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Effect of the CGRP receptor antagonist BIBN4096BS in human cerebral, coronary and omental arteries and in SK-N-MC cells

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Abstract

Several lines of evidence suggest that a calcitonin-gene related peptide (CGRP) receptor antagonist may serve as a novel abortive migraine treatment. Here we present data on a human cell line and isolated human vessels for such an antagonist, BIBN4096BS. On SK-N-MC membranes, radiolabelled CGRP was displaced by both CGRP-(8-37) and BIBN4096BS, yielding pK_i values of 8.5 and 11.4, respectively. Functional studies with SK-N-MC cells demonstrated that CGRP-induced cAMP production was antagonised by both CGRP-(8-37) and BIBN4096BS with pA_2 values of 7.8 and 11.2, respectively. Isolated human cerebral, coronary, and omental arteries were studied with a sensitive myograph technique. CGRP induced a concentration-dependent relaxation that was antagonized by both CGRP-(8-37) and BIBN4096BS in a competitive manner. CGRP was a weaker agonist on coronary arteries as compared to intracranial arteries; however, BIBN4096BS was an equally effective antagonist. In human omental arteries, CGRP did not induce relaxation. BIBN4096 had a pA_2 value of 10.1 in cerebral and 10.4 in coronary arteries. The results of clinical trials with BIBN4096BS for acute migraine attacks are awaited with great interest.

Keywords: CGRP receptor; Cerebral vessel, human; cAMP production; Dilatation

1. Introduction

Calcitonin-gene related peptide (CGRP)-containing nerve fibres supply vessels of different locations, both peripheral and cerebral (Uddman et al., 1986). The intracranial vascular bed is supplied by CGRP fibers that originate in the trigeminal ganglion (Edvinsson, 1985; Uddman et al., 1985). Upon activation, these fibres release CGRP (Goadsby et al., 1988); this occurs both in primary headaches and following subarachnoid haemorrhage (Goadsby et al., 1990; Goadsby and Edvinsson, 1994; Juul et al., 1990, 1995). CGRP has been demonstrated to be a potent vasodilator (Bell and McDermott, 1996), increases heart rate (Franco-Cereceda, 1988) and has positive inotropic effects on isolated human trabeculae (Saetrum Opgaard et al., 2000). CGRP initiates these responses through an interaction with target organ receptors that are primarily coupled to the activation of adenylyl cyclase. CGRP mediates its action via two functional receptor subtypes, the $CGRP_1$ and the $CGRP_2$ receptor (Dennis et al., 1989; Juaneda et al., 2000). Studies of CGRP receptor mechanisms have relied on the use of the antagonist CGRP-(8-37), but now several small molecule non-peptide CGRP antagonists have been reported: Doods et al. (2000) described BIBN4096BS (a Lys–Tyr dipeptide derivative) that had high affinity (picomolar) for CGRP receptors endogenously expressed in SK-N-MC cells and inhibited neurogenic vasodilation evoked by trigeminal ganglion stimulation in marmosets. We have described a related CGRP receptor antagonist (see patent number WO 98/11128, coded Compound 1: 4-(2-oxo-2,3-dihydro-benzoimidazol-1-yl)-piperidine-1-carboxylic acid [1-(3,5-dibromo-4-hydroxy-benzyl)-2-oxo-2-(4-phenyl-piperazin-1-yl)-ethyl]-amide) and used it to characterise CGRP-mediated responses in cranial arteries. Compound 1 blocked CGRP-evoked relaxation in human arteries (Edvinsson et al., 2001). In addition, Aiyar et al. (2001) have revealed that SB-(+)-273779 is a selective non-peptide antagonist at $CGRP_1$ receptors that is also active at rat

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and porcine CGRP receptors. The aim of the present study was to compare the antagonistic effects of BIBN4096BS in various human vascular regions with its affinity in SK-N-MC cells expressing CGRP receptors.

2. Materials and methods

2.1. SK-N-MC cells

2.1.1. Binding studies

The binding of ^{125}I-CGRP to receptors in SK-N-MC cell membranes was carried out essentially as described (Edvinsson et al., 2001). Briefly, membranes (25 μg) were incubated in 1 ml of binding buffer [10 mM HEPES, pH 7.4, 5 mM $MgCl_2$ and 0.2% bovine serum albumin (BSA)] containing 10 pM ^{125}I-CGRP and inhibitor. After incubation at room temperature for 3 h, the assay was terminated by filtration through GFB glass fibre filter plates (Millipore) that had been blocked with 0.5% polyethyleneimine for 3 h. The filters were washed three times with ice-cold assay buffer, then the plates were air dried. Scintillation fluid (50 μl) was added and the radioactivity was counted on a Topcount (Packard Instrument). Non-specific binding was determined by using a final concentration of 500 pM BIBN4096BS. Data analysis was carried out by using Prism and the K_i was determined by using the Cheng–Prusoff equation (Cheng and Prusoff, 1973).

2.1.2. Functional studies

SK-N-MC cells were grown in minimal essential medium (MEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, 100 units/ml penicillin and 100 μg/ml streptomycin at 37 °C, 95% humidity, and 5% CO_2. For cAMP assays, cells were plated at 5×10^5 cells/well in 96-well poly-D-lysine-coated plates (Becton-Dickinson) and cultured for ~18 h before assay.

Cells were washed with phosphate-buffered saline (PBS, Sigma) then pre-incubated with 300 μM isobutylmethylxanthine in serum-free MEM for 30 min at 37 °C. α-CGRP-(S-37) or BIBN4096BS was added and the cells were incubated for 10 min before the addition of CGRP. The incubation was continued for another 15 min, then the cells were washed with PBS and processed for cAMP determination according to the manufacturer's recommended protocol. Maximal stimulation over basal was defined by using 100 nM CGRP. Dose–response curves were generated by using Prism. Dose-ratios (DR) were calculated and used to construct full Schild plots (Arunlakshana and Schild, 1959).

2.2. Isolated human arteries

Human cerebral (cortex) arteries were removed at neurosurgical tumor operations, omental arteries during gut operations, and coronary arteries after heart transplantation (from the explanted heart). All vessels were placed in buffer solution (mM: NaCl, 119; KCl, 4.7; $CaCl_2$, 1.5; $MgSO_4$, 1.17; $NaHCO_3$, 25; KH_2PO_4, 1.18; EDTA, 0.027; glucose, 5.5, pH 7.4) aerated with 5% CO_2 in O_2 (carbogen) and transported to the laboratory for investigation. The study was approved by Lund University Ethics Committee (LU99) and patient's approval.

The arteries were cut into cylindrical segments of 1 mm in length for in vitro pharmacological experiments. Each segment was mounted on two metal prongs, one of which was connected to a force displacement–transducer and attached to a computer, and the other to a displacement device. The position of the holder could be changed by means of a movable unit allowing fine adjustments of vascular tension by varying the distance between the metal prongs (Högestätt et al., 1983). The mounted specimens were immersed in temperature-controlled tissue baths (+37 °C) containing the buffer solution continuously gassed with carbogen, and the artery segments were allowed to equilibrate for approximately 30 min. The vessel tension was continuously recorded and the distance between the pins was adjusted to maintain a resting tone of 4 mN.

Following the 30-min equilibration period, the contractile capacity of each vessel segment was examined by exposure to a potassium-rich (60 mM) buffer solution that had the same composition as the standard solution except that the NaCl was exchanged for an equimolar concentration of KCl.

The vasodilatory effect of human α-CGRP was examined by cumulative application of increasing concentrations of the peptide in the absence or presence of various concentrations of the antagonists. Each segment was precontracted with 3 μM prostaglandin (PG) $F_{2\alpha}$ before CGRP was added. Each segment was exposed to a single cumulative concentration–effect curve and a matched pairs protocol was used where one segment acted as control (no antagonist present), whereas in another, six segments from the same artery; the agonist response was assessed following equilibration (10 min) with various concentrations of the antagonist.

The following materials were used in the in vitro experiments: human α-CGRP and human α-CGRP-(8-37) (Auspep, Australia) and prostaglandin $F_{2\alpha}$ (Dinoprost®, Upjohn). BIBN4096BS was synthesised by Medicinal Chemistry, Merck Sharp and Dohme Research Laboratories, UK and α- CGRP-(8-37) used in the binding and cAMP assays was synthesised by Medicinal Chemistry, Merck Research Laboratories, USA. α-CGRP, α- CGRP-(8-37) and $PGF_{2\alpha}$ were dissolved in water and stored as aliquots at −20 °C. BIBN4096BS was dissolved in dimethylsulphoxide (DMSO) and stored as aliquots at −20 °C. SK-N-MC cells were obtained from ATCC (USA). SK-N-MC membranes were purchased from Receptor Biology (USA). ^{125}I-cAMP direct screening kits were purchased from Amersham (UK).

2.3. Analysis of data

2.3.1. Blood vessel studies

The vasodilatory response was expressed relative to the contraction evoked by prostaglandin $F_{2\alpha}$ (=100%). For each segment, the maximum vasodilatory effect (E_{max}) was calculated. CGRP potency (expressed as pEC_{50}, i.e., negative logarithm of the molar concentration of agonist inducing half maximum response) was determined by non-linear regression analysis (Graph Pad Prism 3.0). Data are expressed as mean values ± S.E.M. and *n* refers to the number of patients from whom the vessels were collected. Statistically significant differences in pEC_{50} values were examined by Mann–Whitney *U*-test. Dose-ratios (DR) were calculated and used to construct full Schild plots (Arunlakshana and Schild, 1959). The dissociation constants were calculated as described by Tallarida et al. (1979).

3. Results

3.1. SK-N-MC binding studies

Saturation binding studies were carried out to characterise the interaction of CGRP with the receptor in SK-N-MC cells. Specific binding of ^{125}I-CGRP was saturable, with a measured dissociation constant (K_D) of 15 pM and a B_{max} of 110 fmol/mg of membrane protein (data not shown). Both CGRP-(8-37) and BIBN4096BS displaced ^{125}I-CGRP with high affinity, yielding pK_i values of 8.5 and 11.4, respectively (Fig. 1).

3.2. Functional studies

3.2.1. SK-N-MC cells

The effect of CGRP-(8-37) and BIBN4096BS on CGRP-induced cAMP production in SK-N-MC cells was investigated. Both compounds caused a rightward shift in the dose–response curve for CGRP. Schild analysis yielded a pA_2 value of 7.8 for CGRP-(8-37) with a slope of 1.2; a pA_2 value of 11.2 was obtained for BIBN4096BS, with a slope of 1.1 (Fig. 2). These results demonstrated that both compounds exhibited competitive antagonism at the CGRP receptor present in SK-N-MC cells.

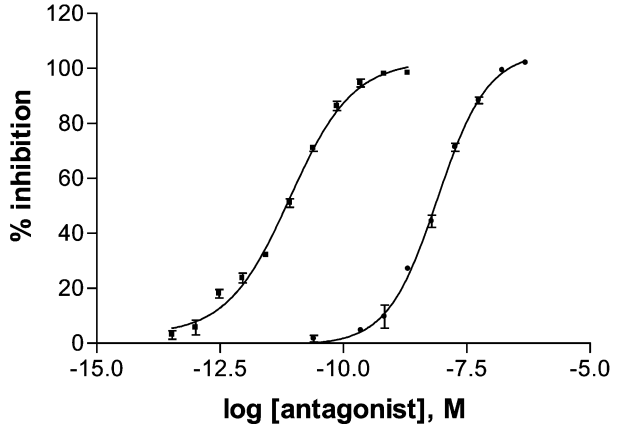

Fig. 1. Displacement of ^{125}I-CGRP binding from SK-N-MC membranes by CGRP-(8-37) (circles) and BIBN4096BS (squares). Values given represent mean ± S.E.M.

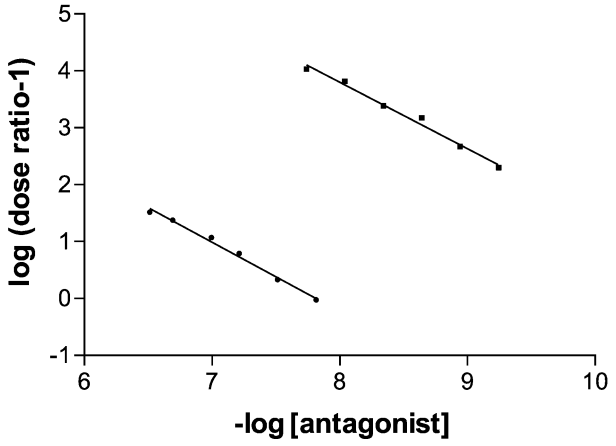

Fig. 2. Schild plots demonstrating the effects of CGRP-(8-37) (circles) and BIBN4096BS (squares) on CGRP-induced cAMP production in SK-N-MC cells. Values given represent mean ± S.E.M.

3.2.2. Human isolated arteries

In human cerebral arteries precontracted with prostaglandin $F_{2\alpha}$, CGRP caused a concentration-dependent relaxation, yielding a pEC_{50} value of 9.4 ± 0.2 and an E_{max} of 99 ± 1%. CGRP-(8-37) caused concentration-dependent parallel shifts to the right of the concentration–effect curve for α-CGRP without changing the maximum relaxant response. The pEC_{50} value for CGRP was significantly reduced from 9.4 ± 0.2 in the absence of CGRP-(8-37) to 6.7 ± 0.3 in the presence of CGRP-(8-37) (Table 1) resulting in a pA_2 value of 7.7. In human cerebral arteries, BIBN4096BS (0.1–10 nM) caused a parallel shift to the right of the concentration–effect curve to CGRP without changing the maximum

Table 1
pD_2 values of α-CGRP in absence and presence of CGRP receptor antagonists in human cerebral arteries

[Antagonist], M	BIBN4096BS	CGRP-(8-37)
0	9.6 ± 0.1	9.5 ± 0.2
10^{-10}	8.8 ± 0.2[a]	
3×10^{-10}	8.1 ± 0.2[a]	
10^{-9}	7.6 ± 0.3[a]	
10^{-8}		
10^{-7}		8.7 ± 0.3[a]
10^{-6}		7.9 ± 0.2[a]
10^{-5}		6.7 ± 0.3[a]

The pD_2 values are given as mean ± S.E.M. (number of individuals = twice number of vessel segments).

[a] pD_2 is significantly different from pD_2 control as evaluated by non-parametric *U*-test (Mann–Whitney *U*).

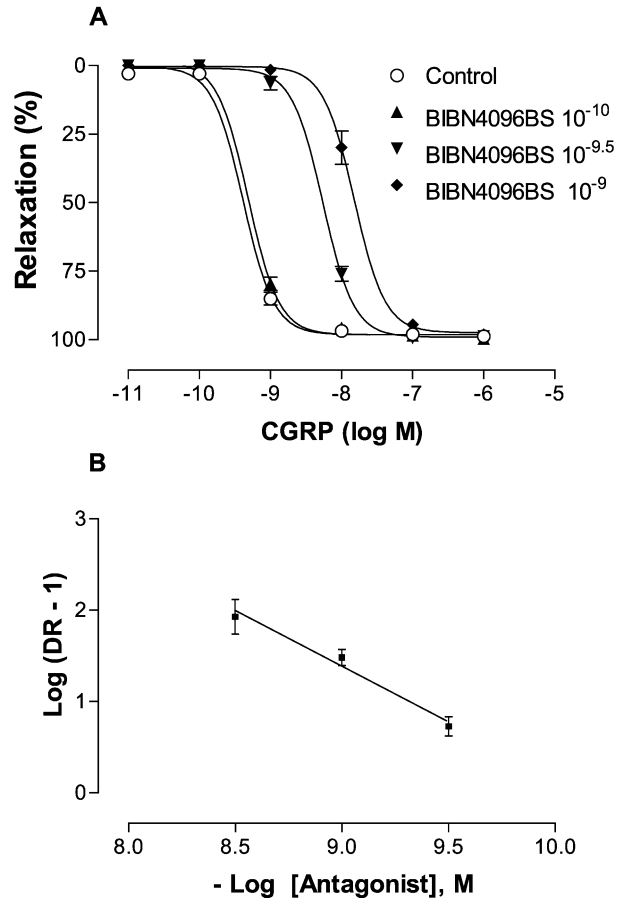

Fig. 3. Effect of CGRP in human cerebral arteries. (A) Concentration–response curves to CGRP in the presence and absence of BIBN4094BS. (B) Schild plot analysis of the antagonistic response of BIBN4096BS in human cerebral arteries. Values given represent mean ± S.E.M.

response (Fig. 3). Schild plot analysis revealed a pA_2 value of 10.1 for BIBN4096BS (the slope, 0.89, was not significantly different from unity).

Human coronary arteries also relaxed upon administration of CGRP in a concentration-dependent manner with a pEC_{50} of 8.3 and an E_{max} of 92.3 ± 5.6% (Fig. 4). CGRP-(8-37) (0.1–10 μM) and BIBN4096BS (0.1–10 nM) inhibited

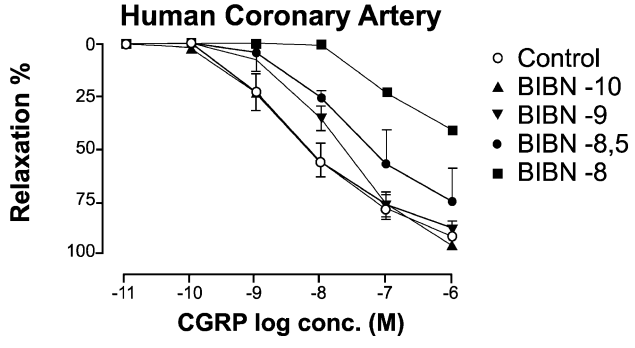

Fig. 4. Relaxant effect of CGRP and the antagonistic effect of BIBN4096BS in human coronary arteries. Values given represent mean ± S.E.M.

this response in a competitive manner. The pA_2 values were 7.3 and 10.4, respectively (regression lines did not differ from unity). In human mesenteric arteries, CGRP failed to induce a reliable relaxation of precontracted vessel segments (maximum effect, 2.7 ± 0.6%).

4. Discussion

In the present study, we have shown that BIBN4096BS is a potent competitive antagonist at human cerebral and coronary artery $CGRP_1$ receptors. The antagonistic properties were clarified both in competition binding experiments in SK-N-MC cells and in a functional cAMP assay. The profile agrees well with our previous study of a related antagonist, Compound 1 (Edvinsson et al., 2001; Hasbak et al., in press), although BIBN4096BS was more potent. As outlined previously, both of these antagonists are more selective for human CGRP receptors than CGRP receptors from experimental laboratory animals (Doods et al., 2000; Edvinsson et al., 2001). This is at some variance with the recently reported CGRP receptor antagonist SB-273779, which was active at submicromolar concentrations and also demonstrated some activity at rodent and porcine CGRP receptors (Aiyar et al., 2001).

CGRP receptors have long been regarded as a useful target for the development of novel antimigraine therapies. In the present study, we have further characterised the receptor for CGRP in human arteries by comparing the effects of CGRP-(8-37) with those of BIBN4096BS. It was previously reported that SK-N-MC cells, which express an endogenous CGRP receptor, display similar pharmacology to a recombinant cell line expressing only CRLR and RAMP1 (McLatchie et al., 1998). We therefore used this cell line to compare the binding and antagonistic properties of CGRP-(8-37), Compound 1 (Edvinsson et al., 2001) and presently of BIBN4096BS. All compounds potently displaced ^{125}I-CGRP from SK-N-MC membranes and functioned as competitive antagonists of CGRP-induced cAMP accumulation in SK-N-MC cells. Based on these observations, it is likely that these compounds all act at CGRP receptors composed of CRLR and RAMP1.

In human isolated cerebral and coronary arteries, BIBN4096BS, Compound 1 and CGRP-(8-37) antagonised the relaxant responses evoked by CGRP, consistent with previous reports (Edvinsson et al., 2001; Hasbak et al., in press; Jansen-Olesen et al., 1996). The pA_2 values were similar to those obtained in the SK-N-MC cell line, suggesting that the receptor mediating the vasodilatory effects of CGRP is similar to the receptor in the cell line, that is, CRLR/RAMP1. This observation is supported by findings from RT-PCR studies showing expression of mRNAs coding for these proteins in human cranial arteries (Sams and Jansen-Olesen, 1998) and in human coronary arteries (Hasbak et al., in press). Interestingly, the human mesenteric or omental arteries did not respond to the application of CGRP.

The reason for this is not clear, but other peripheral arteries such as subcutaneous arteries show good responses to CGRP (see Hasbak et al., in press).

Responses to CGRP in both cerebral and coronary arteries were inhibited by BIBN4096BS with similar pA_2 values, suggesting that these responses are mediated through the same receptor subtype. CGRP was approximately 10-fold less potent on coronary than cerebral arteries (pEC_{50} values of 8.3 and 9.4, respectively). The reason for this difference in agonist potency is not entirely clear, but may be related to differences in receptor density in the two arteries, as has similarly been suggested for the differences in potency of triptans in these two vessel types (Longmore et al., 1998). Other possibilities include potential differences in the coupling pathways or downstream effector ion channels, or compensatory mechanisms in the coronary artery.

The lack of antagonistic effect of Compound 1 on the guinea-pig CGRP receptor (Edvinsson et al., 2001) may reflect species differences in receptor pharmacology for this class of compounds. Indeed BIBN4096BS has been reported to have greater than 200-fold reduced affinity for rat CGRP receptors compared to affinity in the SK-N-MC cell line (Doods et al., 2000). The underlying molecular mechanisms responsible for this species selectivity await clarification.

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References

Aiyar, N., Daines, R.A., Disa, J., Chambers, P.A., Sauermelch, C.F., Quiniou, M.-J., Khandoudi, N., Gout, B., Douglas, S.A., Willette, R.N., 2001. Pharmacology of SB-273779, a nonpeptide calcitonin gene-related peptide 1 receptor antagonist. J. Pharmacol. Exp. Ther. 296, 768–775.

Arunlakshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58.

Bell, D., McDermott, B.J., 1996. Calcitonin gene-related peptide in the cardiovascular system: characterization of receptor populations and their (patho)physiological significance. Pharmacol. Rev. 48, 254–288.

Cheng, Y.-C., Prusoff, W.H., 1973. Relationship between the inhibition constant, (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22, 3099–3108.

Dennis, T.B., Fournier, A., St. Pierrre, S., Quirion, R., 1989. Structure–activity profile of calcitonin gene-related peptide in peripheral and brain tissues: evidence for receptor multiplicity. J. Pharmacol. Exp. Ther. 251, 718–725.

Doods, H., Hallermayer, G., Dongmei, W., Entzeroth, M., Rudolf, K., Engel, W., Eberlein, W., 2000. Pharmacological profile of BIBN4096BS, the first selective small molecule CGRP antagonist. Br. J. Pharmacol. 129, 420–423.

Edvinsson, L., 1985. Functional role of perivascular peptides in the control of cerebral circulation. Trends Neurosci. 8, 126–131.

Edvinsson, L., Sams, A., Jansen-Olesen, I., Tajti, J., Kane, S.A., Rutledge, R.Z., Koblan, K.S., Hill, R.G., Longmore, J., 2001. Characterization of the effects of a non-peptide CGRP receptor antagonist in SK-N-MC cells and isolated human cerebral arteries. Eur. J. Pharmacol. 415, 39–44.

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. 133 (suppl. 569), 1–63.

Goadsby, P.J., Edvinsson, L., 1994. Human in vivo evidence for trigeminovascular activation in cluster headache. Neuropeptide changes and effects of acute attacks therapies. Brain 117, 427–434.

Goadsby, P.J., Edvinsson, L., Ekman, R., 1988. Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system. Ann. Neurol. 23, 193–196.

Goadsby, P.J., Edvinsson, L., Ekman, R., 1990. Vasoactive peptide release in the extracerebral circulation of human during migraine headache. Ann. Neurol. 28, 183–197.

Hasbak, P., Saetrum Opgaard, O., Eskesen, K., Schifter, S., Arendrup, H., Longmore, J., Edvinsson, L., 2001. Effect of "Compound 1" (WO98/11128) a novel non-peptide CGRP receptor antagonist and alpha-CGRP-(S-37) in the human coronary arteries. J. Pharmacol. Exp. Ther. (in press).

Högestätt, E.D., Andersson, K.-E., Edvinsson, L., 1983. Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. Acta Physiol. Scand. 117, 49–61.

Jansen-Olesen, I., Mortensen, A., Edvinsson, L., 1996. Calcitonin gene-related peptide is released from capsaicin-sensitive nerve fibres and induces vasodilation of human cerebral arteries concomitant with activation of adenylyl cyclase. Cephalalgia 16, 310–316.

Juaneda, C., Dumont, Y., Quirion, R., 2000. The molecular pharmacology of CGRP and related peptide receptor subtypes. Trends Pharmacol. Sci. 21, 432–438.

Juul, R., Edvinsson, L., Gisvold, S.E., Ekman, R., Brubakk, A.O., Frederiksen, T.A., 1990. Calcitonin gene-related peptide-LI in subarachnoid haemorrhage in man. Signs of activation of the trigemino-cerebrovascular system? Br. J. Neurosurg. 41, 71–180.

Juul, R., Hara, H., Gisvold, S.E., Brubakk, A.O., Frederiksen, T.A., Waldemar, G., Schmidt, J.F., Ekman, R., Edvinsson, L., 1995. Alterations in perivascular dilatory neuropeptides (CGRP, SP, VIP) in the external jugular vein and in the cerebrospinal fluid following subarachnoid haemorrhage in man. Acta Neurochir. 32, 32–41.

Longmore, J., Razzaque, Z., Shaw, D., Davenport, A.P., Maguire, J., Pickard, J.D., Schofield, W.N., Hill, R.G., 1998. Comparison of the vasoconstrictor effects of rizatriptan and sumatriptan in human isolated cranial arteries: immunohistological demonstration of the involvement of 5-HT_{1B}-receptors. Br. J. Clin. Pharmacol. 46, 577–582.

McLatchie, L.M., Fraser, N.J., Main, M.J., Wise, A., Brown, J., Thompson, N., Solari, R., Lee, M.G., Foord, S.M., 1998. RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 293, 333–339.

Saetrum Opgaard, O., Hasbak, P., de Vries, R., Saxena, P.R., Edvinsson, L., 2000. Positive inotropy mediated via CGRP receptors in isolated human myocardial trabeculae. Eur. J. Pharmacol. 397, 373–382.

Sams, A., Jansen-Olesen, I., 1998. Expression of calcitonin receptor-like receptor (CRLR) and receptor-activity-modifying proteins in human cranial arteries. Neurosci. Lett. 258, 41–44.

Tallarida, R.J., Cown, A., Adler, M.W., 1979. pA_2, and receptor differentiation: a statistical analysis of competitive antagonism. Life Sci. 25, 637–654.

Uddman, R., Edvinsson, L., Ekman, R., McCulloch, J., Kingman, T.A., 1985. Innervation of feline cerebral vasculature by nerve fibres containing calcitonin gene-related peptide: trigeminal origin and coexistence with substance P. Neurosci. Lett. 62, 131–136.

Uddman, R., Edvinsson, L., Ekblad, E., Håkanson, R., Sundler, F., 1986. Calcitonin gene-related peptide (CGRP): perivascular distribution and vasodilatory effects. Regul. Pept. 15, 1–23.