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European Journal of Pharmacology 327 (1997) 209–214

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Involvement of ATP-sensitive K^+ channels in the inhibitory effect of calcitonin gene-related peptide on neurotransmission in rat vas deferens

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Received 4 February 1997; accepted 21 March 1997

Abstract

This study investigated the inhibitory action of human calcitonin-gene-related peptide (CGRP) on neurotransmission in rat isolated vas deferens. The electrically stimulated contractile responses, which were mediated predominantly by activation of postganglionic noradrenergic nerve fibers, were concentration-dependently inhibited by human CGRP (0.1–100 nM, $IC_{50} = 2.15 \pm 0.21$ nM, $n = 17$). Human CGRP at concentrations greater than 3 nM reduced the contractile responses to exogenous noradrenaline and ATP. The inhibitory effect of human CGRP on the electrically stimulated or agonist-induced contractions was antagonized by human CGRP-(8–37), the CGRP receptor antagonist. Glibenclamide (3–10 μM) decreased the effect of human CGRP at a concentration greater than 1 nM whilst glibenclamide did not affect the inhibitory effect of human CGRP on the agonist-induced contractions. These results indicate that human CGRP at low concentrations exerts its inhibitory action mainly by acting on CGRP receptors at the sympathetic nerve terminals supplying rat vas deferens and the activation of ATP-sensitive K^+ channels is at least in part involved in the action of human CGRP on neurotransmission.

Keywords: CGRP (calcitonin gene-related peptide); Electric field stimulation; Glibenclamide; K^+ channel, ATP-sensitive; Neurotransmission; Vas deferens, rat

1. Introduction

Calcitonin-gene-related peptide (CGRP) is a 37-amino-acid peptide, which is primarily localized in the central and peripheral nervous system (reviewed by Ishida-Yamomoto and Tohyama, 1989). The localization of CGRP-like immunoreactivity in primary afferent nerves of various peripheral tissues including vas deferens and wide distribution of CGRP binding sites indicate that CGRP may have a physiological role as a neurotransmitter (Maggi et al., 1987b; Santicioli et al., 1988; Tan et al., 1994). CGRP has been recently shown to inhibit the electric field stimulation-induced contraction of rat and mouse vas deferens (Ohhashi and Jacobowitz, 1985; Al-Kazwini et al., 1986; Goto et al., 1987; Tan et al., 1994; Parlani et al., 1995). However, it is not clear how CGRP could modulate the activity of sympathetic nerve in the periphery. CGRP relaxes arterial smooth muscle in part through activation of ATP-sensitive K^+ channels (Nelson et al., 1990), but the ionic mechanism of the inhibitory action of human CGRP in vas deferens has not been investigated. The present study aims to examine whether human CGRP has a presynaptic inhibitory effect on neurotransmission in rat vas deferens and if ATP-sensitive K^+ channels play a role in the human CGRP-induced response.

2. Materials and methods

2.1. Preparation

The prostatic half (approx. 2 cm) of the vas deferens was prepared from male Sprague-Dawley rats (approx. 250 g) and placed in an organ bath with one end attached to a force-displacement transducer (Grass Instrument). Organ baths were filled with Krebs-Henseleit solution containing (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 25, KH_2PO_4 1.2, D-glucose 11.1, ascorbic acid 0.2. The bath solution was kept at 37 ± 1°C and constantly oxygenated with 95% O_2 + 5% CO_2. The preparations were

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PII S0014-2999(97)00079-4

allowed to equilibrate for about 90 min under an initial tension of 1 g. In the first group of experiments, the monophasic contractions of rat vas deferens were evoked by repetitive electric field stimulation (0.2 Hz, 0.5 ms duration, 60 V). The electrically stimulated contractions were abolished by 3 μM tetrodotoxin or by combination of 10 μM phentolamine and 3 μM α,β-methylene ATP (30 min contact time), suggesting their neurogenic origin. Human CGRP was added cumulatively to induce concentration-dependent inhibition of electrically stimulated contractions in the absence and presence of different concentrations of either the CGRP receptor antagonist, human CGRP-(8–37) or glibenclamide. Glibenclamide at 10 μM increased the electrically stimulated contraction by 12 ± 3.8% ($n = 6$), therefore, the strength of electric stimulation was lowered to between 40–50 V to match the initial level of contractions. Tissues were treated with human CGRP-(8–37) or glibenclamide for 10 min prior to application of the relaxants. The mean amplitude of five consecutive twitches was calculated at the start of application of the next concentration of human CGRP. In the second series of experiments, the preparations were contracted with noradrenaline cumulatively (ranging from 10 nM to 30 μM) to obtain the control concentration–response curve. Once a maximum response to noradrenaline had been reached, tissues were rinsed with Krebs-Henseleit solution every 20 min until the basal tone was restored. The preparations were then incubated with human CGRP (30 nM) for 5 min and another cumulative concentration–response curve to noradrenaline was repeated. The effect of human CGRP on contractions induced by a single concentration of ATP (100 μM) was also examined in the absence and presence of glibenclamide. In some experiments, the epithelial layer attached to the connective tissue was mechanically separated from the muscle layer with fine forceps.

2.2. Drugs

(−)-Noradrenaline bitartrate, human CGRP, human CGRP-(8–37), glibenclamide, tetrodotoxin and α,β-methylene ATP were purchased from Sigma (St. Louis, MO, USA), pinacidil and phentolamine mesylate from Research Biochemicals International (Natick, MA, USA). All drugs were dissolved in Krebs solution except for glibenclamide in dimethyl sulfoxide. Dimethyl sulfoxide at 0.2% (v/v) did not affect the electrically evoked response.

2.3. Statistics

The effect of human CGRP on electrically stimulated contractions was expressed as a percentage of the control value and data were presented as mean ± S.E.M. of n experiments. Cumulative concentration–response relations for human CGRP were analyzed with a non-linear curve fitting by a logistic equation (Grafit Erithacus Software). Student's t-test was used to evaluate a significant difference between mean values ($P < 0.05$).

3. Results

3.1. Antagonism of human CGRP-(8–37) on human CGRP-induced inhibition of evoked contractions

Fig. 1A shows that human CGRP caused a concentration-dependent (0.1–100 nM) inhibition of electrically stimulated contractions of the rat isolated vas deferens ($IC_{50} = 2.15 \pm 0.21$ nM, $n = 17$) and the threshold concentration for the inhibitory action of human CGRP was less than 0.1 nM. The effect of human CGRP was indepen-

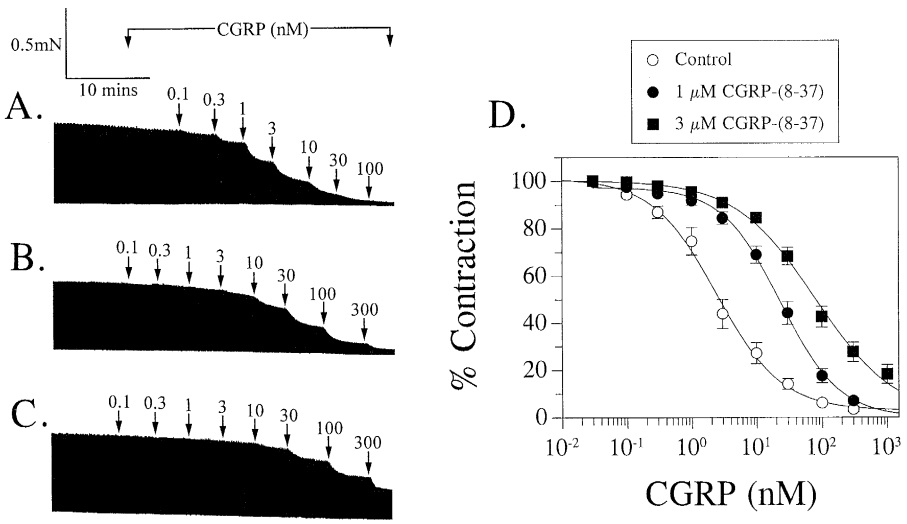

Fig. 1. Representative traces showing the concentration-dependent inhibition by human CGRP of electrically stimulated contractions of rat vas deferens in control (A) and in the presence of 1 μM (B) and 3 μM human CGRP-(8–37) (C). Concentration–response curves (D) for the inhibitory effect of human CGRP in the absence (○, $n = 7$) and presence of human CGRP-(8–37) (●, 1 μM, $n = 7$; ■, 3 μM, $n = 6$). Curves were drawn by fitting the data point to a logistic equation and results are expressed as mean ± S.E.M. of n experiments.

Table 1
Inhibitory effect of human CGRP on noradrenaline- and ATP-induced contraction

	Inhibitory effect			*n*
	First	Second	Ratio (%)	
Noradrenaline (0.3 μM)				
Control	2.15 ± 0.44	2.25 ± 0.61	100.8 ± 9.4	4
Human CGRP	1.93 ± 0.23	0.61 ± 0.16 [a]	31.6 ± 6.9 [a]	4
+1 μM hCGRP-(8–37)	2.58 ± 0.59	1.35 ± 0.27 [b]	53.6 ± 3.6 [a]	4
+3 μM hCGRP-(8–37)	2.88 ± 0.76	2.28 ± 0.86 [b]	72.8 ± 13.1	4
ATP (100 μM)				
Control	4.24 ± 0.46	4.11 ± 0.26	96.9 ± 3.9	5
Human CGRP	4.40 ± 0.36	2.62 ± 0.38 [a]	59.5 ± 7.5 [a]	5
+1 μM hCGRP-(8–37)	4.48 ± 0.65	2.94 ± 0.89 [b]	65.6 ± 6.6 [a]	5
+3 μM hCGRP-(8–37)	4.10 ± 0.72	3.28 ± 0.85 [b]	74.1 ± 9.2 [b]	5

The mean peak amplitude of two contractions induced by noradrenaline (0.3 μM) and ATP (100 μM) was measured and force was presented in mN. The effect of human CGRP (30 nM) was tested by comparing the amplitude of the second contraction in the presence of human CGRP to the first contraction in control. Human CGRP was added 5 min prior to the second application of each agonist. Human CGRP-(8–37) was applied 10 min before addition of human CGRP. Significant difference ([a] $P < 0.01$, [b] $P < 0.05$) between the first and second contractions in paired data. A ratio of the second contraction over the first contraction was calculated and significance was indicated by comparing the control and test groups. Values are mean ± S.E.M. of *n* experiments.

dent of the presence of epithelium ($IC_{50} = 2.02 \pm 0.25$, $n = 4$, without epithelium) but was significantly reduced by human CGRP-(8–37) (1–3 μM), the CGRP receptor antagonist (Fig. 1B and C). The concentration–response curve for the inhibitory effect of human CGRP was shifted to the right in the presence of human CGRP-(8–37). The IC_{50} values were 2.36 ± 0.26 nM ($n = 7$), 23.57 ± 1.76 nM ($n = 7$) and 81.02 ± 7.63 nM ($n = 6$) in the presence of 0, 1 and 3 μM human CGRP-(8–37), respectively (Fig. 1D). The antagonist alone did not affect the electrically stimulated contractions, suggesting that the release of endogenous CGRP may be negligible. On the other hand, human CGRP-(8–37) did not influence the relaxant response to pinacidil ($IC_{50} = 0.41 \pm 0.04$ μM, $n = 5$ in control; 0.39 ± 0.05 μM, $n = 4$, in the presence of 3 μM human CGRP-(8–37)). To further evaluate whether the effect of human CGRP on the electrically stimulated contraction may contain pre- and postjunctional components, the possible inhibition by human CGRP on contractions induced by exogenous noradrenaline (0.3 μM) and ATP (100 μM) was examined. Human CGRP at concentrations smaller than 3 nM did not affect the contractile responses to noradrenaline (0.3 μM, $n = 5$) and ATP (100 μM, $n = 5$), but reduced the agonist-induced response at 30 nM.

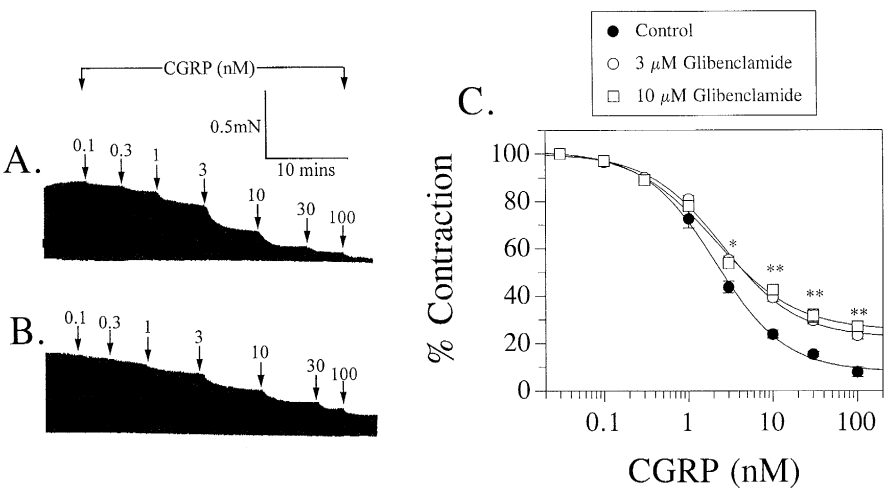

Fig. 2. Representative traces showing the concentration-dependent inhibition by human CGRP of electrically stimulated contractions of rat vas deferens in control (A) and in the presence of 10 μM glibenclamide (B). Concentration–response curves (C) for the effect of human CGRP on electrically stimulated contractions in the absence (●, $n = 10$) and presence of glibenclamide (○, 3 μM, $n = 7$; □, 10 μM, $n = 6$). Curves were drawn by fitting the data point to a logistic equation and results are expressed as mean ± S.E.M. of *n* experiments. Asterisks indicate a significant difference ([*] $P < 0.05$ and [**] $P < 0.01$).

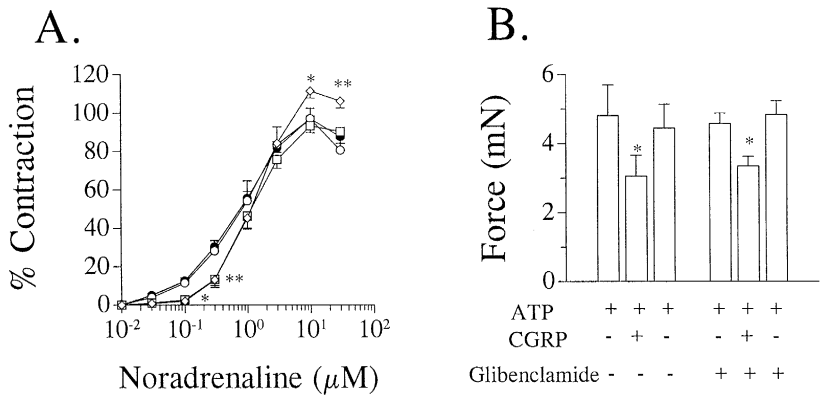

Fig. 3. Concentration–response curves (A) for noradrenaline in the absence (●, $n = 21$ in the first concentration-dependent response and ○, $n = 8$ in the second concentration-dependent response) and presence of human CGRP (□, 30 nM, $n = 7$) and in the presence of 3 μM glibenclamide and 30 nM human CGRP (◇, $n = 6$). Contractile responses (B) to three consecutive applications of ATP (100 μM) with an interval of 45 min in the absence and presence of glibenclamide (3 μM, 10 min contact time, $n = 9$). Human CGRP at 30 nM was added 5 min prior to the second application of ATP. Asterisks indicate a significant difference (* $P < 0.05$ and ** $P < 0.01$).

The inhibitory effect of human CGRP (30 nM) was more profound on electrically stimulated contraction (approx. 86% inhibition) than on the agonist-induced contraction (Table 1). The inhibitory effect of human CGRP on the agonist-induced contractile responses was also reduced by human CGRP-(8–37) (Table 1).

3.2. Effect of glibenclamide on human CGRP-induced inhibition of electrically stimulated contraction

The inhibitory effect of human CGRP at concentrations greater than 1 nM on electrically stimulated contractions was reduced by glibenclamide (Fig. 2A and B). IC_{50} values were 1.94 ± 0.18 nM ($n = 10$) in control, 2.30 ± 0.14 nM ($n = 7$) in 3 μM glibenclamide and 1.98 ± 0.12 nM ($n = 6$) in 10 μM glibenclamide. Glibenclamide (10 μM) caused approximately 22% inhibition of the maximum relaxation induced by human CGRP (100 nM, $n = 4$, Fig. 2C).

3.3. Effect of glibenclamide on human CGRP inhibition of noradrenaline- and ATP-induced contraction

Fig. 3A shows that noradrenaline contracted the preparation in a concentration-dependent manner with an EC_{50} of 1.19 ± 0.27 μM ($n = 21$) for the first concentration–response curve and of 1.27 ± 0.21 μM ($n = 8$) for the second concentration–response curve. Human CGRP (30 nM) did not affect EC_{50} and the maximum response ($EC_{50} = 1.56 \pm 0.47$ μM, $n = 7$). However, human CGRP (30 nM) reduced the contractile response to noradrenaline at 0.1 and 0.3 μM. This inhibitory effect of human CGRP was unaffected by glibenclamide (3 μM, $n = 6$). Fig. 3B shows that human CGRP at 30 nM reduced the ATP (100 μM)-induced contraction by $35.7 \pm 4.7\%$ ($n = 9$) and $26.3 \pm 5\%$ ($n = 9$), respectively, in the absence and presence of 3 μM glibenclamide ($P = 0.152$, unpaired data).

4. Discussion

The present study shows that human CGRP inhibited the contractile response induced by electric field stimulation in the prostatic half of the rat isolated vas deferens. Human CGRP did not affect the noradrenaline- and ATP-induced contractions at 3 nM; this concentration reduced the electrically stimulated contraction by approximately 50%. These results indicate that in the present experimental conditions CGRP at low concentrations may primarily act at the prejunctional level to reduce the release of neurotransmitters. At higher concentrations, human CGRP significantly reduced the contractile responses to noradrenaline and ATP, suggesting that it might affect both pre- and postjunctional sites. The present results are consistent with the earlier reports by Ohhashi and Jacobowitz (1985) and Parlani et al. (1995), who postulated that CGRP receptor existed presynaptically in the rat or mouse vas deferens and that CGRP may inhibit the release of noradrenaline during noradrenergic nerve stimulation, thus raising an interesting possibility that some neuropeptides released from sensory nerves can modulate sympathetic transmission in the peripheral tissues. Inhibition of field stimulation-induced twitches of the rat vas deferens is reportedly mediated through the release of CGRP (Maggi et al., 1987a; Santicioli et al., 1988). These findings would argue against a pure postjunctional site of the inhibitory action for CGRP (Goto et al., 1987). On the other hand, human CGRP did not change the resting membrane potential in freshly isolated single smooth muscle of the rat vas deferens; instead, it reduced the outward K^+ currents and enhanced L-type Ca^{2+} current (Nakazawa et al., 1992). These electrophysiological data imply that CGRP could increase muscle contractility. This report is, however, in sharp contrast to the inhibitory effect on electrically stimulated or agonist-induced contractions of rat vas deferens reported by different groups (Ohhashi and Jacobowitz,

1985; Al-Kazwini et al., 1986; Goto et al., 1987; Maggi et al., 1987a; Tan et al., 1994; Parlani et al., 1995). The modulatory effect of CGRP on ion channels is probably caused by experimental manipulations. Alternatively, CGRP may modulate Ca^{2+} channels in vas deferens smooth muscle but this effect may contribute little to the observed inhibition of evoked contractions. CGRP has so far been shown to promote L-type Ca^{2+} channel activity and accordingly to enhance contractility only in heart muscle (Ono et al., 1989).

The present work also shows that human CGRP-(8–37), the CGRP receptor antagonist, competitively antagonized the effect of human CGRP, indicating that human CGRP exerted its effect probably by activating CGRP receptors in nerve endings or/and smooth muscle. The marked antagonistic effect of human CGRP-induced inhibition of the sympathetic control of mouse vas deferens motility was also observed (Parlani et al., 1995). Maggi et al. (1991) previously reported that human CGRP-(8–37) at 1 μM significantly antagonized the inhibitory effect of human CGRP and fully abolished the relaxant response to capsaicin in rat vas deferens. CGRP receptor heterogeneity has been recently proposed on the basis of differential agonist potencies in brain and peripheral tissues (Dennis et al., 1989). Rat vas deferens is reported to possess $CGRP_1$ and $CGRP_2$ receptor subtypes but $CGRP_2$ receptor was suggested to be relatively insensitive to the antagonism of human CGRP-(8–37) (Dennis et al., 1990). However, the present study shows that human CGRP-(8–37) potently reduced the inhibitory effect of human CGRP on the agonist-induced contractile response in vas deferens. Longmore et al. (1994) also suggested that there is no difference in the ability of human α- and β-CGRP-(8–37) fragments to antagonize a supposedly $CGRP_1$ and $CGRP_2$ receptor-mediated response in the same preparation. These results indicate that human CGRP-(8–37) may not act as a selective antagonist in the rat vas deferens. The different concentration of human CGRP required to inhibit pre- and postjunctionally mediated contractile responses suggests a possible receptor heterogeneity. This possibility can be substantiated only when more selective antagonists for CGRP receptor subtypes become available.

CGRP has been shown to hyperpolarize arterial smooth muscle by opening ATP-sensitive K^+ channels. This glibenclamide- but not charybdotoxin-sensitive hyperpolarization seems to be responsible for part of the observed vasorelaxation (Nelson et al., 1990). The present study demonstrated that glibenclamide, a selective ATP-sensitive K^+ channel blocker, inhibited the relaxant effect induced by human CGRP at concentration higher than 1 nM. This indicates that human CGRP may activate K^+ channels only at high concentrations. It is possible that CGRP receptor subtypes with differential agonist potency may be also present on prejunctional nerve membrane, which are coupled to distinct intracellular second messenger pathways. In addition, glibenclamide did not affect the inhibitory effect of human CGRP on the noradrenaline- and ATP-induced contraction. Taken together, these results show that in addition to other reported mechanisms, the inhibitory action of human CGRP on neurotransmission was at least in part mediated by the activation of ATP-sensitive K^+ channels in sympathetic nerve terminals supplying the rat vas deferens.

Acknowledgements

This work was supported by Hong Kong RGC and UPGC Direct Grants (CUHK 445/95M and A/C 220404750).

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