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Characterisation of calcitonin gene-related peptide receptors in rat atrium and vas deferens: evidence for a [Cys(Et)^{2,7}]hCGRP-preferring receptor

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Abstract

The present study was performed in order to characterise calcitonin gene-related peptide (CGRP) receptor subtypes in rat left atrium and vas deferens by using [*R*-(*R**,*S**)]-*N*-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl]amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2*H*)-quinazolinyl)-,1-Piperidinecarboxamide (BIBN4096BS), a novel CGRP receptor antagonist. When CGRP was used as an agonist, BIBN4096BS exhibited an almost 10-fold higher affinity for CGRP receptors in rat left atrium compared to those in the vas deferens, indicating that CGRP acts through different CGRP receptor subtypes in these two tissues. In addition, BIBN4096BS was almost 10-fold more potent in antagonizing [Cys(Et)^{2,7}]hCGRPα and human adrenomedullin-induced responses than CGRP-induced responses in rat vas deferens. This might indicate receptor heterogeneity in rat vas deferens. Accordingly, the present work provides first experimental evidence that the rat vas deferens contains two CGRP-like receptor subtypes. Namely, the $CGRP_2$ receptor and a ''novel'' receptor that possesses low efficacy for CGRP and that is selectively stimulated by [Cys(Et)^{2,7}]hCGRP or adrenomedullin and which can be blocked with high affinity by BIBN4096BS.

Keywords: Calcitonin gene-related peptide (CGRP); BIBN4096BS; CGRP (8-38); CGRP receptor subtypes; Left atrium, rat; Vas deferens, rat

1. Introduction

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide first described in 1982 by Amara et al. (1982). CGRP is expressed in α- and β-forms that vary by one and three amino acids in rats and humans, respectively (Rosenfeld et al., 1983; Morris et al., 1984; Amara et al., 1985). CGRP exhibits several effects on the cardiovascular, gastrointestinal, bronchotracheal, endocrine systems and on the central nervous system, its most profound action is however vasodilation (Brain et al., 1985; Brain and Cambridge, 1996). The classification of CGRP receptors is in a relatively early stage. The existence of two CGRP receptor subtypes has been proposed on the basis of differential antagonist affinities and agonist potencies in peripheral preparations (Dennis et al., 1989, 1990; Dumont et al., 1997). Thus, the CGRP receptor antagonist CGRP-(8-37) is considered to possess higher affinity for $CGRP_1$ receptors found in the guinea pig atrium (Dennis et al., 1990), whereas the agonists [Cys(Acm)^{2,7}]hCGRPα and [Cys(Et)^{2,7}]hCGRPα are considered to be more potent for the $CGRP_2$ receptor described originally in the rat vas deferens (Dennis et al., 1989, 1990; Dumont et al., 1997).

It has been shown that adrenomedullin, a 52 amino acid peptide, can exert its biological actions via interacting with CGRP receptors, because the vaso-relaxant effects of human adrenomedullin (e.g. in the porcine coronary artery and the isolated rat heart) can be antagonized by the CGRP receptor antagonist CGRP-(8-37), (Entzeroth et al., 1994; Yoshimoto et al., 1998). However, in some other tissues, the actions of adrenomedullin are mediated through specific adrenomedullin receptors, since some effects of adrenomedullin are not antagonized by CGRP-(8-37). For instance, it has been reported that human adrenomedullin

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was as potent as human αCGRP in causing concentration-dependent relaxation in rat aorta and rat pulmonary artery. However, its effects could not be blocked by CGRP-(8-37), suggesting that specific adrenomedullin receptors exist in rat aorta and pulmonary artery (Yoshimoto et al., 1998; Wisskirchen et al., 1998).

The aim of this study was to pharmacologically characterise the CGRP receptor subtypes in rat atrium and vas deferens using several CGRP receptor agonists, the peptide antagonist CGRP-(8-37) and the novel CGRP antagonist *R*-(*R**,*S**)]-*N*-[2-[[5-amino-1-[[4-(4-pyridinyl)-1-piperazinyl]carbonyl]pentyl]amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2*H*)-quinazolinyl)-,1-Piperidinecarboxamide (BIBN4096BS) (Doods et al., 2000).

2. Materials and methods

2.1. Experimental protocol

Male Wistar rats (Chbb: Thom, about 300 g) were exsanguinated under sodium pentobarbitone anaesthesia. The hearts and vas deferens were dissected and immediately placed in oxygenated Krebs buffer. The left atria and vas deferens were carefully prepared and subsequently mounted in 25-ml organ baths containing Krebs solution of the following composition (mmol/l): NaCl 118; KCl 4.7; $MgSO_4$ 1.2; $NaHCO_3$ 25; KH_2PO_4 1.2; glucose 10; $CaCl_2$ 2.5. The Krebs solution was gassed with 95% O_2 + 5% CO_2 and maintained at 37°C. A resting tension of 1 g was applied. Following a 60-min equilibration period, the atria were electrically driven at a frequency of 3 Hz (duration: 0.5 ms; voltage: initial value + 20%) and the vas deferens were stimulated at a frequency of 0.2 Hz (duration: 0.5 ms; voltage: 60 V). Responses were recorded on a polygraph. Following a 30-min period of electrical stimulation, concentration–effect curves to CGRP receptor agonists were obtained in a cumulative fashion, the next concentration being added when the effect of the preceding one had reached a steady state. For experiments using the antagonists, the tissues were incubated for 15 min with antagonist prior to the construction of the concentration–effect curve for CGRP receptor agonists. Only one concentration–effect curve was made in each preparation.

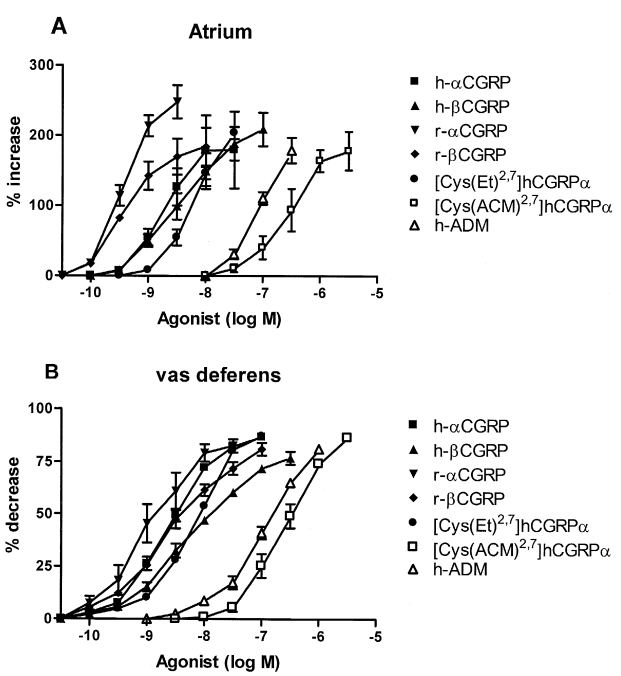

Fig. 1. Concentration–response curves of CGRP receptor agonist-induced positive inotropic effects in rat left atrium and inhibition of electrically evoked twitch response in vas deferens. Data are means ± SEM, $n = 4$.

Table 1
Potencies of CGRP receptor agonists in rat isolated atrium and vas deferens preparations. Results are mean ± SEM, $n = 4$

Compound	Rat atrium EC_{50} (nM)	Rat vas deferens EC_{50} (nM)
h-αCGRP	2.00 ± 0.09	1.91 ± 0.14
h-βCGRP	3.02 ± 0.21	5.62 ± 0.37
r-αCGRP	0.25 ± 0.012	0.78 ± 0.041
r-βCGRP	0.44 ± 0.019	2.04 ± 0.12
$[Cys(Et)^{2,7}]hCGRP_\alpha$	5.50 ± 0.37	8.32 ± 0.45
$[Cys(ACM)^{2,7}]hCGRP_\alpha$	274 ± 13	234 ± 14
h-ADM	83.7 ± 9.1	93.3 ± 5.55

To investigate whether breakdown of peptides by neutral endopeptidase (NEP) is involved, we performed experiments in rat left atrium and vas deferens to compare the effects of BIBN4096BS to human αCGRP-induced responses in the absence and presence of thiorphan. Thiorphan was added to the tissue baths for 15 min prior to the addition of BIBN4096BS.

2.2. Data analysis

Drug-induced effects on rat left atria and vas deferens were calculated as percentage changes from resting levels, measured prior to the first concentration of agonists. Agonist relative potencies were determined by comparing EC_{50} values (molar concentration of the agonist that produced 50% of the maximal response). Antagonist relative potencies were determined by comparing pK_B values. In the presence of an antagonist with a single concentration used, apparent pK_B values were calculated from dose-ratios produced by the stated concentration of CGRP antagonists tested from the equation: $pK_B = \log(DR\text{-}1) - \log[\text{antagonist}]$. Where multiple concentrations of antagonist were used, a Schild plot was constructed. Since the slopes of the Arunlakshana–Schild plots were not significantly different from unity, mean pK_B values were calculated (Kenakin, 1993). All values were expressed as mean ± SEM, $n = 4$–5.

Table 2
pK_B values for the CGRP receptor antagonists h-αCGRP-(8-37), h-βCGRP-(8-37) and BIBN4096BS for CGRP receptors in rat left atrium and vas deferens preparations. Results are mean ± SEM, $n = 4$

		pK_B		
		h-αCGRP-(8-37)	h-βCGRP-(8-37)	BIBN4096BS
atrium	h-αCGRP	7.16 ± 0.05	7.10 ± 0.04	8.52 ± 0.05
	h-βCGRP	7.00 ± 0.01	7.01 ± 0.03	8.13 ± 0.02
	r-αCGRP	7.35 ± 0.03	7.20 ± 0.05	8.73 ± 0.03
	r-βCGRP	6.62 ± 0.01	7.23 ± 0.02	8.23 ± 0.01
	$[Cys(Et)^{2,7}]hCGRP_{\alpha}$	7.38 ± 0.04	7.28 ± 0.05	8.82 ± 0.07
	h-ADM	7.30 ± 0.02	nd	8.63 ± 0.03
vas deferens	h-αCGRP	6.23 ± 0.05	6.29 ± 0.04	7.11 ± 0.04
	h-βCGRP	< 6[a]	< 6[a]	6.74 ± 0.06
	r-αCGRP	6.29 ± 0.02	< 6[a]	7.03 ± 0.04
	$[Cys(Et)^{2,7}]hCGRP_{\alpha}$	7.17 ± 0.03	7.30 ± 0.06	8.38 ± 0.03
	h-ADM	6.95 ± 0.05	nd	8.12 ± 0.05

nd — not determined.
[a]A rightward shift less than twofold.

2.3. Drugs used

The following drugs were used: $[Cys(Et)^{2,7}]hCGRP\alpha$, human αCGRP-(8-37), human αCGRP, human βCGRP and rat αCGRP, human adrenomedullin and $[Cys(ACM)^{2,7}]hCGRP\alpha$ were purchased from Polypeptide, Wolfenbüttel, Germany; rat βCGRP and human βCGRP-(8-37) were purchased from Neosystem, Strasbourg, France; BIBN4096BS was synthesized by Boehringer Ingelheim Pharma, Biberach/Riss, Germany. All peptides were dissolved in distilled water. BIBN4096BS was dissolved with a small volume (20 μl) of 1 N HCl, further diluted with saline, then adjusted to pH 6.5–7.0 by 1 N NaOH. Solutions were diluted to the final concentration with Krebs buffer.

3. Results

3.1. Effect of CGRP-related peptide agonists

All CGRP receptor agonists produced concentration-dependent positive inotropic effects in rat left atrium and inhibited the electrically evoked twitch responses in vas deferens (Fig. 1A and B). EC_{50} values are summarised in

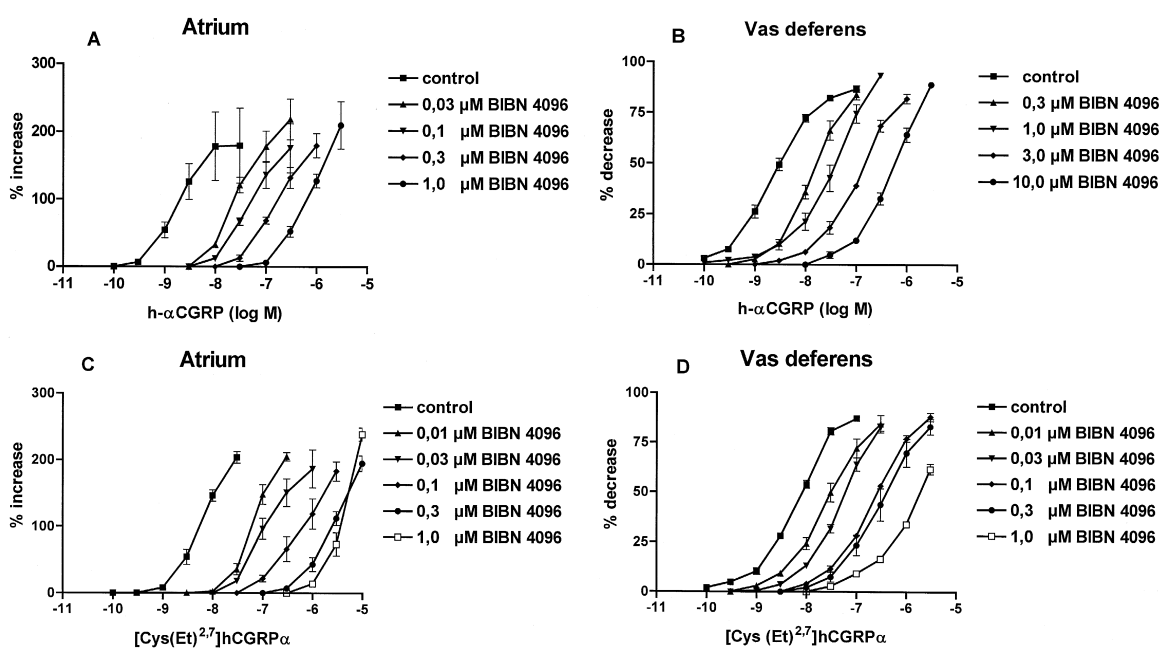

Fig. 2. Concentration–response curves of human αCGRP and $[Cys(Et)^{2,7}]hCGRP\alpha$ in the absence and presence of different concentrations BIBN4096BS in rat left atrium and vas deferens. Data are means ± SEM, $n = 4$.

Table 1. None of the CGRP agonists clearly discriminated between CGRP receptors on rat left atrium and rat vas deferens. Both α and β rat CGRP were slightly more potent than the human forms.

3.2. Effect of CGRP antagonists

Human αCGRP-(8-37), human βCGRP-(8-37) and BIBN4096BS at a concentration of 1 μM antagonized the CGRP receptor agonist-induced responses in rat left atrium and vas deferens, without depression of the maximal responses. Antagonist potencies were determined by comparing their pK_B values (Table 2). BIBN4096BS was in general approximately 10 times more potent in blocking CGRP receptor agonist-induced responses in rat left atrium and in vas deferens than human αCGRP-(8-37) and human βCGRP-(8-37). In the rat atrium, BIBN4096BS as well as human αCGRP-(8-37) and human βCGRP-(8-37) were unable to discriminate between the receptors stimulated by the different agonists. The pK_B values for BIBN4096BS were between 8.13 and 8.82, and for human αCGRP (8-37) and human β-CGRP (8-37) between 6.62 and 7.35, respectively.

The antagonists were at least 10-fold less potent in blocking the rat/human α-and β-CGRP induced responses in vas deferens compared to rat left atrium. However, in the vas deferens, the antagonists were approximately 10-fold more effective in blocking [Cys(Et)^{2,7}]hCGRPα and human adrenomedullin-induced responses than rat/human α-and β-CGRP induced responses.

In order to further investigate the antagonism of BIBN4096BS to human αCGRP and [Cys(Et)^{2,7}]hCGRPα-induced responses in rat left atrium and vas deferens, Arunlakshana–Schild plot analysis was performed. BIBN4096BS induced a concentration-dependent rightward shift of the dose–response curves of human αCGRP and [Cys(Et)^{2,7}]hCGRPα in rat left atrium and vas deferens, without depression of the maximal responses (Fig. 2A–D). The slopes of the Schild plots were not significantly different from unity (Table 3, Fig. 3A and B), and for this reason mean pK_B values were calculated (Table 3). In contrast to the rat left atrium, there was a significant difference in the estimated pK_B values for BIBN4096BS when human αCGRP or [Cys(Et)^{2,7}]hCGRPα was used as the agonist in the vas deferens. The calculated pK_B values were 7.23 ± 0.04 ($n = 16$) and 8.37 ± 0.05 ($n = 20$) for h-αCGRP and [Cys(Et)^{2,7}]hCGRPα, respectively.

Table 3
pKb values as determined by Schild plot analysis of BIBN4096BS to antagonize h-αCGRP and [Cys(Et)^{2,7}]hCGRPα mediated responses in rat left atrium and vas deferens. Results are mean ± SEM, $n = 5$

	Agonist	$pK_B \pm$ SEM mean	(n)[a]	Slope[b]
atrium	h-αCGRP	8.48 ± 0.03	16	1.00
	[Cys(Et)^{2,7}]hCGRP$_\alpha$	8.97 ± 0.07	20	1.00
vas deferens	h-αCGRP	7.23 ± 0.04	16	1.09
	[Cys(Et)^{2,7}]hCGRP$_\alpha$	8.37 ± 0.05	20	0.95

[a] (n) = (total number of data points).
[b] Slopes of the Schild regression were not significantly different from unity ($p < 0.01$).

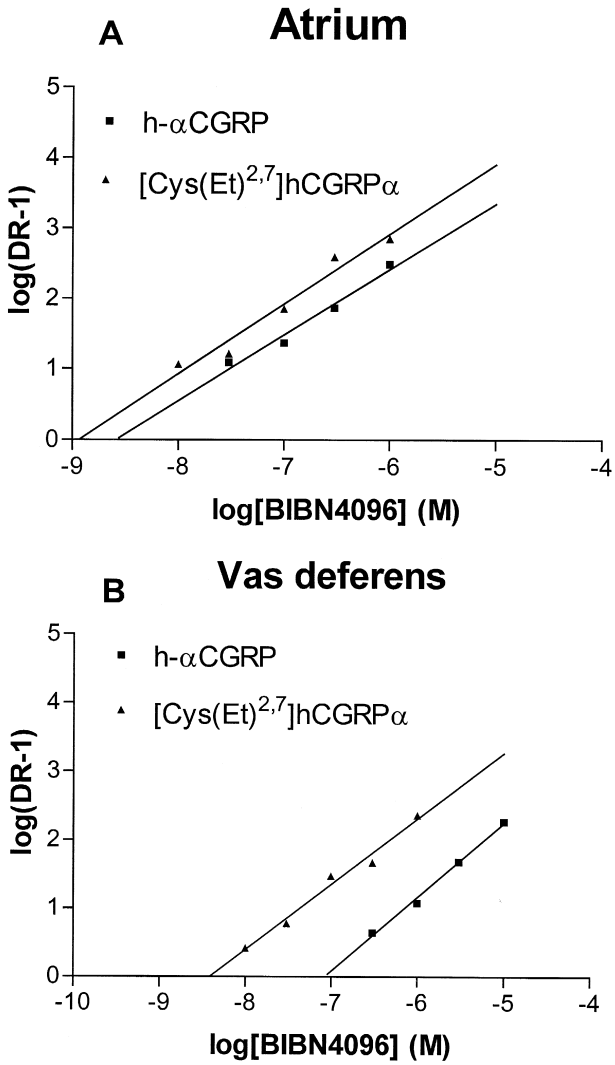

Fig. 3. Schild regression analysis for the antagonism of BIBN4096BS to human αCGRP and [Cys(Et)^{2,7}]hCGRPα induced responses in rat atrium and rat vas deferens, respectively.

3.3. Effect of thiorphan

The effects of BIBN4096BS to human αCGRP-induced responses in the absence and presence of thiorphan in rat left atrium and vas deferens were similar. The dose–response curves were superimposed (data not shown).

4. Discussion

CGRP receptor classification is based on the observation that the peptidic CGRP receptor antagonist, CGRP-(8-

37), is more potent to antagonize the effects of CGRP on guinea pig atrium, compared to the rat vas deferens (Dennis et al., 1989, 1990; Dumont et al., 1997). In addition, two CGRP analogues, [Cys(ACM)^{2,7}]hCGRPα and [Cys(Et)^{2,7}]hCGRPα appear to preferentially stimulate CGRP receptors in rat vas deferens (Dennis et al., 1989, 1990; Dumont et al., 1997). Accordingly, it has been proposed that CGRP receptors should be subdivided into $CGRP_1$ and $CGRP_2$ receptors. Receptors that can be blocked by CGRP-(8-37) with a pK_B value of approximately 7.0 are designated as $CGRP_1$ receptors (e.g. atrium), and those at which CGRP-(8-37) is ineffective or less effective ($pK_B \leq 6.0$) are designated as $CGRP_2$ receptors (e.g. vas deferens) (Quirion et al., 1992; Poyner, 1995).

In the present study, the agonists investigated produced concentration-dependent positive inotropic effects in the rat left atrium, and an inhibition of electrically evoked twitch responses in the vas deferens. However, in contrast to literature data [Cys(Et)^{2,7}]hCGRPα and [Cys(ACM)^{2,7}]-hCGRPα showed no selectivity between CGRP-mediated responses in left atrium and vas deferens. Especially [Cys(Et)^{2,7}]hCGRPα showed potent positive inotropic effects in left atrium and inhibition of the electrically evoked twitch responses in the vas deferens. Since in the original paper the rat vas deferens and the guinea pig atrium was used, we investigated whether a species difference is involved. Therefore, we also tested [Cys(Et)^{2,7}]hCGRPα on the guinea pig atrium. However, [Cys(Et)^{2,7}]hCGRPα also evoked potent concentration-dependent positive inotropic effects in guinea pig left atrium. The ED_{50} value of 22 nM (data not shown) was only fourfold lower compared to the value obtained in the rat (5.5 nM). The results obtained suggest that [Cys(ACM)^{2,7}]hCGRPα and [Cys(Et)^{2,7}]-hCGRPα are not $CGRP_2$ receptor selective, in contrast to the data reported by Dumont et al. (1997). The differences in results might be due to different strains of animals used or different experimental conditions.

For unequivocal receptor classification, the use of antagonists is a prerequisite. The present study provided further experimental evidence for a CGRP receptor subclassification by using BIBN4096BS. This novel antagonist possesses an affinity (Ki) of 14.4 ± 6.3 pM for human CGRP receptors on SK-N-MC cells and an affinity (Ki) of 3.4 ± 0.5 nM for rat CGRP receptors in spleen (Doods et al., 2000). BIBN4096BS was about 10-fold more potent in blocking rat/human α- and β-CGRP-mediated responses in both rat left atrium and vas deferens compared to human αCGRP-(8-37) and human βCGRP-(8-37). BIBN4096BS, human αCGRP-(8-37)and human βCGRP-(8-37) proved about 10-fold more potent in antagonizing rat/human α-and β-CGRP-mediated responses in rat left atria than in the vas deferens. These findings clearly indicate that rat/human α-and β-CGRP act through different receptors in rat atrium and vas deferens, and support the original sub-classification of CGRP receptors into $CGRP_1$ and $CGRP_2$ receptors (Quirion et al., 1992).

Several publications have described a discrepancy between affinity data of CGRP-(8-37) for CGRP receptors in the vas deferens obtained from receptor binding studies and functional studies. Although CGRP-(8-37) is 10-fold less potent in antagonizing human αCGRP-induced responses in rat vas deferens than in rat left atrium, it displaces [^{125}I]CGRP with high affinity from vas deferens membranes (Dennis et al., 1989, 1990; Poyner et al., 1999). Also [^{125}I-Tyr]hCGRP-(8-37) failed to detect receptor heterogeneity in rat brain and peripheral tissues (Van Rossum et al., 1994). We observed similar results with BIBN4096BS. BIBN4096BS is not able to discriminate between ^{125}I-CGRP binding sites in guinea pig vas deferens and atrium (data not shown). Accordingly, the $CGRP_{1/2}$ sub-classification so far is only based on functional data.

It seems of interest that all three antagonists did not discriminate between the effects of [Cys(Et)^{2,7}]hCGRPα or human adrenomedullin and rat/human α-or β-CGRP in rat atrium, whereas the three antagonists displayed an approximately 10-fold higher potency in antagonizing the responses of [Cys(Et)^{2,7}]hCGRPα and human adrenomedullin in the vas deferens. These findings suggest that rat vas deferens contains an additional receptor besides the $CGRP_2$ receptor.

In order to investigate the antagonism of BIBN4096BS towards human αCGRP and [Cys(Et)^{2,7}]hCGRPα-induced responses in rat left atrium and vas deferens in more detail, we performed Schild-plot analysis. BIBN4096BS induced a concentration-dependent rightward shift of the dose–response curves of human αCGRP and [Cys(Et)^{2,7}]hCGRPα in rat left atrium and vas deferens. The slopes of the Schild plots were not significantly different from unity, thus indicating that BIBN4096BS is a competitive antagonist for human αCGRP and [Cys(Et)^{2,7}]hCGRPα mediated receptors in both tissues. This also ruled out the possibility that human αCGRP and [Cys(Et)^{2,7}]hCGRPα interact with two different receptors, named low/high affinity for BIBN4096BS in vas deferens. From the Schild plot analyses of CGRP it is obvious that this agonist interacts with either a single class of receptors or a mixed receptor population that cannot be discriminated by BIBN4096BS. Accordingly, it is rather unlikely that vas deferens possesses a heterogeneous population of $CGRP_1$ and $CGRP_2$ receptors. It has been suggested that differences in enzyme distribution may reflect differential responses of CGRP analogues in functional assays (Longmore et al., 1994). In the present study, thiorphan, an inhibitor of the enzyme NEP, did not interfere with the effects of BIBN4096BS to human αCGRP-induced responses in rat left atrium and vas deferens. Wisskirchen et al. (1998) also reported that the properties of the CGRP receptor antagonist CGRP-(8-37) were not altered by pretreatment with peptidase inhibitors. Accordingly, the question remains what is the nature of the receptor that is activated by [Cys(Et)^{2,7}]-hCGRPα and human adrenomedullin and potently antagonized by BIBN4096BS.

One possibility could be that [Cys(Et)^{2,7}]hCGRPα and human adrenomedullin stimulate adrenomedullin receptors, since it has been suggested that guinea pig vas deferens possesses adrenomedullin receptors (Poyner et al., 1999). Adrenomedullin shows approximately 30% of structural homology with CGRP, and it has been reported that adrenomedullin can act through CGRP receptors as well as specific adrenomedullin receptors. However, several lines of evidences do not support that [Cys(Et)^{2,7}]hCGRPα or human adrenomedullin stimulates adrenomedullin receptors in the rat vas deferens. First, the effects of adrenomedullin in the rat vas deferens can be blocked by CGRP-(8-37), which is not observed in the guinea pig vas deferens (Poyner et al., 1999). Second, radioligand binding studies showed that BIBN4096BS has very low affinity (IC_{50} of 10.3 μM) for adrenomedullin receptors (Doods et al., 2000). The adrenomedullin binding assay was performed using ^{125}I-rat adrenomedullin (50 pM) and tissue from rat spinal cord. In this assay, adrenomedullin, amylin and CGRP possess an affinity (IC_{50}) of 1.4, 492 and 877 nM, respectively. Accordingly, the receptor stimulated by adrenomedullin in the rat vas deferens appears not to be identical with the adrenomedullin receptor. It has also been suggested that the CGRP receptor in the rat isolated thoracic aorta is different from the $CGRP_1$ and $CGRP_2$ receptors (Wisskirchen et al., 1998). This suggestion is based on the lack of effects of both the agonist [Cys(ACm)^{2,7}]hCGRPα and the antagonist CGRP (8-37) in this preparation. However, although these findings partly contradict with the report by Yoshimoto et al. (1998), this putative different CGRP receptor is not related to the novel receptor in the rat vas deferens since in the rat vas deferens both [Cys(Et)^{2,7}]hCGRP as well as CGRP (8-37) showed activity. Therefore, it seems that human adrenomedullin and [Cys(Et)^{2,7}]hCGRPα act through a novel, non-adrenomedullin receptor in the rat vas deferens. It cannot be excluded that the abovementioned agonists also stimulate this novel receptor in rat atrium, since the antagonists investigated do not discriminate between the $CGRP_1$ receptor and the receptor stimulated by adrenomedullin or [Cys(Et)^{2,7}]hCGRPα. Quirion et al. (1992) proposed that human αCGRP acts through $CGRP_1$ receptors in rat left atrium and via $CGRP_2$ receptors in vas deferens. Based on the data obtained from the present study, we propose that [Cys(Et)^{2,7}]hCGRPα and human adrenomedullin act through a third CGRP/adrenomedullin-like receptor in the rat vas deferens. CGRP itself seems to possess low efficacy for this receptor. Recently, it has been shown that receptor activity modifying proteins (RAMPs; McLatchie et al., 1998) determine the specificity of the calcitonin-receptor like receptor (Aiyar et al., 1996). Co-expression of calcitonin receptor-like receptor with $RAMP_1$ gives a pharmacological profile in line with a CGRP receptor and $RAMP_2$ co-expression results in an adrenomedullin-receptor. Although we mentioned before that the novel receptor shows different characteristics compared to the CGRP and adrenomedullin receptor, it would be of interest to evaluate BIBN4096BS in cell lines expressing the different RAMPs and the calcitonin receptor-like receptor.

In conclusion, the present study has demonstrated that BIBN4096BS is a potent and competitive CGRP antagonist and possesses a 10-fold higher affinity for $CGRP_1$ than for $CGRP_2$ receptors. Moreover, CGRP and adrenomedullin or [Cys(Et)^{2,7}]hCGRPα seem to stimulate different receptor subtypes in rat vas deferens. CGRP is an agonist for the $CGRP_2$ receptor and adrenomedullin or [Cys(Et)^{2,7}]hCGRP for a novel CGRP or adrenomedullin-like receptor. The novel CGRP receptor antagonist BIBN4096BS exhibits a high affinity for this receptor. In order to further characterise whether this postulated receptor is indeed a novel receptor subtype or for example a second binding site on the $CGRP_2$ receptor further receptor binding studies are in progress employing [^{3}H]-BIBN4096BS.

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