Electrophysiological and mechanical effects of calcitonin gene-related peptide on guinea-pig atria

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- 1 The effects of calcitonin gene-related peptide (CGRP) on mechanical and electrophysiological responses were studied in the guinea-pig atrial muscle preparations and in single cells.
- 2 CGRP ($>10^{-9}$ M) enhanced the twitch contraction in a concentration-dependent manner in electrically driven left atria and increased heart rate in spontaneously beating right atria. The positive inotropic and chronotropic effects of CGRP were not inhibited by propranolol but were attenuated by reduction of the calcium concentration in the bathing medium.
- 3 In single left atrial cells, CGRP slightly hyperpolarized the resting potential but did not affect the other action potential parameters significantly.
- 4 Under whole-cell voltage-clamp conditions, CGRP increased the calcium inward current. The peptide also increased the steady inward current elicited by hyperpolarization and the late outward current by depolarization.
- 5 These results suggest that CGRP may produce the positive inotropic and presumably chronotropic effects by increasing calcium inward current. CGRP also increases the potassium permeability. Such effects on ionic currents may not produce any apparent change in the action potential conformation, due to their opposite directional actions and relatively weak potencies.

Introduction

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide, which has been encoded by the same gene as calcitonin and is generated in neural cells by an alternative tissue specific splicing of the mRNA (Amara *et al.*, 1982; Craig *et al.*, 1982; Rosenfeld *et al.*, 1983). CGRP-immunoreactivity is found in heart particularly around the sinoatrial and atrioventricular node, as well as in periaortic nerves in association with blood vessels (Manson *et al.*, 1984; Wisenfeld-Hallin *et al.*, 1984; Mulderry *et al.*, 1985; Gennari & Fischer, 1985).

CGRP shows potent positive chronotropic and inotropic effects on the atrial muscles of rats and guinea-pigs (Franco-Cereceda & Lundberg, 1985; Marshall *et al.*, 1986; Saito *et al.*, 1987). Since CGRP enhances adenylate cyclase activity resulting in an elevation of adenosine 3':5'-cyclic monophosphate (cyclic AMP) content, it is suggested that the positive inotropic effect of CGRP may be attributable to an increase in calcium influx (Ishikawa *et al.*, 1988). However, there is no direct evidence for the effect of CGRP on ionic currents. In the present study, we examined the electrophysiological and mechanical effects of CGRP.

Methods

Male guinea-pigs weighing 250–350 g were anaesthetized with sodium pentobarbitone (30 mg kg⁻¹, i.p.), exsanguinated from the carotid arteries and the hearts were quickly isolated. Atria were dissected and mounted vertically in 20 ml organ baths containing modified Tyrode solution of the following composition (in mM): NaCl 136.9, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.0, NaHCO₃ 23.8, NaH₂PO₄ 0.21 and glucose 5.6, gassed with a mixture of 95% O₂ and 5% CO₂ at 30°C. Square wave pulses of 1 ms duration and suprathreshold voltage were applied at a frequency of 1 Hz in left atria. In right atria, spontaneous beating was recorded. Contractile force was measured isometrically by a force displacement transducer (T7-30-240, Toyo Baldwin, Japan) and recorded on a strip chart recorder (RJG-4128, Nihon Kohden, Japan). The preparations were

equilibrated at a resting tension of 1 g for 90 min before experiments started.

In some experiments, the calcium concentration in the bathing medium was reduced to a half (0.9 mM) or a quarter (0.45 mM) of the normal calcium concentration.

Single atrial myocytes from guinea-pig hearts were prepared as described in a previous paper (Ohmura *et al.*, 1990). A patch clamp amplifier (CEZ-2100, Nihon Kohden) was used to study action potentials and membrane currents (for details see Nishio *et al.*, 1988). Patch electrodes were filled with a solution of following composition (in mM): potassium aspartate 100, KCl 5, MgCl₂ 5, HEPES (hydroxyethylpiperazinyl-ethane-sulphonic acid) 5, EGTA (ethylene-glycol-bis(β -aminoethyl-ether)N,N,N',N'-tetraacetic acid) 2.5, adenosine 5'-triphosphate (ATP) 5 and phosphocreatine 5. The pH was adjusted to 7.3 with KOH. The solution for superfusing the cells was as follows (in mM): NaCl 136.9, KCl 5.4, CaCl₂ 1.8, MgCl₂ 0.5, glucose 10 and HEPES 5. The pH was adjusted to 7.3 with NaOH.

Calcitonin gene-related peptide (CGRP, human) was supplied by Peptide Institute Inc. (Osaka, Japan) and dissolved in saline solution containing 2% gelatin.

Data and analysis

Experimental values are given as mean $\pm$ s.e.mean. The effects of drugs were considered significant if $P < 0.05$ by Student's *t* test for unpaired data.

Results

Effects of CGRP on mechanical responses in atrial preparations

CGRP produced a positive inotropic response in electrically driven left atria (1 Hz) and a positive chronotropic response in spontaneously beating right atria. Figure 1 shows the concentration-response curves to CGRP, in which CGRP was applied cumulatively and the maximum response at each concentration was plotted. The responses were concentration-dependent; the EC₅₀ values being $(9.7 \pm 1.5) \times 10^{-9}$ M for the

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Figure 1 Effects of calcitonin gene-related peptide (CGRP) on the twitch contraction of left atria (a) and on the heart rate (HR) of right atria (b). Left atria were driven at a frequency of 1 Hz. Net increases in contractile force and heart rate were plotted against the concentration of CGRP. Each value indicates the mean with s.e.mean indicated by vertical lines ($n = 5-6$).

positive inotropic effect and $(1.3 \pm 0.3) \times 10^{-8}$ M for the positive chronotropic effect, respectively. The maximal inotropic response of CGRP corresponded to 81.5% of that of isoprenaline in left atria (mean of 5 experiments). The effects of CGRP were not affected by propranolol (10^{-6} M) or atropine (10^{-6} M). However, the positive inotropic and chronotropic responses were markedly attenuated in low calcium medium. Figure 2 shows the effects of 10^{-7} M CGRP at various calcium concentrations. Reduction of calcium concentration from 1.8 mM to 0.45 mM significantly attenuated the responses to CGRP.

Figure 2 Influence of extracellular calcium concentration on the positive inotropic effect in left atria (a) and the positive chronotropic effect in right atria (b) of calcitonin gene-related peptide (CGRP, 10^{-7} M). Left atria were driven at a frequency of 1 Hz. Net increases in contractile force and heart rate were plotted against the concentration of CGRP. Each value indicates the mean with s.e.mean indicated by vertical lines ($n = 5-6$).

Effects on resting and action potentials in single atrial cells

Single atrial cells were stimulated at a frequency of 1 Hz and the membrane potentials were recorded before and after perfusion of 10^{-7} M CGRP. No apparent change in the membrane potential parameters was observed before and after 10^{-7} M CGRP, except that the resting potential was slightly hyperpolarized in all 4 cells tested (Figure 3 and Table 1).

Effects of CGRP on ionic currents in single atrial cells

Whole-cell voltage clamp experiments were carried out on single left atrial cells of guinea-pig. The membrane potential was held at the resting potential (-85 mV in Figure 4) and the depolarizing or hyperpolarizing pulses of 300 ms duration to various potentials were delivered every 5 s. Figure 4a shows representative current recordings. When the membrane was

Figure 3 Effects of calcitonin gene-related peptide (CGRP) on the action potentials of a single atrial cell. The cell was driven at a frequency of 1 Hz. Action potentials before (control) and 5 min after the treatment of 10^{-7} M CGRP are superimposed.

Figure 4 Effects of calcitonin gene-related peptide (CGRP 10^{-7} M) on ionic currents in a single atrial cell. Upper traces indicate the voltage commands. (a1) Effect on the inward current induced by a hyperpolarization from a resting potential (-85 mV) to -125 mV. (a2) Effects on the inward and outward currents induced by a depolarization to 5 mV. (b) Effects on inward calcium current, which was elicited by double pulse protocol (see text for further explanation).

Table 1 Effects of calcitonin gene-related peptide (CGRP) on resting and action potentials of guinea-pig atrial myocytes

	RP	APA	APD ₃₀	APD ₅₀	APD ₉₀
Control	-80.3 ± 1.0	106.0 ± 1.8	63.8 ± 8.0	89.3 ± 10.5	135.0 ± 12.4
CGRP 10^{-7} M	-82.3 ± 0.8 ($2.0 \pm 0.2^*$)	108.5 ± 3.5	62.5 ± 9.2	85.6 ± 10.3	127.5 ± 7.8

RP, resting potential; APA, action potential amplitude; APD₃₀, APD₅₀ and APD₉₀; action potential duration at 30%, 50% and 90% repolarization. Each value is mean $\pm$ s.e.mean of 4 experiments.

* All four cells hyperpolarized after CGRP perfusion; the net value of hyperpolarization is shown.

Figure 5 Current-voltage relationships for the inward calcium current (circles) and the current measured at the end of 300 ms clamp pulses (triangles); closed symbols: before 10^{-7} M calcitonin gene-related peptide (CGRP), open symbols: after 10^{-7} M CGRP. The membrane was held at -85 mV.

hyperpolarized to -125 mV (Figure 4a1), a steady state current flowed during the application of the pulse, which was significantly increased by 10^{-7} M CGRP ($18 \pm 9\%$ increase in 3 cells tested). On the other hand, a depolarizing pulse to $+5$ mV produced a transient inward current followed by an outward current. CGRP (10^{-7} M) apparently slowed down the decaying phase of inward current and increased the outward current at the end of depolarizing pulse (Figure 4a2). Such responses were not affected by propranolol 10^{-6} M.

In order to observe the effect on calcium current clearly, we used a double pulse protocol, in which the membrane was depolarized to -45 mV for 20 ms before application of test pulses. Under such conditions, sodium channels were inactivated during the prepulse and calcium current was seen in the early phase of the test pulse. CGRP (10^{-7} M) significantly increased the calcium current (Figure 4b).

Figure 5 shows the current-voltage relationships in which the calcium currents (circles) were elicited with the use of double pulse protocol (see Figure 4b) and the late currents (triangles) were measured at the end of 300 ms single pulses (see Figure 4a). CGRP increased the inward calcium current and the steady state current induced by hyperpolarizing pulses. The outward currents elicited by depolarization to less negative potentials than -35 mV were also increased by CGRP.

Discussion

CGRP produced positive inotropic and chronotropic effects in the guinea-pig atria with a potency comparable to isoprenaline. Since the responses were markedly attenuated by reducing the calcium concentration in the bathing medium from 1.8 mM to 0.45 mM, it was likely that the positive inotropic and chronotropic effects of CGRP were dependent on the

extracellular calcium concentration, probably on the calcium influx. When we examined the electrophysiological parameters in single atrial cells, no apparent change in the action potential parameters was observed, except a slight but consistent hyperpolarization of the membrane potential in each cell after CGRP application. Such lack of significant change by CGRP in action potential parameters has also been found in the multicellular preparations of guinea-pig atria (Ishikawa *et al.*, 1988).

Since the atrial action potential is constructed under the sophisticated balance of ionic currents (Beeler & Reuter, 1977; Trautwein & McDonald, 1978), we examined the effects of CGRP on the individual ionic currents by using the voltage-clamp method. Three effects were observed: (1) an increase in inward calcium current, (2) an increase in inward current which was elicited by the application of hyperpolarizing pulses from the resting potential and (3) an increase in the late outward current upon depolarization to the membrane potential over -40 mV. However, the extent of such effects was relatively small. Furthermore, the directions of the first effect and the second and third effects mentioned above were opposite in terms of their contribution to the action potential duration, resulting in an apparent lack of change in the action potential.

The effects of CGRP on cardiac ionic currents is reminiscent of the responses to β -adrenoceptor agonists (Reuter, 1974; Kass & Wieggers, 1974). Like isoprenaline, CGRP has been recently reported to increase cyclic AMP content in the guinea-pig atria (Ishikawa *et al.*, 1988). Since CGRP responses were not inhibited by propranolol, it is likely that the mechanisms subsequent to the receptor are common to the responses to CGRP and β adrenoceptor agonists.

The guinea-pig heart is innervated by various peptide-containing nerves. CGRP-immunoreactivity has been found in the sensory nerves. Since sensory nerves can act peripherally (Muramatsu, 1987), CGRP may be released from the sensory nerves and then produce positive inotropic and chronotropic effects (Franco-Cereceda & Lundberg, 1985; Saito *et al.*, 1987). In contrast, somatostatin is distributed in the parasympathetic nerves (Franco-Cereceda *et al.*, 1986). Recently, we have found that somatostatin inhibits the inward calcium current and then produces negative inotropic responses in the guinea-pig atria (Ohmura *et al.*, 1990). This evidence, together with the present results, suggests that the calcium channels of atrial cells may be reciprocally regulated by both peptides (CGRP and somatostatin), in addition to the classical adrenergic and cholinergic control.

Note added in proof

After this work was submitted, a paper by Ono, Delay, Nakajima Irisawa and Giles was published in *Nature* (340, 721–724, 1989). Their results are similar to ours in demonstrating that CGRP increases calcium current in frog atrial myocytes.

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