Pharmacology of SB-273779, a Nonpeptide Calcitonin Gene-Related Peptide 1 Receptor Antagonist


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ABSTRACT
Calcitonin gene-related peptide (CGRP), a potent vasodilatory and cardiotonic peptide, has a potential role for CGRP in diverse physiologic and pathophysiologic situations such as congestive heart failure, diabetes, migraine, and neurogenic inflammation. Although a peptide CGRP receptor antagonist, CGRP8-37, is available, its utility presents significant limitations for these indications. Here, we describe the properties of SB-(+)-273779 [N-methyl-N-[2-(methylphenyl)-3-nitro-4-(2-thiazolylsulfinyl)nitrobenzani]de], a selective nonpeptide antagonist of CGRP1 receptor. SB-(+)-273779 inhibited 125I-labeled CGRP binding to SK-N-MC (human neuroblastoma cells) and human cloned CGRP1 receptor with IC50 values of 310 ± 40 and 250 ± 15 nM, respectively. SB-(+)-273779 also inhibited CGRP (3 nM)-activated adenyly cyclase in these systems with IC50 values of 390 ± 10 nM (in SK-N-MC) and 210 ± 16 nM (recombinant human CGRP receptors). Prolonged treatment (>30 min) of SK-N-MC cells with SB-(+)-273779 followed by extensive washing resulted in reduction in maximum CGRP-mediated adenyly cyclase activity, suggesting that this compound has irreversible binding characteristics. In addition, SB-(+)-273779 antagonized CGRP-mediated 1) stimulation of intracellular Ca2+ in recombinant CGRP receptors in HEK-293 cells, 2) inhibition of insulin-stimulated [14C]deoxyglucose uptake in L6 cells, 3) vasodilation in rat pulmonary artery, and 4) decrease in blood pressure in anesthetized rats. SB-(+)-273779 tested at 3 μM had no significant affinity for calcitonin, endothelin, angiotensin II, and α-adrenergic receptors under standard ligand binding assays. SB-(+)-273779 also did not inhibit forskolin and pituitary adenylate cyclase-activating polypeptide. These results suggest that SB-(+)-273779 is a valuable tool for studying CGRP-mediated functional responses in complex biological systems.

Calcitonin gene-related peptide (CGRP) is a 37-amino acid peptide that is synthesized from alternate processing of the calcitonin gene mRNA (Amara et al., 1982). CGRP is produced in sensory neurons and is transported peripherally to terminal areas in visceral organs and centrally to the dorsal horn. In the cardiovascular system, CGRP is a potent vaso- dilator in several species (Bell and McDermott, 1996) and produces an increase in heart rate (Franco-Cereceda, 1988). CGRP has a positive inotropic effect on isolated rat ventricular cardiomyocytes (Bell and McDermott, 1994) and in isolated trabeculae from porcine right atria and left ventricles (Saetrum Opgaard et al., 1999). CGRP is also a potent inhibitor of insulin-mediated glycogen synthesis in skeletal muscle with effects in the low nanomolar range (Poyner, 1992; Aiyar et al., 1996; Bell and McDermott, 1996; Wimalawansa, 1997; Franco-Cereceda and Liska, 1999).

CGRP initiates responses through an interaction with target organ receptors that are primarily coupled to the activation of adenyly cyclase. CGRP receptors have been identified and characterized from several tissues of neuronal and peripheral origin. CGRP mediates its action via two functional receptors subtypes, the CGRP1 and the CGRP2 receptor (Dennis et al., 1989). The fragment CGRP8-37 is a selective functional antagonist for CGRP1 receptors. Reduction of the disulfide bond of CGRP, which destroys the N-terminal ring structure of the peptide, yields a linear analog, diaacetamidomethyl cysteine CGRP ([Cys (ACM)2,7]CGRP), that is a selective agonist for CGRP2 receptors. CGRP1 receptors have been cloned from human (Aiyar et al., 1996), rat (Han et al., 1997), and pig (Elshourbagy et al., 1998). They show 91 to 95% identity at the amino acid level among species. The receptor is a seven-transmembrane domain receptor protein, which belongs to a subgroup of the G-protein-coupled receptor family, and is predominantly expressed in heart and lung.
with limited success because of its peptidic nature. CGRP8-37 CGRP are present in the circulation (Wimalawansa, 1997).

including migraine, type 2 diabetes, inflammation, and actions in vitro (Wimalawansa, 1997). In skeletal muscle, CGRP impairs glycogen synthesis and enhances glycogenolysis, glycolysis, and lactate production. CGRP, like amylase, decreases basal and insulin-stimulated glycogen synthesis in muscle and stimulates lactate production from isolated soleus muscle. Taken as a whole, these results indicate a potential role for CGRP to the pathophysiology of type 2 diabetes.

Evidence is accumulating that inappropriate release of CGRP is a potential causative factor in several diseases, including migraine, type 2 diabetes, inflammation, and congestive heart failure. These postulations are derived from the findings that increased concentrations of immunoreactive CGRP are present in the circulation (Wimalawansa, 1997). To determine the endogenous role of CGRP, investigators have used the CGRP receptor peptide antagonist, CGRP8-37, with limited success because of its peptidic nature. CGRP8-37 displays a wide range of affinities against CGRP responses across different species and tissues. The apparent pA2 values of CGRP8-37 in various assays are highly variable, with values ranging between 6.0 to greater than 9.0. It is also known that CGRP8-37 had vasodilator activity at micromolar concentrations in the pulmonary artery, indicating partial agonistic property (Wisskirchen et al., 1998). Identification of a non-peptide CGRP antagonist would provide an excellent tool to study the role of CGRP. Such compounds will be a promising lead for novel therapeutic agents for the treatment of type-2 diabetes, migraine, and pain. Based on these considerations, we screened the SmithKline Beecham (SB) chemical collection bank for novel CGRP receptor ligands, and identified a peptide CGRP antagonist, CGRP8-37, and test compound by itself at 1 and 10 μM, and test compound by itself at 10 and 100 μM. After the binding assay was done for 30 min at 25°C, the reaction mixture was rapidly diluted with 2 ml of cold wash buffer (0.9% NaCl) followed by rapid filtration over Skatron Filter Mates precoated in 0.2% polyethyleneimine using a Skatron cell harvester (Skatron Instruments, Lier, Norway). All binding assays were done in duplicate, and each experiment was repeated three to four times. Analysis of all binding data (i.e., the determination of Kd, Bmax, and Kd values) were performed by computer-assisted nonlinear least square fitting using PRIZM (GraphPad Software, Inc., San Diego, CA). Nonspecific binding was defined using hCGRP at a final concentration of 1 μM. The radioactivity was determined using a gamma counter (Packard Instrument, Meriden, CT).

**Adenylyl Cyclase Assay.** Membrane-bound adenylyl cyclase activity was determined as the rate of conversion of [α-32P]ATP to [32P]cAMP as previously described (Elshourbagy et al., 1998). Membranes (40–60 μg of protein), various concentrations of test compounds (10 nM to 10 μM), and 5 nM CGRP were incubated in triplicate tubes in buffer containing 50 mM Tris-HCl, pH 7.4, 10 mM MgCl2, 1.2 mM ATP, 1.0 μC of [32P] ATP, 0.1 mM CAMP, 2.8 mM phosphoenolpyruvate, and 5.2 μg/ml myokinase in a final volume of 100 μl for 20 min at 30°C. Experiments were also done to measure the adenylyl cyclase activity in the absence of CGRP (basal activity) and test compound by itself at 1 and 10 μM. The reactions were stopped with 1 ml of a solution containing 0.28 mM cAMP, 0.33 mM ATP, and 22,000 cpm [3H]cAMP. [32P]cAMP was separated using sequential chromatography on Dowex and alumina columns (Salmon et al., 1974). The results are expressed as percentage of inhibition of CGRP-mediated adenylyl cyclase activity at various concentrations of test compounds.

**Intracellular [Ca2+]i, Mobilization.** CGRP-mediated intracellular [Ca2+]i, mobilization was studied using recombinant porcine CGRP receptor stably expressed in HEK-293 cells as discussed (Aiyar et al., 1999). The effect of SB-273779 (at 0.1, 1.0, and 10 μM) was studied on basal and CGRP-mediated [Ca2+]i mobilization in recombinant porcine CGRP receptor.

**[14C]Deoxyglucose Uptake.** L6 cells were obtained from the American Type Culture Collection and grown in 6-well plates in MEM containing 2% bovine serum. Experiments were done following the procedure of Kreutter et al. (1989). In brief, cells were serum-
depleted for 5 h in aMEM. During the final 60 min of this period, the cells were treated with insulin (1 μM) or insulin (1 μM) plus SB-273779 (1.0 μM) or CGRP (10 nM) or insulin (1 μM) plus CGRP (10 nM) and SB-273779 (1 μM). The cells were washed, and uptake of 0.1 mM [1-14C]2-deoxyglucose was initiated. Incubations were performed in triplicate for 10 min at room temperature. Uptake was terminated by aspirating the buffer and washing the cells with ice-cold PBS. Cells were dissolved in 0.1 N NaOH and counted in a liquid scintillation counter (Beckman Instruments, Fullerton, CA).

**Vasorelaxation Measurements in Pulmonary Artery.** All animals were housed in an accredited laboratory animal facility, and all procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health, Education, and Welfare; Department of Health and Human Services publication NIH 85-23). The Animal Care and Use Committee at SmithKline Beecham Pharmaceuticals approved all procedures. Male Wistar rats (275–300 g, Janvier, Le Genest, France) were anesthetized with sodium thiopentone (50 mg/kg i.p., Nesdonal, Rhône Mérieux, Lyon, France) before excision of the pulmonary artery. The vessel placed in cold Krebs-Henseleit solution oxygenated with 95% O2/5% CO2 mixture at 37°C. The composition of the Krebs-Henseleit solution was as follows (in mM): NaCl (95), KCl (5), CaCl2 (2.6), MgSO4 (1.2), KH2PO4 (1.2), NaHCO3 (24.9), and D-glucose (10), pH 7.4. The tension was measured isometrically using a force transducer (EMKA Technologies, Paris, France) and recorded on chart paper (RS-3400, Gould Instruments, Ballainvilliers, France).

After equilibration for a minimum period of 45 min, at an optimal resting tension of 0.7 g, each ring was precontracted with repeated application of phenylephrine (0.1 μM). The functional integrity of the endothelium was evaluated by assessing the relaxation induced by 1.0 μM acetylcholine in precontracted vessels. Test drugs were then applied at the maximum contraction induced by phenylephrine, and the vasorelaxing effect of CGRP (3.0 nM) and SB-273779 (1.0 μM) or CGRP (10 nM) or insulin (1 μM) plus CGRP (10 nM) and SB-273779 (1 μM). The reversibility of inhibitory action caused by phenylephrine. The inhibitors were applied 20 min

**Results**

High-throughput screening of SB-compound libraries led to the identification of a low-affinity lead, SB-211973 (3 μM), that served as a starting point for subsequent chemical modifications. Optimization of this activity by structural modifications of the lead compound resulted in a series of nitrobenzamides as represented by a racemic compound, SB-268262 (thiazolylsulfoxide nitrobenzanilide) (Table 1). The individual enantiomers of SB-268262 were separated via chiral HPLC (SB-(+)-273779 and SB-(−)-273780) and evaluated. SB-(+)-273779 was 5-fold more active than SB-(−)-273780 in the cyclase assay and 2-fold more active than

**TABLE 1**

Chemical structure of selected nonpeptide CGRP receptor antagonists

The values represent the inhibitory concentrations obtained both in [125I]CGRP binding and CGRP-mediated adenylyl cyclase stimulation in SK-N-MC cell membranes.

<table>
<thead>
<tr>
<th>SB-Number</th>
<th>Compound</th>
<th>Binding IC50</th>
<th>Cyclase IC50</th>
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</thead>
<tbody>
<tr>
<td>SB-211973</td>
<td><img src="https://example.com/image1.png" alt="Image" /></td>
<td>1.93 ± 0.47</td>
<td>0.9 ± 0.37</td>
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<tr>
<td>SB-(±)-268262</td>
<td><img src="https://example.com/image2.png" alt="Image" /></td>
<td>0.24 ± 0.03</td>
<td>0.83 ± 0.29</td>
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<tr>
<td>SB-(+)-273779</td>
<td><img src="https://example.com/image3.png" alt="Image" /></td>
<td>0.31 ± 0.04</td>
<td>0.39 ± 0.01</td>
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<tr>
<td>SB-(−)-273780</td>
<td><img src="https://example.com/image4.png" alt="Image" /></td>
<td>0.69 ± 0.19</td>
<td>2.07 ± 0.17</td>
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</tbody>
</table>
SB-(±)-268262, SB-(+)

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evaluate the affinity for SB-273779 (10 μM) for Ca²⁺ receptor antagonist, CGRP 8-37, by itself had no effect on stimulated the release of intracellular [Ca²⁺].

Due to lack of selective CGRP receptor antagonist, we could not selectivity. These data suggest that SB-273779 had no significant affinity for the following receptors: recombinant human endothelin A and B and α-adrenoreceptors (α₁A, α₂A) (in Chinese hamster ovary cells), calcitonin (T47D), and angiotensin II (rat adrenal cortex) when tested at 3 μM concentration with standard ligand binding assays. SB-273779 also did not inhibit forskolin or pituitary adenylyl cyclase-activating polypeptide-stimulated cAMP in SK-N-MC cells. In contrast, the compound inhibited adrenomedullin binding and cAMP response with Kᵢ value of 1.5 μM in human calcitonin receptor-like receptor/receptor activity modifying protein-2 (RAMP2) in HEK-293 cells (data not shown). These data suggest that SB-273779 is a selective CGRP receptor antagonist. Due to lack of selective CGRP₂ receptor system (native or recombinant), we could not evaluate the affinity for SB-273779 in CGRP₂ receptor.

In addition to the activation of adenyl cyclase, CGRP stimulated the release of intracellular [Ca²⁺] in HEK-293 cells expressing recombinant human and porcine CGRP receptors with an EC₅₀ value of 1.7 ± 0.4 nM. The CGRP receptor antagonist, CGRP₈-₃₇, by itself had no effect on [Ca²⁺] release but inhibited the CGRP-stimulated [Ca²⁺] release (10 nM). The nonpeptide antagonist, SB-273779, at 1.0 μM effectively blocked the CGRP-mediated cytosolic [Ca²⁺] increase. The inhibitory effect was specific, since the compound has no effect on endothelin-1-mediated [Ca²⁺] release in these cells (Fig. 6).

To investigate the effect of SB-273779 on CGRP-mediated carbohydrate metabolism, we used L6 skeletal muscle cells. L6 cells have functional CGRP receptors that are linked to stimulation of adenyl cyclase, and this can be competitively antagonized by CGRP₈-₃₇. Treatment of L6 cells with 1 μM insulin for 60 min stimulated [¹⁴C]2-deoxyglucose uptake by 2-fold. Inclusion of CGRP during the incubation period suppressed insulin-mediated deoxyglucose uptake. The maximum inhibition reached ~70% after incubation with 10 nM CGRP. Both CGRP₈-₃₇ and SB-273779 have no direct effect on insulin-stimulated deoxyglucose uptake, but are able to reverse the CGRP-mediated suppression in a concentration-dependent manner (Fig. 7).

The effect of SB-273779 was also investigated in vasorelaxation experiments in vitro. Application of the solvent DMSO before CGRP, for 20 min, did not alter the vasorelaxing efficacy of the peptide. CGRP (3 nM) caused 21.6 ± 2.9% vasorelaxation in phenylephrine-contracted vessel rings. This effect was reduced by 76 ± 6% following application of the peptide antagonist, CGRP₈-₃₇, at 1.0 μM (20 min before CGRP, Fig. 8). Pretreatment with SB-273779 from 0.1 to 1.0 μM caused a concentration-dependent inhibitory effect on the CGRP-mediated vasorelaxation. The inhibitory effect of SB-273779 reached 12 ± 9% (0.1 μM) and 35 ± 8% (0.3 μM). At higher concentration (1.0 μM), the effect of CGRP was reduced by 66 ± 4% (n = 5) (Fig. 9). The inhibitory effect of SB-273779 demonstrated a long duration of action. As shown in Fig. 9, initial recovery of vasorelaxing response to CGRP started to occur 40 min after removal of SB-273779 at 0.3 μM but not at 1.0 μM. In contrast, the effect of CGRP₈-₃₇ was completely reversed after 20 min of washout. We also investigated the effect of SB-273779 on the hypotensive effect mediated by bolus administration of CGRP. Intravenous administration of CGRP (100 or 300

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**Fig. 5.** SK-N-MC cells were pretreated with vehicle (control) or SB-273779 (10 μM) for 10 or 60 min at room temperature. Adenylyl cyclase activity was measured in membranes prepared from washed cells. A, CGRP activation of adenylyl cyclase in control and SB-273779 pretreated cells. B, CGRP (100 nM), vasoactive intestinal peptide (100 nM), isoproterenol (1 μM), and forskolin (10 μM)-activated adenylyl cyclase. Data are presented as percentage change over basal from a representative of three independent experiments done in duplicate.

**Fig. 6.** Effect of SB-273779 on CGRP-mediated Ca²⁺ release in HEK-293 cells expressing recombinant porcine CGRP receptor. Cells were incubated with SB-273779 (0.3 or 1.0 μM) for 10 min before adding 10 nM CGRP. Endothelin-1-mediated Ca²⁺ release used as positive control was not altered by pretreatment with SB-273779. The data shown are mean ± S.E.M. from three independent experiments.
pmol/kg) to the anesthetized rat pretreated with atropine and propranolol produced a dose-related reduction in arterial blood pressure (Fig. 10, A and B). Pretreatment with SB-(+)-273779 (10 min before the administration of CGRP) at 10 mg/kg antagonized the hypotensive effects of CGRP induced by both 100 (A) and 300 (B) pmol/kg (i.v.). No inhibitory effect was observed at a dose of 1 mg/kg of SB-(+)-273779. SB-273779 treatment had no effect on the transient hypotensive effects produced by sodium nitroprusside (30 ug/kg i.v.) administration (data not shown).

Discussion

In the present study, we have demonstrated that SB-(+)-273779, a submicromolar-selective nonpeptide antagonist of the CGRP receptor, shows in vitro and in vivo activities. SB-(+)-273779 is a selective inhibitor of [125I]CGRP binding to membranes isolated from human neuroblastoma cells (SK-N-MC) and HEK-293 cells expressing recombinant CGRP receptor with submicromolar (200–300 nM) affinity. The compound inhibited [125I]CGRP binding to membranes isolated from porcine or rat tissues by ~40% only, even at 10 μM concentration. The specificity of SB-(+)-273779 for CGRP receptors was demonstrated by its lack of activity (IC_{50} > 3 μM) in other radioligand binding assays, including recombinant human endothelin (A and B), α-adrenergic receptors as well as calcitonin receptors in T47D cells and rat angiotensin II receptors. To determine whether SB-(+)-273779 interacts reversibly or irreversibly with CGRP receptors, [125I]CGRP binding in SK-N-MC cell membranes was determined in the presence and absence of SB-(+)-273779. In the Scatchard analysis of the binding data, SB-273779 decreased the slope without affecting the intercept. Thus, the interaction of SB-(+)-273779 with the CGRP receptor is reversible and competitive. Recently, Dodds et al. (2000) reported a novel CGRP receptor antagonist, BIBN 4096BS, with picomolar affinity for [125I]CGRP binding to SK-N-MC cell membranes. Based on [125I]CGRP radioligand binding studies performed in human (SK-N-MC) and rat (spleen) membranes, BIBN 4096BS has been characterized as a human-selective antagonist (Dodds et al., 2000). Thus, SB-273779 is further distin-
A. CGRP (100 pmol/kg, iv)

![Graph showing change in MAP vs. time](image)

B. CGRP (300 pmol/kg, iv)

![Graph showing change in MAP vs. time](image)

Fig. 10. Inhibition of CGRP-mediated hypotensive effect of SB-([+])-273779. CGRP was administered at time 0 (100 and 300 pmol/kg i.v. in A and B, respectively). SB273779 was administered 10 min before CGRP administration, and mean arterial pressure (MAP) was measured. *Differs significantly from the vehicle control (p < 0.05). n = 4 to 5 per group.

SB-273779 is a potential antagonist of the rat and porcine CGRP receptors. Several functional and in vitro studies were conducted to characterize the nature of interaction of SB-([+])-273779 with the CGRP receptor. SB-([+])-273779 inhibited CGRP-mediated adenylyl cyclase activity in SK-N-MC cell membranes and recombinant CGRP1 receptor (human and porcine) in HEK-293 cell membranes, with IC50 values of 200 to 300 nM. In these assays, SB-([+])-273779 (0.1 and 1.0 μM concentrations) produced a parallel shift in the CGRP concentration-response curve to the right without changing the maximum response suggesting competitive antagonism. However, prolonged treatment (>30 min) of SK-N-MC cells with SB-([+])-273779 (3 μM) followed by extensive washing revealed an apparent irreversible property on the compound. SB-([+])-273779 had no effect on forskolin or pituitary adenylyl cyclase-activating polypeptide-mediated adenylyl cyclase activity in SK-N-MC cell membranes. Additionally, SB-([+])-273779 inhibited CGRP-mediated intracellular [Ca2+]i release from cloned CGRP1 receptor (human and porcine) expressed in HEK-293 cells.

In skeletal muscle, CGRP impairs glycogen synthesis and enhances glycogenolysis and lactate production. CGRP, like amylin, decreases basal and insulin-stimulated glycogen synthesis in muscle and stimulates lactate production from isolated soleus muscle (Cooper, 1994). Taken as a whole, these results indicate a potential role for CGRP for the pathophysiology of type 2 diabetes. Muscle has been suggested as an ideal tissue for glucose-uptake study because of its involvement in glucose utilization in vivo. However, the use of skeletal muscle tissue has several disadvantages for glucose-uptake studies (limited and inconsistent exposure of fibers to substrate). Alternatively, skeletal muscle cells in culture have proven to be a good model system for glucose-transport studies. Kreutter et al. (1989) have reported CGRP-mediated deoxyglucose uptake studies using L6 skeletal muscle cells. L6 cells have functional CGRP receptors that are linked to stimulation of adenylyl cyclase, and this can be competitively antagonized by CGRP8-37.

CGRP inhibited insulin-stimulated glucose uptake in a concentration-dependent manner. The maximal inhibition (70–80%) was observed at 100 nM CGRP. CGRP had no effect on basal glucose uptake in the cells. CGRP8-37 blocked CGRP effect at a concentration of 300 nM. It was less potent in blocking CGRP effect on glucose uptake compared with its effect on CGRP-mediated adenylyl cyclase activation. SB-([+])-273779 also reversed the CGRP effect by >90% at 3 μM. As a negative control, several inactive compounds from benzanilide analogs were tested both in cyclase and binding assays (data not shown), which also had no effect on CGRP-mediated inhibition of glucose uptake.

The vasorelaxant activity of CGRP isoforms has been well documented within the mammalian vasculature. Indeed, such responses have been reported in a diverse range of vessels, including those isolated from rat (Mannan et al., 1995; Wisskirchen et al., 1998; Kawasaki et al., 1999), hamster (Hall and Brain, 1999), pig (Wisskirchen et al., 1999), and human (Thom et al., 1987).

To this end, the present study examined the ability of SB-273779 to attenuate the CGRP1-mediated vasodilator actions of CGRP in the isolated rat pulmonary artery. According to the ability of SB-([+])-273779 to attenuate CGRP-induced cAMP accumulation in SK-N-MC cells (IC50 300 ± 18 nM), this benzanilide moiety inhibited the endothelial CGRP1 receptors responsible for transducing the relaxant responses induced by CGRP in the rat isolated pulmonary artery preconstricted with phenylephrine. Inhibition was concentration-dependent and was observed over a concentration range (0.1–1.0 μM) entirely consistent with a CGRP1-selective antagonist. As shown in Fig. 9, SB-273779 exhibited a poor reversibility of inhibitory action on CGRP-mediated relaxation in pulmonary artery. This effect of SB-273779 was also reported in experiments in vitro on the inhibition of CGRP-mediated adenylyl cyclase stimulation but was not observed with the peptide antagonist, CGRP8-37. It may be suggested that the functional antagonistic action of SB-273779 is mediated via interaction with a binding site on the receptor, which is distinct from that of CGRP itself. This hypothesis is supported by the low potency of SB-273779 to displace [125I]CGRP binding from rat membranes.

In vivo, the intravenous administration of CGRP has been
shown to decrease arterial blood pressure, in a dose-dependent manner, by reducing total peripheral resistance (Lappe et al., 1987). CGRP-mediated reductions in arterial blood pressure are inhibited by the reversible peptide antagonist, CGRP9-37. In the present study, we found that the intravenous administration of SB-(+)-273779 significantly reduced the dose-related hypertensive effects elicited by CGRP. The actions of SB-(+)-273779 were selective and did not alter direct nitrate-mediated smooth muscle relaxation and hypertension induced by the intravenous administration of sodium nitroprusside. In addition, the weak in vivo potency of SB-(+)-273779 precluded a more vigorous in vivo characterization. Doses greater than 10 (or 30 if any) mg/kg i.v. were not well tolerated, and a dose of 10 mg/kg i.v. did not inhibit significantly the CGRP-mediated hypertension. However, we did attempt to evaluate the role of CGRP in modulating insulin sensitivity in the Zucker rat, but we were not able to observe a consistent effect of CGRP on insulin sensitivity (CGRP9-37, CGRP receptor antagonist failed to reduce insulin sensitivity in the model). Furthermore, we also attempted to evaluate the effect of SB-273779 on CGRP-induced hyperlactemia in normal rats. SB-273779 at 0.1 and 1.0 μmol/kg/min had no effect. At higher concentrations, the compound precipitated out. Preliminary studies also suggest that the pharmacokinetic profile of SB-273779 (poor oral bioavailability and short half-life of ~10 min) makes it unsuitable for use in chronic animal studies where repeated enteral administration is preferred.

In summary, SB-(+)-273779 is a selective CGRP receptor antagonist with functional activities. SB-273779 effectively inhibits CGRP-mediated vasorelaxation in vitro and in vivo. Although the apparent irreversibility of this interaction, at least in vitro, may not represent the optimal therapeutic profile for a CGRP antagonist, the nature of this interaction does not reduce the potential experimental utility of SB-273779. This antagonist, the first nonpeptide antagonist of “cross-species” (human, porcine, rat) CGRP receptors with in vitro antagonist activity at submicromolar concentration is likely to serve as a valuable pharmacological tool in the future. These data suggest that SB-(+)-273779, a prototype nonpeptide CGRP receptor antagonist, may help in elucidating specific CGRP receptor-mediated functions in complex biological systems, including in vivo pharmacological models.

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References


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