic and skeletal muscle arteries (Pernow, 1989). In contrast, the relaxation induced by CGRP in PC arteries was almost totally dependent on the endothelium, which is partly consistent with that reported for rat aorta (Brain *et al.*, 1985; Kubota *et al.*, 1985; Grace *et al.*, 1987), where the response to CGRP was completely abolished after removal of the endothelium. It seems, therefore, that the action of CGRP in rat PC arteries may be a transition between that in aorta and small intramyocardial arteries. The transition may be attributed to the differential morphogenesis of the proximal and distal part of the coronary circulation which is derived from aortic and myocardial mesenchyme, respectively (Conte & Pellegrini, 1984).

The endothelial factor released by CGRP in PC arteries appears to be a cyclo-oxygenase product since indomethacin but not methylene blue had an inhibitory effect on the relaxation. The concentration of indomethacin used (3×10^{-6} M) did not antagonize the CGRP-induced relaxation to the same extent as did endothelium-denudation. This is probably due to a partial inhibition of the cyclo-oxygenase by indomethacin. We could not test this since unspecific relaxation of PC arteries occurs at higher concentrations of indomethacin (Nyborg & Mikkelsen, 1990).

There is much evidence in the literature that CGRP mediates its action through adenosine 3',5'-cyclic monophosphate (cyclic AMP) generation in endothelial and vascular smooth muscle cells (Edvinsson *et al.*, 1985; Kubota *et al.*, 1985; Grace *et al.*, 1987; Hirta *et al.*, 1988; Crossmann *et al.*, 1990). In addition, some of these studies demonstrated also that CGRP has no effect on cyclic GMP generation, which in part supports the lack of effect of methylene blue on the CGRP-induced relaxation of PC and DC arteries in the present study. However, Nelson *et al.* (1990) recently demonstrated that CGRP hyperpolarizes rabbit mesenteric resistance artery smooth muscle by opening K^+-channels, and that the hyperpolarization as well as the CGRP-induced relaxation of these arteries was reversed by glibenclamide, a blocker of ATP-sensitive K^+-channels (Standen, 1989). The present experiments do not confirm the involvement of glibenclamide-sensitive K^+-channels in the CGRP-induced relaxation of rat coronary arteries. However, this does not exclude the involvement of other types of K^+-channels in the direct CGRP-mediated smooth muscle relaxation in rat coronary arteries; this requires further studies on second messengers and on the effect of other K^+-channel inhibitors.

D.P. and S.B. were supported by the Spanish Ministry of Education and Science. This study was supported by the Danish Medical Research Council, Grant No. 12-7744 and 12-8717.

References

AL-KAZWINI, S.J., CRAIG, R.K., HOLMAN, J.J. & MARSHALL, I. (1985). Human and rat calcitonin gene related peptides (CGRP) are vasodilators in coronary and mesenteric vasculature. *Br. J. Pharmacol.*, **86**, 544P.

AL-KAZWINI, S.J., CRAIG, R.K., HOLMAN, J.J. & MARSHALL, I. (1986). Different potencies of calcitonin gene-related peptides (CGRP) on mesenteric vasculature constricted by noradrenaline or potassium. *Br. J. Pharmacol.*, **88**, 439P.

AMARA, S.G., JONAS, V., ROSENFELD, M.G., ONG, E.S. & EVANS, R.M. (1982). Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. *Nature*, **298**, 240–244.

BÉNY, J.L., BRUNET, P.C. & HUGGEL, H. (1989). Effects of substance P, calcitonin gene-related peptide and capsaicin on tension and membrane potential of pig coronary artery *in vitro*. *Regul. Pept.*, **25**, 25–36.

BUCK, S.H. & BURKS, T.F. (1986). The neuropharmacology of capsaicin: Review of some recent observations. *Pharmacol. Rev.*, **38**, 179–226.

BRAIN, S.D., WILLIAMS, T.J., TIPPINS, J.R., MORRIS, H.R. & MACINTYRE, I. (1985). Calcitonin gene-related peptide is a potent vasodilator. *Nature*, **313**, 54–56.

CONTE, G. & PELEGRINI, A. (1984). On the development of the coronary arteries in human embryos, stage 14–19. *Anat. Embryol.*, **169**, 209–218.

CROSSMAN, D.C., DASHWOOD, M.R., BRAIN, S.D., McEWAN, J. & PEARSON, J.D. (1990). Action of calcitonin gene-related peptide upon bovine vascular endothelial and smooth muscle cells grown in isolation and co-culture. *Br. J. Pharmacol.*, **99**, 71–76.

EDVINSSON, L., FREDHOLM, B.B., HAMEL, E., JANSEN, I. & VERRECCHIA, C. (1985). Perivascular peptides relax arteries concomitant with stimulation of cyclic AMP accumulation or release of an endothelium derived relaxing factor. *Neurosci. Lett.*, **58**, 213–220.

EDVINSSON, L., GULBENKIAN, S., JANSEN, I., WHARTON, J., CERVANTES, C. & POLAK, J.M. (1989). Comparison of peptidergic mechanisms in different parts of the guinea-pig superior mesenteric artery: immunocytochemistry at the light and ultrastructural levels and responses *in vitro* of large and small arteries. *J. Auton. Nerv. Syst.*, **28**, 141–154.

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.*, Suppl. **569**, 1–63.

FRANCO-CERECEDA, A. & LUNDBERG, J.M. (1987). Potent effects of neuropeptide Y and calcitonin gene-related peptide on human coronary vascular tone "*in vitro*". *Acta Physiol. Scand.*, **131**, 159–160.

FRANCO-CERECEDA, A., RUDEHILL, A. & LUNDBERG, J.M. (1987). Calcitonin gene-related peptide but not substance P mimics capsaicin induced coronary vasodilation in the pig. *Eur. J. Pharmacol.*, **142**, 235–243.

FRANCO-CERECEDA, A., SARIA, A. & LUNDBERG, J.M. (1989). Differential release of calcitonin gene-related peptide and neuropeptide Y from the isolated heart by capsaicin, ischemia, nicotine, bradykinin and ouabain. *Acta Physiol. Scand.*, **135**, 173–187.

GRACE, G.C., DUSTING, G.J., KEMP, B.E. & MARTIN, T.J. (1987). Endothelium and the vasodilator action of rat calcitonin gene-related peptide. *Br. J. Pharmacol.*, **91**, 729–733.

GREENBERG, B., RHODEN, K. & BARNES, P. (1987). Calcitonin gene-related peptide (CGRP) is a potent non-endothelium-dependent inhibitor of coronary vasomotor tone. *Br. J. Pharmacol.*, **92**, 789–794.

HIRATA, Y., TAKAGI, Y., TAKATA, S., FUKUDA, Y., YOSHIMI, H. & FUJITA, T. (1988). Calcitonin gene-related peptide receptor in cultured vascular smooth muscle and endothelial cells. *Biochem. Biophys. Res. Commun.*, **151**, 1113–1121.

HOLMAN, J.J., CRAIG, R.G. & MARSHALL, I. (1986). Human α- and β-CGRP and rat α-CGRP are coronary vasodilators in the rat. *Peptides*, **7**, 231–235.

KUBOTA, M., MOSELEY, J.M., BUTERA, L., MACDONALD, P.S. & MARTIN, T.J. (1985). Calcitonin gene-related peptide stimulates cyclic AMP formation in rat aortic smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **132**, 88–94.

MAGGI, C.A., SANTOCIOLI, P., GEPPETTI, P., PARLANI, M., ASTOLFI, M., PRA DELLES, P., PATACCHINI, R. & MELI, A. (1988). The antagonism induced by Ruthenium Red of the actions of capsaicin on the peripheral terminals of sensory neurons: further studies. *Eur. J. Pharmacol.*, **154**, 1–10.

MAGGI, C.A., PATACCHINI, R., SANTOCIOLI, P., GIULIANI, S., BIANCO, E.D., GEPPETTI, P. & MELI, A. (1989). The 'efferent' function of capsiacin-sensitive nerves: Ruthenium Red discriminates between different mechanisms of activation. *Eur. J. Pharmacol.*, **170**, 167–177.

McEWAN, J., LARKIN, S., DAVIES, G., CHIERCHIA, S., BROWN, M., STEVENSON, J., MCINTYRE, I. & MASERI, A. (1986). Calcitonin gene-related peptide: a potent dilator of epicardial coronary arteries. *Circulation*, **74**, 1243–1247.

MULDERRY, P.K., GHATEI, M.A., RODRIGO, J., ALLEN, J.M., ROSENDFELD, POLAK, J.M. & BLOOM, S.R. (1985). Calcitonin gene-related peptide in cardiovascular tisues of the rat. *Neurosci.*, **14**, 947–954.

MULVANY, M.J. & NYBORG, N. (1980). An increased calcium sensitivity of mesenteric resistance vessels in young and adult spontaneously hypertensive rats. *Br. J. Pharmacol.*, **71**, 585–596.

NELSON, M.T., HUANG, Y., BRAYDEN, J.E., HESCHELER, J. & STANDEN, N.B. (1990). Arteriolar dilations in response to calcitonin gene-related peptide involve citivation of K^+-channels. *Nature*, **344**, 770–773.

NYBORG, N.C.B. (1990). Action of noradrenaline on isolated rat proximal and distal coronary arteries: Selective release of endothelium-derived relaxing factor in proximal arteries. *Br. J. Pharmacol.*, **100**, 552–556.

NYBORG, N.C.B., BAANDRUP, U., MIKKELSEN, E.O. & MULVANY, M.J. (1987). Active, passive and myogenic characteristics of isolated rat intramural coronary resistance arteries. *Pflügers Arch./Eur. J. Physiol.*, **410**, 664–670.

NYBORG, N.C.B. & MIKKELSEN, E.O. (1990). 5-Hydroxytryptamine does not induce release of endothelium-derived relaxing factor in rat coronary arteries. *Eur. J. Pharmacol.*, **186**, 295–300.

PERNOW, J. (1989). Actions of constrictor (NPY and endothelin) and dilator (substance P, CGRP and VIP) peptides on pig splenic and human skeletal muscle arteries: involvement of the endothelium. *Br. J. Pharmacol.*, **97**, 983–989.

PRIETO, D., BENEDITO, S., SIMONSEN, U. & NYBORG, N.C.B. (1990). Neuropeptide Y contracts and potentiates noradrenaline and 5-hydroxytryptamine induced vasoconstriction of rat isolated coronary arteries. *Eur. J. Pharmacol.* **183**, 2219–2220.

SHOJI, T., ISHIHARA, H., ISHIKAWA, T., SAITO, A. & GOTO, K. (1987). Vasodilating effects of human and rat calcitonin gene-related peptides in isolated porcine coronary arteries. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **336**, 438–444.

STANDEN, N.B., QUALE, J.M., DAVIES, N.W., BRAYDEN, J.E., HUANG, Y. & NELSON, M.T. (1989). Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science*, **245**, 177–180.

THOM, S.M.J., HUGHES, A.D., GOLDBERG, P., MARTIN, G., SCHACHTER, M. & SEVER, P.S. (1987). The actions of calcitonin gene-related peptide and vasoactive intestinal polypeptide as vasodilators in man *in vivo* and *in vitro*. *Br. J. Clin. Pharmacol.*, **24**, 129–134.

(Received October 30, 1990
Revised February 4, 1991
Accepted February 27, 1991)