ELSEVIER

European Journal of Pharmacology 351 (1998) 53–59

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Caliber dependent calcitonin gene-related peptide-induced relaxation in rat coronary arteries: effect of K^+ on the tachyphylaxis

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Received 8 January 1998; accepted 7 April 1998

Abstract

The influence of vessel caliber on rat calcitonin gene-related peptide (rat-αCGRP)-induced responses and the reproducibility of rat-αCGRP concentration–response curves were investigated in the left intramural coronary artery of Sprague–Dawley rats. Rat-αCGRP (10^{-11}–10^{-7} M) induced concentration-dependent relaxations with a pD_2-value equal to 8.43 ± 0.05 ($n = 44$) and maximal relaxation equal to $52 \pm 3\%$ ($n = 44$). Both the maximal relaxation and the sensitivity to rat-αCGRP were significantly and inversely correlated with vessel lumen diameter. The coronary arteries developed tachyphylaxis in response to rat-αCGRP, which was concentration dependently decreased by activating the vessels twice with a buffer containing 36 or 125 mM K^+. The rat-αCGRP-curve became fully reproducible after activation of the arteries twice with 125 mM K^+. These results indicate a caliber-related dependency of both the effect of and sensitivity to rat-αCGRP in intramural rat coronary arteries because the arteries become more sensitive and reactive to rat-αCGRP with decreasing caliber. Tachyphylaxis can be avoided by repeated activation with 125 mM K^+.

Keywords: CGRP, (Calcitonin gene-related peptide); Vessel caliber; Tachyphylaxis; K^+; Coronary artery

1. Introduction

Calcitonin gene-related peptide (CGRP) is a 37-amino acid residue peptide generated by alternative splicing of calcitonin gene transcripts (Amara et al., 1982; Rosenfeld et al., 1983). CGRP immunoreactivity is widely distributed in neural tissue and co-localized with substance P in sensory nerves in the body (Franco-Cereceda et al., 1987b). CGRP-containing nerve fibers are found in high density in cardiac tissue and they are particularly abundant in the atrial muscle and the ventricular vasculature, indicating an important function of CGRP in the regulatory processes of coronary blood flow and myocardial contractility (Franco-Cereceda et al., 1987b).

In vitro studies have shown that CGRP causes a positive inotropic action in guinea pig atria and potent vasodilatation in various mammalian coronary arteries (Franco-Cereceda and Lundberg, 1985; McEwan et al., 1986; Franco-Cereceda et al., 1987a; Shoji et al., 1987; Prieto et al., 1991a). CGRP can be released during hypoxia and by low pH levels in the myocardium (Franco-Cereceda and Lundberg, 1992), thus suggesting a vasodilator role during ischemic conditions. Recent functional studies in rat, porcine and human coronary vascular preparations have shown variations in CGRP vasodilator activity and tachyphylaxis, depending on the size and the location of the vessels used (Foulkes et al., 1991; Gray and Marshall, 1991; Ludman et al., 1991; Prieto et al., 1991a). The distribution of CGRP-binding sites and receptor subtypes has also been shown to exhibit species dependent variability as well as regional variation in different vascular beds (Ludman et al., 1991; Knock et al., 1992).

We have previously shown development of tachyphylaxis as well as deviation in CGRP-mediated relaxation between epicardial and intramural rat coronary arteries (Prieto et al., 1991a), but a possible variability in the effect of CGRP within the intramural coronary arteries was not investigated. Since the intramural coronary artery has a general heterogeneous receptor distribution (Nyborg and Mikkelsen, 1988; Nyborg, 1991), we aimed in the present study to further investigate the influence of vessel caliber on rat-αCGRP-induced responses and the reproducibility of rat-αCGRP concentration–response curves in intramural ring segments of the left anterior descending coronary artery from 3-month-old Sprague–Dawley rats. The in-

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0014-2999/98/$19.00

volvement of endothelium in rat-αCGRP-induced relaxation was not investigated in these coronary arteries since we (Prieto et al., 1991a) previously have shown that rat-αCGRP induces an endothelium-independent relaxation in rat intramural coronary arteries.

2. Materials and methods

2.1. Dissection and mounting

Intramural segments (1–2 mm long) of the left anterior descending coronary artery were isolated from the hearts of 3-month-old male Sprague–Dawley rats, as previously described (Nyborg and Mikkelsen, 1985, 1988). The arteries were mounted as rings on two 40-μm stainless steel wires connected to a force transducer and a micrometer, respectively, in the organ bath of a double myograph (Mulvany and Halpern, 1977; Mulvany and Nyborg, 1980). This allowed direct determination of the isometric wall tension while the internal circumference of the vessels was controlled.

2.2. Experimental procedure

After mounting, the arteries were equilibrated at 37°C for 30 min in oxygenated (95% O_2 and 5% CO_2) physiological salt solution (PSS) with the following composition (in mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.18, $MgSO_4 \cdot 7H_2O$ 1.17, $CaCl_2 \cdot 2H_2O$ 21.5, ethylene diamine tetraacetic acid (EDTA) 0.027 and glucose 5.5; pH was adjusted to 7.4. The vessels were then stretched to their optimal lumen diameter $l_1 = 0.9 \times l_{100}$, where l_{100} is an estimate of the diameter the vessel would have under a passive transmural pressure of 13.3 kPa (N m^{-2}) (100 mm Hg) in order to obtain optimal conditions for active tension development (Nyborg et al., 1987). Each experiment was initiated by contracting the vessels repeatedly with K-PSS (similar to PSS except that NaCl was replaced by KCl on an equimolar basis) until reproducible wall tensions were recorded. The maximal contractile response of the vessels (ΔT_{max}) was then determined by measuring the differences in vessel wall tension (newton per meter of vessel wall, N m^{-1}) when the vessel was maximally contracted with K-PSS to which 10^{-5} M serotonin (5-HT) and 10^{-5} M prostaglandin $F_{2\alpha}$ were added and when maximally relaxed in Ca^{2+}- free PSS (Nyborg, 1991; Prieto et al., 1991a). Ca^{2+}-free PSS was similar to PSS except that $CaCl_2$ was replaced with 0.01 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA). Vessels were accepted only if the maximal active pressure (calculated according to Laplace's law: $\Delta P_{max} = 2 \times \Delta T_{max}/l_1$) exceeded 9 kPa.

Three protocols were used to investigate the development of tachyphylaxis and the rat-αCGRP response in relation to caliber in 10^{-5} M prostaglandin $F_{2\alpha}$-precontracted coronary arteries. In all protocols, rat-αCGRP concentration–response curves were made by precontracting the coronary arteries with 10^{-5} M prostaglandin $F_{2\alpha}$ and then adding rat-αCGRP to the organ bath in a cumulative manner (10^{-11}–10^{-7} M).

In protocol A, time-dependent changes in vessel responsiveness were investigated by dividing the organ bath in two, which enabled parallel experiments to be performed with the two mounted vessels on the myograph. One vessel was used to record two consecutive rat-αCGRP concentration–response curves (10^{-11}–10^{-7} M) with a 15-min wash-out period with PSS between the two curves. The other vessel served as time-control and was kept in normal PSS. It was used only once to make a rat-αCGRP concentration–response curve at the same time as the second concentration–response curve was made with the first vessel.

In protocol B, the vessels were activated twice for 3 min with either 36 mM K^+ or 125 mM K^+ within the 15-min washout period before the second rat-αCGRP concentration–response curve was recorded. The activation of vessels with K^+ was performed in order to investigate if there is a relationship between the concentration of K^+ in the activating solution and the reduction of rat-αCGRP-tachyphylaxis. In the third protocol (protocol C) we used the same procedure as protocol B except that the vessels were activated twice with 10^{-5} M 5-HT in order to investigate the effect of receptor-mediated contractions on the development of tachyphylaxis to rat-αCGRP. The contractions induced by prostaglandin $F_{2\alpha}$ were stable throughout the time needed to make the rat-αCGRP concentration–response curves.

2.3. Drugs

Drugs used were rat-αCGRP, 5-hydroxytryptamine (5-HT) HCl (Sigma, St. Louis, MO, USA) and prostaglandin $F_{2\alpha}$ (Dinoprost®, UpJohn, Belgium). 5-HT and rat-αCGRP were dissolved in distilled water. Stock solutions of 5-HT (10^{-2} M) and rat-αCGRP (10^{-4} M) were stored at -20°C and dilutions were made just before experimentation.

2.4. Data analysis and statistics

Vessel responses are expressed as either active vessel wall tension (N m^{-1}), calculated as the increase in vessel wall force divided by twice the length of the vessel segment, or as a percentage of the response induced by prostaglandin $F_{2\alpha}$.

Sensitivity to rat-αCGRP is expressed in terms of pD_2-values, where $pD_2 = -\log(EC_{50}\ [M])$ and is the concentration of agonist required to produce half-maximum relaxation. EC_{50}[M] was estimated by using iterative nonlinear regression analysis (GraphPAD program GraphPAD corp, San Diego, CA, USA) fitting the data to a sigmoid equation: $R/R_{max} = A[M]^n/(A[M]^n + EC_{50}\ [M]^n)$, where R_{max} is the maximum response developed to the agonist,

A[M] is the concentration of agonist and n is a curve-fitting parameter, the Hill coefficient (Kenakin, 1986). Results are given as means ± S.E.M., (n = number of vessels). Differences between mean values were analyzed by using a two-tailed Student's t-test for paired or unpaired observations where appropriate. One-way analysis of variance (ANOVA) was used to compare the contractions (% of ΔT_{max}) of the first and second rat-αCGRP-curves. The level of significance was for all tests set to P-values less than 0.05.

3. Results

3.1. Effect of time on vessel response to rat-αCGRP

In protocol A, in which the time dependency of the rat-αCGRP-response was investigated, there was no significant difference either in maximal rat-αCGRP-induced relaxation or in sensitivity to rat-αCGRP between the first curves (Fig. 1B). The maximal relaxation obtained for the first curves was 58 ± 4% (n = 10) vs. 57 ± 6% (n = 10) and the corresponding sensitivity was 8.50 ± 0.10 (n = 10) vs. 8.35 ± 0.08 (n = 10) for the twice-exposed vessels and the time-control vessels, respectively. However, the maximal rat-αCGRP-induced relaxation in the twice-exposed vessels was significantly ($P < 0.05$) reduced 44 ± 4% (n = 10) in the second rat-αCGRP-curve (Fig. 1A). Mean lumen diameter (l_1) of the coronary arteries was 208 ± 10 μm (n = 10) for twice-exposed vessels and 209 ± 9 μm (n = 10) for the time-control vessels, respectively.

3.2. Effect of K^+- and 5-HT-induced contraction on the reproducibility of rat-αCGRP-induced responses

When the coronary arteries were washed twice with PSS containing 5.9 mM K^+, they developed a pronounced tachyphylaxis to rat-αCGRP, as indicated in Fig. 2A. The maximal response to rat-αCGRP was significantly ($P <$ 0.01) reduced 44 ± 4% (n = 16) in the second curve, the maximal relaxation being 48 ± 5% (n = 16) and 27 ± 4% (n = 16) for the first and second curves, respectively. The sensitivity of the vessel to rat-αCGRP was not altered, the pD_2-value being 8.32 ± 0.10 (n = 16) and 8.20 ± 0.07 (n = 14) for the first and second curves, respectively. Mean lumen diameter (l_1) of the coronary arteries was 247 ± 18 μm (n = 16).

When the vessels were activated twice with 36 mM K^+ between the two rat-αCGRP dose–response curves, there was still a significant ($P < 0.05$) 22 ± 7% (n = 6) reduction in the maximal rat-αCGRP-induced relaxation (Fig. 2B). Maximal relaxation was 54 ± 11% (n = 6) and 40 ± 8% (n = 6) for the first and second curves, respectively. Again, the vessel sensitivity to rat-αCGRP was not significantly altered, the pD_2 value being 8.50 ± 0.10 (n = 6) and 8.32 ± 0.07 (n = 6) for the first and second curves, respectively. Mean lumen diameter (l_1) of the coronary arteries was 236 ± 20 μm (n = 6).

When 125 mM K^+ was used in the activating solution, there was no significant reduction in the maximal rat-αCGRP-induced relaxation between the first and the second curves. Maximal relaxation was 53 ± 9% (n = 6) for the first curve and 50 ± 9% (n = 6) for the second curve (Fig. 2C). There was no significant difference in sensitivity to rat-αCGRP between the first and the second curves, pD_2 value being 8.47 ± 0.15 (n = 6) and 8.32 ± 0.15 (n = 6) for the first and second curve, respectively. Mean lumen diameter (l_1) of coronary arteries was 249 ± 25 μm (n = 6).

When the data from these three experiments were combined, an inverse linear relationship ($r = 0.74$; $P = 0.0001$, n = 28) was found between the concentration of K^+ in the activating solution and the reduction in maximal rat-αCGRP-induced relaxation (Fig. 3).

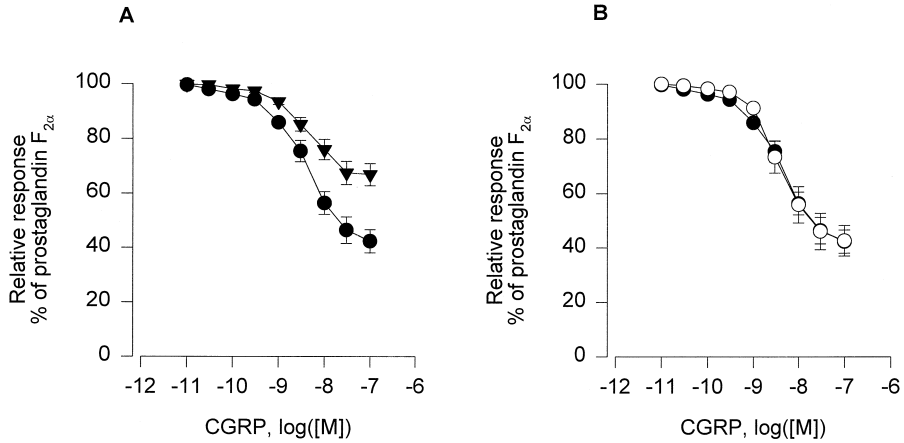

Fig. 1. The effect of time on rat-αCGRP concentration–response curve (10^{-11}–10^{-7} M) recorded in 10^{-5} M prostaglandin $F_{2\alpha}$-precontracted coronary arteries from Sprague–Dawley rats using protocol A. Closed symbols are used for twice-exposed vessels (1st Curve, ●; 2nd Curve, ▼) and open circle is used for time-control vessels. Points represent mean values for 10 vessels and vertical bars indicate ± S.E.M. where this value exceeds the size of symbol. Responses are given as percentages of the initial response to prostaglandin $F_{2\alpha}$ (10^{-5} M) just before the vessels were challenged with rat-αCGRP.

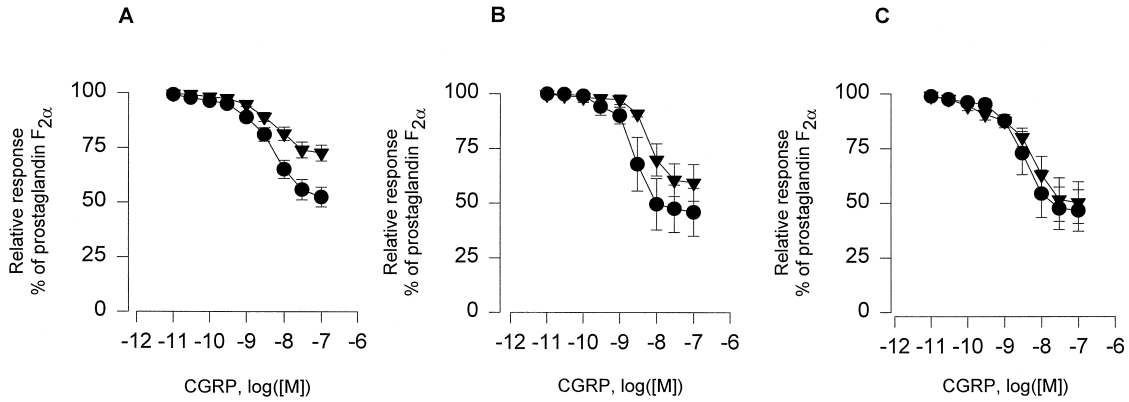

Fig. 2. The effect of different concentrations of extracellular K^+ on the reproducibility of rat-αCGRP concentration–response curves (10^{-11}–10^{-7} M) recorded in 10^{-5} M prostaglandin $F_{2\alpha}$-precontracted coronary arteries from Sprague–Dawley rats using protocol B. Closed circles represent the first curves and closed triangles represent the second curves. (A) PSS containing 5.9 mM K^+ ($n = 16$), (B) K-PSS containing 36 mM K^+ ($n = 6$) and (C) K-PSS containing 125 mM K^+ ($n = 6$). Points represent mean values and vertical bars indicate ±S.E.M. where this value exceeds the size of symbol. Responses are given as percentages of the initial response to prostaglandin $F_{2\alpha}$ (10^{-5} M) just before the vessels were challenged with rat-αCGRP.

In protocol C, in which 10^{-5} M 5-HT was used as intermediary activator, the maximal rat-αCGRP-induced relaxation was significantly ($P < 0.01$) reduced 57 ± 4% ($n = 6$), from 55 ± 6% ($n = 6$) in the first curve to 24 ± 4% ($n = 6$) in the second curve (Fig. 4). The vessel sensitivity to rat-αCGRP was not significantly altered, the pD_2-value being 8.75 ± 0.12 ($n = 6$) and 8.50 ± 0.10 ($n = 6$) for the first and second curves, respectively. Mean lumen diameter (l_1) of coronary arteries was 202 ± 15 μm ($n = 6$).

The contractions induced by 36 mM K^+, 125 mM K^+ and 10^{-5} M 5-HT as percentage of ΔT_{max} were 36 ± 4% ($n = 6$), 88 ± 3% ($n = 6$) and 71 ± 6% ($n = 6$), respectively ($P < 0.001$; 36 mM K^+ vs. 125 mM K^+, $P < 0.05$; 125 mM K^+ vs. 5-HT-induced contraction, one-way ANOVA with Bonferorroni correction).

3.3. Relation between vessel caliber and maximal rat-αCGRP-induced relaxation

The compiled average maximal relaxation induced by rat-αCGRP in prostaglandin $F_{2\alpha}$-precontracted arteries was 52 ± 3% ($n = 44$). The sensitivity (pD_2-value) of the ves-

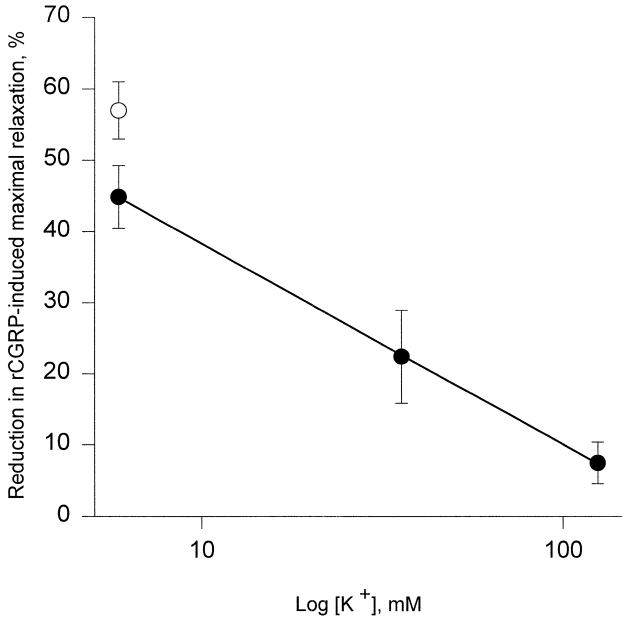

Fig. 3. Relationship between the concentration of K^+ (closed circles) in the activating solution and the reduction in maximal rat-αCGRP-induced relaxation between the first and second curves recorded in 10^{-5} M prostaglandin $F_{2\alpha}$-precontracted coronary arteries from male Sprague–Dawley rats. Open circle shows reduction in maximal rat-αCGRP-relaxation in the vessels contracted with 10^{-5} M 5-HT in the interval between the recording of two rat-αCGRP-curves. Points represent mean values of 6–16 vessels and vertical bars indicate ±S.E.M. where this value exceeds the size of symbol. Reduction in maximal rat-αCGRP-induced relaxation is given as percentage change in maximal relaxation from the first curve to the second curve.

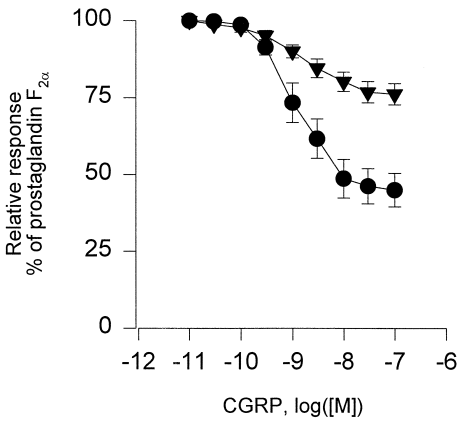

Fig. 4. The effect of contraction elicited by 10^{-5} M 5-HT on the reproducibility of rat-αCGRP concentration–response curves (10^{-11}–10^{-7} M) recorded in prostaglandin $F_{2\alpha}$-precontracted coronary arteries from Sprague–Dawley rats using protocol B. Closed circles represent the first curves and closed triangles represent the second curves. Points represent mean values for 6 vessels and vertical bars indicate ±S.E.M. where this value exceeds the size of symbol. Responses are given as percentages of the initial response to 10^{-5} M prostaglandin $F_{2\alpha}$ just before the vessels were challenged with rat-αCGRP.

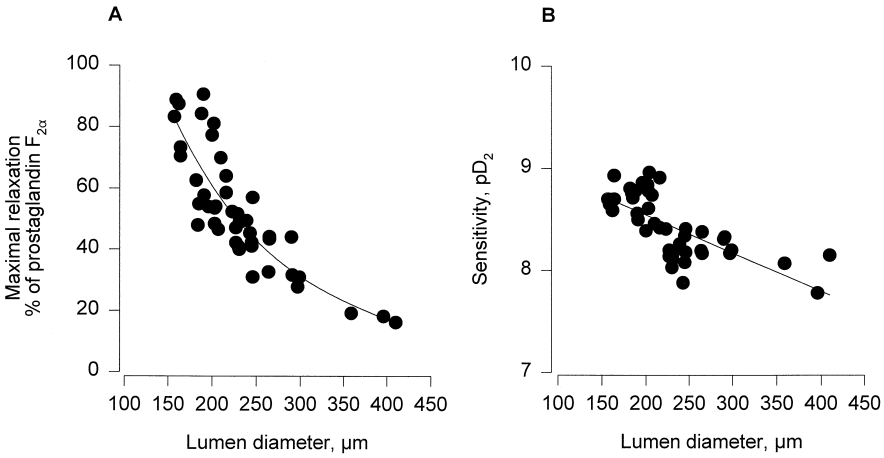

Fig. 5. Relationship between the lumen diameter and maximal rat-αCGRP-induced relaxation (A) and sensitivity to rat-αCGRP (B) in 10^{-5} M prostaglandin $F_{2\alpha}$-precontracted coronary arteries ($n = 44$) from male Sprague–Dawley rats.

sels to rat-αCGRP was 8.43 ± 0.05 ($n = 44$) and the mean lumen diameter (l_1) of arteries was 231 ± 9 μm ($n = 44$). The mean vessel response to prostaglandin $F_{2\alpha}$ was 1.56 ± 0.08 N m^{-1} ($n = 44$), equal to $75 \pm 1\%$ ($n = 44$) of the maximal contractile response of vessels (ΔT_{max}), which was 2.08 ± 0.12 N m^{-1} ($n = 44$).

Further analysis of these data showed that there was a significant correlation between the arterial lumen diameter (relative to the maximal rat-αCGRP-induced relaxation) and the vessel sensitivity to rat-αCGRP, which was best fitted to an exponential decay function ($r = 0.87$; $P < 0.0001$; $n = 44$) and a straight line function ($r = 0.70$; $P < 0.0001$; $n = 44$), respectively (Fig. 5A and B).

The caliber of the coronary arteries had no influence neither on the level of preconstriction tone (% of ΔT_{max}) induced by prostaglandin $F_{2\alpha}$ ($r = 0.02$; $P = 0.89$; $n = 44$) nor on the attenuation of the maximal rat-αCGRP-induced response in vessels kept in PSS in the interval between recording of the two concentration–response curves ($r = 0.01$; $P = 0.97$; $n = 16$), since both regression lines had slopes not significantly different from zero.

4. Discussion

The present study shows that relaxations induced by rat-αCGRP in rat intramural coronary arteries as well as vessel sensitivity to rat-αCGRP depends on the caliber of the vessels, as both response and sensitivity increased with deceasing vessel diameter. Our finding indicates that CGRP exerts its major vasodilator effects in coronary arteries with lumen diameters less than 200 μm, in which the major part of the precapillary flow resistance resides (Chillian, 1988). Vessel caliber has been shown to influence the response of rat coronary arteries to other vasoactive compounds. The contractile response to 5-HT decreases with decreasing vessel caliber (Nyborg and Mikkelsen, 1988) and is possibly related to the 5-HT receptor reserve in these arteries (Nyborg, 1991). Noradrenaline causes contraction in rat epicardial and dilation in intramural small arteries (Nyborg, 1990), and the magnitude of dilatation induced by the K^+ channel opener, levcromakalim, increases with decreasing vessel diameter (Sato et al., 1994). A recent study has demonstrated that the mechanisms of the coronary microvascular responses to pertussis toxin-sensitive G protein activation depends on vessel caliber (Komaru et al., 1997). These studies indicate that receptor distribution, ion channels and second messenger systems may be finely adjusted within the coronary circulation, probably reflecting the physiological demands on the vascular segment.

Tachyphylaxis or attenuation of the vascular response to peptides is often encountered. We have previously described this for neuropeptide Y-induced contractions and rat-αCGRP-induced relaxations in Wistar rats (Prieto et al., 1991a,b). Tachyphylaxis to CGRP also develops in porcine coronary arteries (Gray and Marshall, 1991; Marshall, 1992). Our data showed that the development of tachyphylaxis to rat-αCGRP was pronounced during recording of the second concentration–response curve for rat-αCGRP in the experiments where the vessels were kept in buffer containing a normal concentration of 5.9 mM K^+. The development of tachyphylaxis was not time-dependent or dependent on the caliber of the vessels. However, activation of the arteries twice with 36 or 125 mM K^+ between recording of the two rat-αCGRP concentration–response curves reduced the development of tachyphylaxis and the rat-αCGRP concentration–response curve became fully reproducible after activation of the arteries twice with 125 mM K^+. These findings could indicate that rat-αCGRP mediates its vasodilator action partly via a mechanism that is inactivated during the first exposure of the vessels to rat-αCGRP and which is reactivated by the depolarization of the smooth muscle cell membrane with K^+. This is further supported by our finding that 5-HT was without an opposing effect on the development of tachy-

phylaxis even though the magnitude of contractions induced by this receptor-mediated agonist was close to that induced by 125 mM K^+, thus indicating that it is not the contraction itself that opposes the development of tachyphylaxis.

The tachyphylaxis to CGRP and capsaicin was prevented in guinea pig hearts by using colchicine, an inhibitor of tubulin polymerization and internalization of membrane-bound proteins (Franco-Cereceda, 1990). It could be speculated that membrane depolarization and/or high extracellular K^+ concentrations mimic the action of colchicine, preventing internalization of the CGRP receptors on the vascular smooth muscle cells in the rat coronary arteries.

It is known that CGRP mediates its vasorelaxing effect through different and combined mechanisms of action (Kline and Pang, 1997). The direct action of CGRP on vascular smooth muscle is generally thought to be mediated by an increase in the intracellular cAMP level, which subsequently alters protein kinase A-dependent kinase activity and hence causes relaxation of smooth muscle cell (Crossman et al., 1987; Edwards et al., 1991; Gray and Marshall, 1991). Repeated application of CGRP can therefore bring about desensitization of one or two biochemical pathways involved in vasodilator mechanisms.

Patch-clamp and electrophysiological experiments have shown that CGRP can hyperpolarize rabbit arterial smooth muscle cell membrane by opening K^+_{ATP} channels which are inhibited by glibenclamide (Standen et al., 1989; Nelson et al., 1990; Kitazono et al., 1993). Since the graded depolarization of the smooth muscle cell membrane with 36 or 125 mM K^+ reduced the attenuation of the rat-αCGRP-induced response in the coronary arteries, this procedure could reactivate ion channels in the smooth muscle cell membrane. We have no indication of which type of channels these are, but they are not likely to be K^+_{ATP} channels or other K^+ channels since glibenclamide and high concentrations of tetraethylammonium have no inhibitory effect at all on CGRP-induced relaxations in intramural coronary arteries in Wistar rats (Prieto et al., 1991a).

The sensitivity of Sprague–Dawley coronary arteries to rat-αCGRP in our experiments was similar to that reported in a previous study with coronary arteries from Wistar rats (Prieto et al., 1991a) and close to that of many other vascular preparations (Foulkes et al., 1991; Lei et al., 1994). The inverse linear correlation between response and sensitivity to rat-αCGRP and vessel caliber may indicate that the rat-αCGRP-induced response is limited by the receptor density and/or intermediary intracellular messengers in the arteries used in our study.

In conclusion, rat coronary arteries become more sensitive and reactive to rat-αCGRP with decreasing caliber, indicating that CGRP exerts its major vasodilator action in the flow-regulating coronary arteries. The results also show that only repeated exposure of the arteries to 125 mM K^+ can overcome the development of tachyphylaxis; however, the mechanism behind the development of tachyphylaxis and the caliber-dependent response to rat-αCGRP remains to be elucidated in intramural coronary arteries.

Acknowledgements

This work is supported by the Danish Heart Foundation, Grant No. 96-1-3-64/22386 and Novo Nordisk Research Foundation.

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