European Journal of Pharmacology 670 (2011) 347–355

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

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

Molecular and Cellular Pharmacology

In vitro and in vivo pharmacological characterisation of the potent and selective vasopressin V_{1A} receptor antagonist 4-[4-(4-Chloro-phenyl)-5-[1,2,3] triazol-2-ylmethyl-4H-[1,2,4]triazol-3-yl]-piperidin-1-yl-(3,5-difluoro-phenyl) methanone (PF-00738245)

Rachel Russell, Rachel Doyle, Jamie Turner, Neil Attkins, Simeon Ramsey, Laura Weibley, Lucy Bateman, Magda Bictash, Stevie Neal-Morgan, Magnus Ivarsson, Nick Pullen *

Pfizer Global Research & Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, United Kingdom

ARTICLE INFO

Article history:
Received 16 May 2011
Received in revised form 5 September 2011
Accepted 11 September 2011
Available online 21 September 2011

Keywords:
PF-00738245
Arginine vasopressin
Smooth muscle contraction
Vasopressin antagonist

ABSTRACT

The dysregulation of arginine vasopressin (AVP) release and activation of vasopressin receptors plays an important role in disease conditions including polycystic kidney disease, congestive heart failure and dysmenorrhoea. The development of potent and selective vasopressin receptor ligands is needed to help dissect the function of the specific subtypes in disease pathogenesis. Here we report the pharmacological characterisation of PF-00738245 in in vitro binding and functional assays using cells expressing vasopressin V_{1A}, V_{1B} or V_2 receptors. PF-00738245 inhibited AVP binding to the recombinant human vasopressin V_{1A} receptor ($K_i = 2.85$ nM) and blocked AVP-induced rat aortic ring and human myometrial contraction ($pK_B = 7.35$ and 8.62 respectively). PF-00738245 was selective for the vasopressin V_{1A} receptor by demonstrating minimal binding to vasopressin V_{1B} (3.6% inhibition at 10 μM) or functional activity at vasopressin V_2 receptors (8.1% agonist and −8.4% antagonist activity at 10 μM) as well as the oxytocin receptor (46.3% antagonist activity at 10 μM). The in vivo pharmacological properties were tested orally in the rat and PF-00738245 dose dependently blocked the effect of AVP on a capsaicin-induced cutaneous flare response. Taken together the data support the use of PF-00738245 as a potent and selective vasopressin V_{1A} receptor antagonist which may have utility in the treatment of disease conditions which are propagated by elevation in vasopressin V_{1A} receptor signalling.

1. Introduction

Arginine vasopressin (AVP) is synthesised in the neurosecretory cells of the paraventricular and supraoptic nuclei of the hypothalamus and stored in the posterior pituitary gland. AVP is a cyclical nonapeptide which in the periphery exerts broad pharmacological action on vasopressin V_{1A}, V_{1B} and V_2 receptors, but also the oxytocin receptor. Through vasopressin V_{1A} and V_{1B} receptors, AVP triggers the production of Ca^{2+} fluxes, through the $G_{q/11}$ activation of phospholipase C. In contrast, vasopressin V_2 receptor activation triggers a G_s response and production of cyclic adenosine monophosphate (cAMP) (Schoneberg et al., 1998). In the control of osmolality and blood pressure, AVP release from the posterior pituitary stimulates water resorption through vasopressin V_2 receptors in the renal distal convoluted tubules and medullar collecting ducts. Additional cardiovascular control is determined by the effects of AVP on vasopressin V_{1A} receptors expressed on cardiac myocytes, renal and vascular smooth muscle cells as well as platelets (Aoyagi et al., 2009). AVP also functions as a neurotransmitter on centrally expressed vasopressin V_{1B} receptors, mediating adrenocorticopin release on pituitary cortiocotrophs as well as additional behavioural and endocrine responses triggered by anxiety and stress, where there appears to be a contribution from vasopressin V_{1A} receptors (Egashira et al., 2009).

The pathophysiological consequences of deregulated AVP secretion have been studied in a number of systems. For instance, in polycystic kidney disease, elevated AVP is hypothesised to stimulate accumulation of cAMP by the vasopressin V_2 receptor, triggering epithelial cell growth and fluid secretion culminating in renal cyst formation (Torres et al., 2004). Similarly, the effects of AVP on activating the renin angiotensin system as well as triggering peripheral vascular resistance, oedema and hypertrophic cardiac responses and the role of vasopressin V_{1A} and V_2 receptors have been highlighted as causally associated with congestive heart failure (Nemerovski and Hutchinson, 2010; Veeraveedu et al., 2010). Additionally, the expression of vasopressin V_{1A} receptors on vascular smooth muscle has highlighted the involvement on AVP in propagating the abnormal

* Corresponding author. Tel.: +44 1304 644507.
E-mail address: dr.nick.pullen@gmail.com (N. Pullen).

0014-2999/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2011.09.034

vasoactive response in Raynaud's syndrome (Hayoz et al., 2000). Primary dysmenorrhoea is a common gynaecological complaint, characterised by cyclical cramping pelvic pain. Symptoms are manifested at a time of markedly increased myometrial activity and ischemia caused by associated reduction in uterine blood flow (Akerlund, 1994; Stromberg et al., 1984). Vasopressin levels appear to be raised in women with dysmenorrhoea both prior to and during menstruation (Ekstrom et al., 1992; Hauksson et al., 1987; Hauksson et al., 1988; Stromberg et al., 1984), the effects of which appear to increase myometrial smooth muscle contraction (Hauksson et al., 1988; Liedman et al., 2006). Several pilot clinical studies have examined the hypothesis that blocking the effects of vasopressin on uterine myometrial smooth muscle may have utility in the treatment of dysmenorrhoea as well as other conditions, such as pre-term labour. Both atosiban, a moderately selective intravenous vasopressin V_{1A} receptor and oxytocin receptor antagonist as well as the selective vasopressin V_{1A} receptor antagonist, relcovaptan/SR-49059, have been shown to reduce intrauterine contractions elicited by AVP and reduce dysmenorrhoeic symptoms in subjects with moderate to severe dysmenorrhoea (Bossmar et al., 1997; Brouard et al., 2000; Liedman et al., 2006; Steinwall et al., 2004; Steinwall et al., 2005).

In recent years, a class of vasopressin V_2 receptor antagonists, the 'vaptans' has emerged as an important class of agents (Favory et al., 2009; Veeraveedu et al., 2010) with potential benefit in treating hyponatremia, congestive heart failure and polycystic kidney disease. The development of selective agents has been challenging due to both the peptidic nature of the ligand and high receptor homology. Several other touted selective vasopressin V_{1A} receptor antagonists are in development but few published information are available on their pharmacological properties. Here we present the first in vitro and in vivo pharmacological profiling data on PF-00738245, an orally active, potent and selective vasopressin V_{1A} receptor antagonist.

2. Materials and methods

PF-00738245 (4-[4-(4-Chloro-phenyl)-5-[1,2,3]triazol-2-ylmethyl-4H-[1,2,4]triazol-3-yl]-piperidin-1-yl-(3,5-difluoro-phenyl) methanone) (Fig. 1) was discovered as part of a screening campaign for vasopressin V_{1A} receptor antagonists and synthesised at Pfizer, Sandwich. The discovery and synthesis will be described elsewhere. [^{3}H]-arginine vasopressin was obtained from PerkinElmer. Arginine vasopressin (AVP), oxytocin, β-mercapto-β,β-cyclopentamethylenepropionyl^1, OMe-Tyr^2, Arg^8]-vasopressin (βMCPVP), lysine vasopressin and probenecid were obtained from Sigma-Aldrich. β-lactamase reagents were obtained from Aurora Biosciences. The CHO-hV_2-CRE and CHO-hOT-NFAT β-lactamase reporter cell lines were generated using standard molecular techniques. PF-00738245 was screened against a broad spectrum of 72 different binding assays containing native and recombinant receptors, transporters, ion channels and enzymes at Cerep SA (http://www.cerep.com).

2.1. Binding affinity of PF-00738245 at human recombinant vasopressin V_{1A} and V_{1B} receptors

The binding affinity of PF-00738245 at human recombinant vasopressin V_{1A} and V_{1B} receptors was determined by its ability to inhibit binding of [^{3}H]-arginine vasopressin ([^{3}H]-AVP) to respective membrane preparations made from Chinese Hamster Ovary (CHO) cell lines stably expressing either the human recombinant vasopressin V_{1A} or V_{1B} receptors. Briefly, membranes from CHO cells expressing either vasopressin V_{1A} or V_{1B} receptors (V_{1A} 150 μg/ml; V_{1B} 100 μg/ml) were incubated in assay buffer (50 mM Tris pH 7.4, 5 mM $MgCl_2$, 0.05% BSA) with 0.5 nM [^{3}H]-AVP and increasing concentrations of PF-00738245 (0.3 nM to 10 μM) in a total assay volume of 250 μl with a final assay concentration of 1% (v/v) dimethyl suphoxide (DMSO). Total [^{3}H]-AVP binding was defined in the presence of 1% DMSO vehicle. Non-specific binding was defined using βMCPVP (10 μM) for vasopressin V_{1A} and lysine-vasopressin (1 μM) for vasopressin V_{1B} receptors in 1% DMSO. Assay was incubated for 60 min at room temperature. After this time receptor bound [^{3}H]-AVP was separated from free by rapid vacuum filtration of samples over 96-well GF/B UniFilter plates pre-soaked in 0.5% polyethyleneimine using a 96 well Brandel Harvester. Filterplates were washed (3×1 ml) with ice cold wash buffer (50 mM Tris pH 7.4 5 mM $MgCl_2$). Plates were dried prior to the addition of scintillation cocktail and read on a suitable protocol on a PerkinElmer TopCount NXT. Data points were converted to specific binding and IC_{50} values were converted to K_i using the Cheng–Prusoff equation (Yung-Chi and Prusoff, 1973).

2.2. In vitro cell based potency of PF-00738245 at vasopressin V_2 and oxytocin receptors

The agonist and antagonist properties of PF-00738245 at the recombinant human vasopressin V_2 and oxytocin receptors were assessed using β-lactamase assay technology. CHO-hV_2-CRE and CHO-hOT-NFAT cells were grown to 80% confluency (growth medias: CHO-hV_2-CRE; 90% Dulbecco's Modified Eagle Media with GlutaMAX, 25 mM HEPES, 10% FBS, 1 mM sodium pyruvate, 1× non essential amino acids, 400 μg/ml geneticin and 200 μg/ml zeocin. CHO-hOT-NFAT; 90% nutrient mixture F-12 Ham, 10% FBS, 2 mM L-glutamine, 15 mM HEPES, 400 μg/ml geneticin, 200 μg/ml zeocin) harvested and plated at 12000 cells/well in growth media (30 μl) in 384-well black clear bottom plates and incubated at 37 °C, 5% CO_2 16–20 h prior to experimentation.

2.2.1. Agonist format (vasopressin V_2 receptor only)

On the day of experimentation a dilution series of 11 concentrations of PF-00738245 was prepared in assay buffer (0.05% pluronic acid in PBS). Compound (10 μl) and assay buffer (10 μl) was added to duplicate wells yielding a final assay concentration of 1.25% DMSO. Maximum control stimulation was achieved by substitution of PF-00738245 (10 μl) with a small molecule vasopressin agonist which was discovered as part of the screening programme (10 μl) yielding a final assay concentration of 10 μM. Minimum control stimulation was achieved by substituting PF-00738245 (10 μl) with assay buffer. Plates were incubated for 4.5 h at 37 °C, 5% CO_2 after which time Aurora β-lactamase reagents were added. Cell plates were incubated for a further 1.5 h at room temperature prior to reading on an LJL Analyst with 405 nm excitation and 450 nm and 530 nm emissions.

2.2.2. Antagonist format (vasopressin V_2 and oxytocin receptors)

Assay was performed as above but with the substitution of assay buffer with either AVP (10 μl, 10 pM final assay concentration in assay buffer) for the vasopressin V_2 receptor assay or oxytocin (10 μl, 2 nM final assay concentration in assay buffer) for oxytocin receptor assay. PF-00738245 was pre-incubated for 15 min at 37 °C, 5% CO_2 prior to the addition of agonist. Maximum control receptor stimulation was also determined with AVP and oxytocin as described.

The maximum agonist stimulation window was calculated by subtracting minimum control wells from maximum agonist control stimulation. Specific PF-00738245 activity was expressed as a percentage of this window. Percentage activity was plotted against PF-00738245 concentration and a sigmoidal non-linear regression fitted. EC_{50} and IC_{50} values were calculated. Separate AVP and oxytocin concentration effect curves were generated for conversion of antagonist IC_{50} to K_i using the Cheng–Prusoff equation (Yung-Chi and Prusoff, 1973).