Cardiovascular Drug Reviews
Vol. 23, No. 1, pp. 31–42

The Preclinical Pharmacology of BIBN4096BS, a CGRP Antagonist

Debbie L. Hay and *David Poyner

*School of Biological Sciences, University of Auckland, Auckland, New Zealand and *School of Life and Health Sciences, Aston University, Birmingham, UK*

Keywords: Adrenomedullin — BIBN4096BS — Calcitonin receptor — CGRP — CGRP antagonist — CGRP receptor — $CGRP_{8–37}$ — Migraine.

ABSTRACT

CGRP is an important neuropeptide found throughout the cardiovascular system. However, until recently it has been difficult to define its pharmacology or physiological role because of the lack of suitable antagonists. BIBN4096BS is a high-affinity, non-peptide antagonist that shows much greater selectivity for human $CGRP_1$ receptors compared to any other drug. Its pharmacology has been defined with studies on transfected cells or cell lines endogenously expressing receptors of known composition. These have allowed confirmation that in many human blood vessels, CGRP is working via $CGRP_1$ receptors. However, it also interacts with other CGRP-activated receptors, of unknown composition. *In vivo*, clinical studies have shown that BIBN4096BS is likely to be useful in the treatment of migraine. It has also been used to define the role of CGRP in phenomena such as plasma extravasation and cardioprotection following ischemia.

INTRODUCTION

BIBN4096BS is a new calcitonin gene-related peptide (CGRP)-selective antagonist. As a stable, non-peptide antagonist of high affinity and selectivity it represents a marked improvement on the previous CGRP antagonist, $CGRP_{8–37}$ and as such, it may be expected to greatly facilitate the study of this peptide. It is also starting to leave its mark in the clinic, for the treatment of migraine. This article reviews its pharmacology.

CHEMISTRY

The structure of BIBN4096BS (1-piperidinecarboxamide, 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(2H)-quinazolinyl)) is shown in Fig. 1. This

Address correspondence and reprint requests to: Dr. David Poyner, School of Life and Health Sciences, Aston University, Birmingham, B4 7ET, UK.
Tel: +41 (121) 204-3997, Fax: +41 (121) 359-5142, E-mail: D.R.Poyner@aston.ac.uk

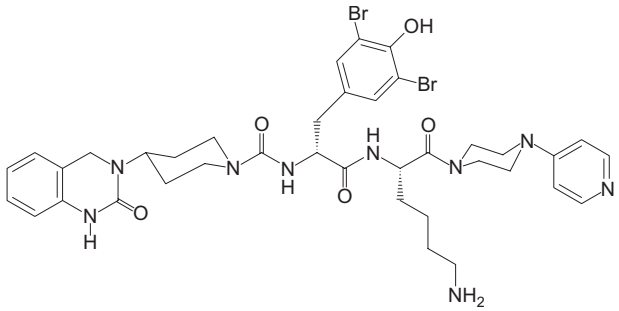

FIG. 1. Structure of BIBN4096BS.

drug was developed at Boehringer Ingelheim Pharmaceuticals, Inc. from a dipeptide lead. It is a white solid substance with a molecular weight of 867. Boehringer Ingelheim scientists developed also a radiolabeled version of the drug, [^{3}H]BIBN4096BS. BIBN4096BS is best dissolved in 1 M HCl, thereafter it may be diluted with buffer and then adjusted to pH 6.5–7.0 with 1 M NaOH. It is soluble in aqueous solution at concentrations well in excess of 1 mg/mL. The solution may be conveniently stored in frozen aliquots.

PHARMACOKINETICS

The pharmacokinetics of BIBN4096BS following i.v. infusion in humans has been described in several reports (22,33,49). Its distribution is best described by a three-compartment model, where a central compartment with a volume of distribution of 8.4 L is in equilibrium with shallow and deep compartments of 4.4 and 15.6 L (49). The non-compartmental studies estimated the volume of distribution as about 20 L (22). Overall plasma clearance was approximately 12 L/h. As renal clearance was only 2 L/h, the authors concluded that the kidney played only a minor role in the removal of the unmetabolized drug (22). The pharmacokinetics did not appear to depend on the dose of drug (22,33).

PHARMACOLOGY OF CGRP RECEPTORS

Introduction

To understand the pharmacology of BIBN4096BS, it is necessary to briefly provide some background information on the role of CGRP and allied peptides in the cardiovascular system and their pharmacological profiles.

CGRP and Related Peptides in the Cardiovascular System

CGRP is a 37 amino acid peptide; it is the main neurotransmitter released by capsaicin-sensitive sensory nerve fibers (C-fibers). It is found in most branches of the cardiovascular system where it exerts a wide range of effects (4). Its receptors are found throughout the cardiovascular system (4,16,29). It has positive chronotropic and inotropic effects on the heart and is a very potent vasodilator. Activation of C-fibers during myocardial ischemia releases it, causing local vasodilation and reducing the effects of any infarction

(16). CGRP release also plays a major part in the cardioprotective effect of ischemic preconditioning (28). In congestive heart failure, CGRP infusion increases cardiac output, mainly due to vasodilation (44). In chronic stable angina, it increases exercise tolerance (5,50). CGRP is also of potential use in peripheral circulatory diseases. Beneficial effects have been reported in Raynaud's disease where it produces a long-lasting increase in hand skin blood flow and promotes healing of ulcers (6,42). Early clinical trials involving CGRP to treat the vasospasm that follows subarachnoid hemorrhage gave promising results but were not followed up as the peptide frequently caused hypotension. However, recent gene transfer experiments in animal models have given positive results, suggesting that the peptide will be useful if it can be targeted appropriately (15,48). Gene transfer of CGRP in animal models has also been used successfully to treat pulmonary hypertension (7). CGRP overproduction can be significant in a number of pathological conditions. In septic shock, CGRP release is triggered by endotoxin and this results in inappropriate vasodilation (1). CGRP is also released in neurogenic inflammation; the resulting excessive vasodilation may be important in conditions such as migraine and chronic pelvic pain (27,45). Studies on CGRP knockout mice have not always given consistent results; there are reports that some animals have elevated blood pressure but its importance in physiological (as opposed to pathophysiological) conditions is at best unclear (34).

CGRP is related to the peptide adrenomedullin (AM) (20). Human (h) AM is a 52 amino acid peptide; however, there is about 25% homology with CGRP over residues 13–52 and this has essentially the same biological activity as full length AM. There is cross-reactivity between CGRP and AM at their receptors (8). AM functions as an autocrine/paracrine factor and is released by vascular endothelial cells. It has similar pharmacological properties to CGRP on the heart and vasculature. Data from AM knockout mice suggest that it is important in the physiological control of blood pressure, blood flow and vascular development (21). In severe heart failure in man, there is a reduction in coronary and pulmonary AM release (20). Components of the receptors for both CGRP and AM are also reduced in models of heart failure (20).

CGRP and AM are also related to two other peptides, calcitonin (CT) and amylin (AMY). Both of them have distinct receptors although they are of minor significance in the cardiovascular system. However, CGRP can activate AMY receptors, at least pharmacologically. An additional recently discovered member of this family is intermedin (IMD) or adrenomedullin 2 (AM2) (39,47). It can activate CGRP, AM and AMY receptors.

Is the CGRP Receptor Heterogenic?

The serious study of CGRP receptor pharmacology began with the discovery that N-terminally truncated fragments of CGRP, particularly $CGRP_{8–37}$, could act as antagonists (9,11,12). Quirion and co-workers observed that some receptors (typified by those on the rat atria) were antagonized more potently by these fragments than those on the tissues such as the guinea pig vas deferens. By contrast, ring-opened derivatives of CGRP such as $(Cys[ACM])^{2,7}$-CGRP and $(Cys[Et])^{2,7}$-CGRP were more potent agonists at the vas deferens than at the atria. The antagonist sensitive receptors were designated the $CGRP_1$-subtype, as opposed to the $CGRP_2$-subtype typified by the vas deferens (11,23).

The $CGRP_1$/$CGRP_2$ receptor classification has been very fruitful in furthering research. There is abundant evidence that CGRP can interact with several receptors, at least pharmacologically if not physiologically. However, it is questionable whether a single $CGRP_2$ receptor exists. A wide range of pA_2 estimates for $CGRP_{8–37}$ against different

tissues has been reported (see ref. 38 for review). (Cys[ACM])^{2,7}-CGRP is a partial agonist, which complicates its use in receptor classification (53). Whilst the $CGRP_1$ receptor has been defined molecularly, the $CGRP_2$ receptor remains elusive. Accordingly, it has been suggested that the "$CGRP_2$ receptor" may represent the action of CGRP at certain subtypes of AM or AMY receptor which have a high affinity for CGRP but a low affinity for $CGRP_{8–37}$ (38). This is an area of controversy.

Molecular Pharmacology of CGRP and AM

The $CGRP_1$ receptor is a G-protein coupled receptor (GPCR), but most unusually it is a heterodimer. The 7-transmembrane component is termed calcitonin receptor-like receptor (CRLR or CL). This is a secretin-like GPCR. However, by itself it will not respond to CGRP. It interacts with a single transmembrane accessory protein called receptor activity modifying protein 1 (RAMP1). This gives a complex that corresponds to the $CGRP_1$ receptor (29).

There are two proteins homologous to RAMP1: RAMP2 and RAMP3. RAMP2/CL gives the AM_1 receptor; this has high selectivity for AM over CGRP. RAMP3/CL gives the AM_2 receptor; this shows less discrimination between AM and CGRP, particularly β-CGRP (23,29). Both of these receptors have a low affinity for $CGRP_{8–37}$. RAMPs will also associate with other GPCRs, particularly the CT receptor. Here they produce three AMY receptors, depending on which RAMP is involved. The AMY_1 (RAMP1/CT-receptor) and to some extent, the AMY_3 (RAMP3/CT-receptor) have significant affinities for CGRP. These appear to have low affinity for $CGRP_{8–37}$ but can be activated by analogs of (Cys[ACM])^{2,7}-CGRP. Thus they could contribute to "$CGRP_2$" receptor pharmacology, although other factors may also be at work (38).

Receptor Pharmacology of BIBN4096BS

The interaction of BIBN4096BS with CGRP receptors

An interesting property of BIBN4096BS is the great selectivity it shows for human (or more strictly, primate) receptors over rodent receptors (see below) (13). This property has been used to study its interaction with $CGRP_1$ receptors. Receptors formed from mixed human/rat RAMP1 and CL demonstrated that the species selectivity was determined by the RAMP. Site directed mutagenesis has shown that it can be traced to a single amino acid at position 74; this is tryptophan in humans and lysine in rats. Presumably the tryptophan stabilizes a hydrophobic interaction required for high affinity BIBN4096BS binding (30). It is not known whether there is a direct interaction between RAMP1 and the antagonist; this is possible but equally it might promote a fold in CL necessary for binding. Regardless of the role of RAMPs, it has been reported that BIBN4096BS interacts with CL (40). It is usual to find that there is at best only partial overlap between the binding sites of peptide and non-peptide ligands at GPCRs and this would be expected to be true of BIBN4096BS. As will be seen below, there are some reports of non-competitive antagonism with BIBN4096BS; until the binding site is mapped in more detail it is difficult to know if they are consequences of examining the antagonist under non-equilibrium conditions or whether there is true non-competitive antagonism.

The role of RAMP1 in promoting high affinity binding of BIBN4096BS raises the possibility that the AMY_1 receptor, a complex between the CT-receptor and RAMP1, might also have some affinity for the antagonist. This remains to be tested.

Pharmacology on recombinant receptors and cell lines

The binding of BIBN4096BS has been studied either directly using [^{3}H]BIBN4096BS or indirectly, in competition with [^{125}I]iodohistidyl-hαCGRP (Table 2). For hCL/hRAMP1 complexes expressed in Cos 7 cells, [^{3}H]BIBN4096BS has a pK_d of 10.05 ± 0.03 (n = 3) (10). This is in fair agreement with a pK_i of 10.74 estimated on the same complex expressed in 293 EBNA cells in a competition study (30). For rat (r) CL/rRAMP, the pK_i in competition studies was 8.67 (30).

Several studies have looked at the binding of BIBN4096BS to SK-N-MC cells. These express CL and RAMP1 and provide an endogenous system to study hCGRP$_1$ receptors. In direct binding, BIBN4096BS had a pK_d of 10.35. This also illustrated that the ligand has slow kinetics; at 50 pM it required about 2 h to reach equilibrium and the $t_{1/2}$ for dissociation was 357 min (i.e., k_{off} = 0.0018 min^{-1}) (41). The slow dissociation would be expected for a high affinity ligand. In competition studies at SK-N-MC cells, pK_i values cover a range from 11.4 to 10.8, with a mean of 11.2 (Table 2). Pooling all direct and indirect binding data on exogenous or endogenous expressed human CL/RAMP1 complexes gives a mean pK_i/pK_d estimate of 10.8 ± 0.22 (n = 6).

The pA_2/pK_d has also been estimated in a number of functional studies on SK-N-MC cells (Table 1). The mean value is 10.9 ± 0.22 (n = 3), in excellent agreement with the radioligand binding data. In one study (18), the Schild slope was significantly greater than one, indicating that the binding was not strictly competitive; the pA_2 derived from the x intercept on the Schild plot was 9.95. The apparent non-competitive behavior was considered to be an artifact due to the slow kinetics of the antagonist; the slope of the plot was influenced by the two concentrations at the extreme ends of the range used, where the kinetic effects would be most pronounced. If the slope was constrained to unity, an apparent pK_b of 10.47 could be calculated.

The same study measured pA_2/pK_b values in two other cell lines (18). Rat L6 cells are a model of the rCGRP$_1$ receptor, expressing CL and RAMP1 (albeit with RAMP2 as well). On these cells, the Schild slope (0.89) was just significantly less than unity, giving a pA_2 of 9.25. As with the SK-N-MC cells, this was considered to be the result of the slow kinetics of the antagonist (again, the slope of the Schild plot was largely skewed by the very highest and lowest antagonist concentrations) and when constrained to unity, an apparent pK_b of 9.1 was calculated. Human Col 29 cells have been reported to express a CGRP$_2$-like receptor, although the molecular nature of this is unknown. Here, the Schild plot had a slope that was not different from unity, allowing a pK_b of 9.75 to be calculated. This was significantly different from the pK_b for BIBN4096BS on SK-N-MC cells. BIBN4096BS has also been examined for activity against Rat 2 cells, which are a model for rAM$_1$ receptors (CL/RAMP2). It had no antagonist effects at concentrations up to 10 μM (18). It was similarly inactive on Cos 7 cells expressing hAM$_1$ and AM$_2$ (CL/RAMP3) or rAM$_2$ receptors (19).

In summary, a clear picture has emerged of the pharmacology of BIBN4096BS against molecularly defined receptors expressed exogenously or endogenously in cell lines. It is a very potent (pK_d/pK_b of 10 to 11) antagonist of hCGRP$_1$ receptors. As might be predicted from this affinity, its kinetics are correspondingly slow. It is much less potent (100–1000 fold) against rCGRP$_1$ receptors. It seems to be able to discriminate between CGRP-activated receptors, as evidenced by its lower affinity for the CGRP-responsive receptor on Col 29 cells. However it has virtually no affinity for AM$_1$ or AM$_2$ receptors. As will be seen below, this profile can also be seen in tissues.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.