Vascular Pharmacology 90 (2017) 36–43

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Binding and functional pharmacological characteristics of gepant-type antagonists in rat brain and mesenteric arteries

Majid Sheykhzade [a,*], Nilofar Amandi [a], Monica Vidal Pla [a], Bahareh Abdolalizadeh [a], Anette Sams [b], Karin Warfvinge [b], Lars Edvinsson [b], Darryl S. Pickering [a]

[a] Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
[b] Department of Clinical Experimental Research, Glostrup Research Institute, Glostrup University Hospital, DK-2600 Glostrup, Denmark

ARTICLE INFO

Article history:
Received 23 November 2016
Received in revised form 13 January 2017
Accepted 5 February 2017
Available online 10 February 2017

Keywords:
Binding affinity
CGRP receptor
Schild plot
Gepants
Brain
Mesenteric arteries

ABSTRACT

Aim: The neuropeptide calcitonin gene-related peptide (CGRP) is found in afferent sensory nerve fibers innervating the resistance arteries and plays a pivotal role in a number of neurovascular diseases such as migraine and subarachnoid bleedings. The present study investigates the binding and antagonistic characteristics of small non-peptide CGRP receptor antagonists (i.e. gepants) in isolated rat brain and mesenteric resistance arteries.
Methods: The antagonistic behavior of gepants was investigated in isolated rat mesenteric arteries using a wire myograph setup while binding of gepants to CGRP receptors was investigated in rat brain membranes using a radioligand competitive binding assay. Furthermore, the histological location of the key components of CGRP receptor (RAMP1 and CLR) was assessed by immunohistochemistry.
Results: Our functional studies clearly show that all gepants are reversible competitive antagonists producing Schild plot slopes not significantly different from unity and thus suggesting presence of a uniform CGRP receptor population in the arteries. A uniform receptor population was also confirmed by radioligand competitive binding studies showing similar affinities for the gepants in rat brain and mesenteric arteries, the exception being rimegepant which had 50-fold lower affinity in brain than mesenteric arteries. CLR and RAMP1 were shown to be located in both vascular smooth muscle and endothelial cells of rat mesenteric arteries by immunohistochemistry.
Conclusion: The present results indicate that, despite species differences in the CGRP receptor affinity, the antagonistic nature of these gepants, the distribution pattern of CGRP receptor components and the mechanism behind CGRP-induced vasodilation seem to be similar in resistance-sized arteries of human and rats.

1. Introduction

Calcitonin gene-related peptide (CGRP) is a naturally occurring 37 amino acid neuropeptide with a potent vasodilator effect [3]. CGRP is widely distributed throughout the central and peripheral nervous systems [25] and belongs to the family of related hormones which also includes calcitonin, adrenomedullin, intermedin and amylin [1]. Because of its wide distribution, it participates in many physiological functions. In addition, CGRP plays an important role in various circulatory and neurovascular diseases such as hypertension, ischemic heart diseases, Raynaud's phenomenon, subarachnoid haemorrhage and migraine [7].

The functional CGRP receptor has been reported to be a complex of three well-defined components, calcitonin receptor-like receptor (CLR) and a specific chaperone protein called receptor activity modifying protein 1 (RAMP1) and receptor component protein (RCP), which is responsible for receptor-effector coupling and onset of intracellular signaling pathways. Three RAMPs (RAMP1, RAMP2 and RAMP3) have been identified as chaperones escorting CLR to the plasma membrane to generate either CGRP (when associated with RAMP1) or adrenomedullin (AM) receptors (when associated with RAMP2 or RAMP3) [21]. In addition, the amino acid sequence of RAMP1 determines the species selectivity; in particular the amino acid residue Trp74 modulates the affinity of small molecule antagonists for CRLR/RAMP1 [18]. RAMP1 and CLR together seem to create a binding pocket for CGRP. The C-terminal of CGRP first binds with high affinity to the N-terminal regions of CLR and RAMP1 forming an affinity trap and then, as local concentration of CGRP increases, the N-terminal of the peptide interacts with the juxtamembrane region of CLR which activates the receptor leading to accumulation of cAMP [23]. As mentioned earlier, the CLR component of the CGRP receptor is also a component of the AM receptors while the RAMP1 component is part of the amylin 1 (AMY1) receptor complex [5,23,34]. Thus, the heteromeric nature of the CGRP receptor makes discovery of a selective CGRP receptor

* Corresponding author at: Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Jagtvej 160, DK-2100 Copenhagen Ø, Denmark.
E-mail address: mash@sund.ku.dk (M. Sheykhzade).

http://dx.doi.org/10.1016/j.vph.2017.02.001
1537-1891/

antagonist difficult because a compound that binds exclusively to CLR will also likely antagonize AM1 and AM2, while a compound that binds to RAMP1 will likely antagonize the AMY1 receptor. The ideal antagonist from a selectivity perspective would be one that makes contact with both components of the CGRP receptor and therefore displays acceptable selectivity against the related receptors. Several of the known CGRP receptor antagonists exhibit this feature. Antagonists such as telcagepant or olcegepant bind to a hydrophobic pocket formed by CLR and RAMP1 preventing the initial CGRP binding and subsequent receptor activation [23,31]. Most of the studies found in the available literature database have mainly focused on investigating the inhibitory effect of small non-peptide CGRP receptor antagonists (i.e. gepants) on primate CGRP receptors both in vitro and in vivo. Therefore, there was a huge gap in the available literature between published studies investigating the effect of small non-peptide CGRP receptor blockers on primate receptors and those involving other species, such as rodents.

In support of a central role for CGRP and activation of its receptor in migraine pathology, the non-peptide CGRP receptor antagonists, the so-called gepants (e.g. olcegepant and telcagepant) have been shown to be effective in the acute phase of migraine attack. Unfortunately, due to liver toxicity, the compounds are no longer being pursued as frontline preventive migraine drugs. Indeed, there is a dire need to back-translationally re-explore the CGRP receptors in non-primate species using these small non-peptide CGRP antagonists as tool compounds in order to gain more detailed knowledge about presence of possible receptor subtypes and/or allosteric binding sites.

The present study was therefore designed to characterize the gepants' pharmacology in the rat by examining their binding in rat brain and their effects in isolated rat resistance mesenteric arteries. Furthermore, the purpose of the study was to locate the key receptor components for the neuropeptide CGRP in rat mesenteric resistance arteries. By conducting these studies we will gain knowledge about nature of these small non-peptide antagonists, existence of possible allosteric binding sites and receptor subtypes with different histological locations in rodents. The picture might be different in rats compared to primates.

2. Material and methods

2.1. Animals

All animal procedures were carried out in accordance with national laws and guidelines. A total of 40 animals were used in the experiments described here. Male Sprague-Dawley rats (200–300 g) (Taconic Europe, Denmark) were housed in our local animal facility in a temperature (22–23 °C) and humidity controlled environment with a 12-hour light and 12-hour dark cycle and ad libitum access to standard chow (Chr. Petersen A/S, Ringsted, Denmark) and water.

2.2. Materials

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) was obtained from Pfizer as Dinolytic Vet®, 5 mg/ml Dinoprost containing 5 × 10 ml injection vials (for intramuscular injection) and stored frozen in small aliquots at −20 °C until use. Acetylcholine chloride (ACh) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Rat-αCGRP was purchased from BACHEM (Rhein, Germany). Telcagepant (MK0974), olcegepant (BIBN4096BS), MK3207 and rimegepant (BMS927711) were purchased from MedChem Express (MCE, Monmouth Junction, NJ, USA). All CGRP receptor antagonists were dissolved in anhydrous DMSO, except rat-αCGRP and acetylcholine chloride that were dissolved in distilled H_2O. Stock solutions of the drugs (CGRP at 1 mM, ACh and CGRP receptor antagonists at 10 mM) were stored frozen in small aliquots at −20 °C and dilutions were prepared just before experimentation.

Physiological salt solution (PSS) had the following composition (in mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 1.5, KH_2PO_4 1.18, $MgSO_4 \cdot 7H_2O$ 1.17, ethylenediaminetetraacetic acid (EDTA) 0.027 and glucose 5.5, with pH adjusted to 7.4. Ca^{2+}-free PSS was similar to PSS except that $CaCl_2$ was replaced by 0.01 mM ethylene glycol-bis(2-aminoethyl ether)-N,N,N′N′-tetraacetic acid (EGTA). K-PSS was prepared by replacing all sodium with an equimolar amount of potassium resulting in a total K^+ concentration of 125 mM.

2.3. Vascular force measurement

Rats were sedated with CO_2 and euthanized by guillotining followed by exsanguination. The mesenteric arcade was immediately excised and immersed in ice-cold PSS (see composition above: Materials). Following pinning out the mesenteric arcade in a silicon-covered petri-dish, 2nd order mesenteric branches (mean lumen diameter ≈ 225 μm) were isolated and immersed in ice-cold PSS. Depending on the length, each branch was then cut into two to four, 1–2 mm long, cylindrical arterial segments for in vitro pharmacology experiments.

Vasomotor properties of isolated arteries are studied using a wire myograph that records isometric tension [15,24]. Mesenteric arterial segments (1–2 mm long) were mounted on two stainless steel wires (40 μm diameter) in the organ bath of a small vessel wire myograph (Danish Myo Technology A/S, Aarhus, Denmark). One wire is connected to a micrometer screw enabling adjustments of the distance between the wires. The other wire is connected to a force displacement transducer attached to an analogue-digital converter unit (ADInstruments, Chalgrove, UK). Measurements of vascular tone are recorded using a Power Lab unit (ADInstruments). Each segment was immersed in 37 °C bicarbonate buffer solution (physiological saline solution (PSS)) (see Materials), which is continuously aerated with 5% CO_2/95% O_2 resulting in pH 7.4. The vessels were stretched to 90% of the normal internal circumference each vessel would have under a passive transmural pressure of 100 mm Hg [24] thereby ensuring maximal force development. Following an equilibration period of approximately 20 min each segment was exposed thrice to a potassium rich bicarbonate buffer solution, KPSS, containing 125 mM K^+ (see Materials) to examine the viability and reproducibility of contractions in the vessels.

2.4. Functional assay

Due to development of CGRP-induced desensitization in isolated rat mesenteric arteries, the parallel experimental design were chosen where control ring segments were incubated with vehicle while other consecutive ring segments isolated from the same 2nd order mesenteric branch were incubated with increasing concentrations of CGRP-receptor antagonists (see below). Cumulative concentration–response curves with rat-αCGRP (10 pM–100 nM) were performed either in control segments (in absence of antagonist: only vehicle was added) or in the vessel segments that were pre-incubated (30 min) with small non-peptide CGRP receptor antagonists: telcagepant (MK0974: 0.1 μM, 1 μM, 10 μM and 100 μM), olcegepant (BIBN4096BS: 0.1 nM, 1 nM, 10 nM, 0.1 μM and 1 μM), MK3207 (0.1 nM, 1 nM, 10 nM, 0.1 μM and 1 μM) and BMS927711 (rimegepant: 1 nM, 10 nM, 0.1 μM, 1 μM and 3 μM). The cumulative concentration–response curves (CRCs) with rat-αCGRP were all made in the mesenteric arterial segments which were pre-contracted to a steady (or stable) tension level by 3–10 μM $PGF_{2\alpha}$. In the rat-αCGRP concentration–response experiments, where the vessels were pre-incubated with the CGRP-receptor antagonists, the last concentration of rat-αCGRP in the organ bath (i.e. 300 nM, 1 μM and 3 μM) was adjusted to that of antagonist in order for a maximum response to be achieved.

2.5. Assessment of endothelial function and endothelial dependency of CGRP-induced vasodilation

In order to assess the endothelium function in isolated mesenteric resistance arteries, a stable contraction was first induced in these vessels by applying 10 μM prostaglandin $F_{2\alpha}$. The vessels were then challenged