BIOCHEMICAL PHARMACOLOGY 73 (2007) 198–205

available at www.sciencedirect.com

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

Mechanism of action of AZD0865, a K^+-competitive inhibitor of gastric H^+,K^+-ATPase

Karin Gedda *, *Carin Briving, Karin Svensson, Ingela Maxvall, Kjell Andersson*

AstraZeneca R&D, Mölndal S-431 83, Sweden

ARTICLE INFO

Article history:
Received 5 July 2006
Accepted 20 September 2006

Keywords:
H^+,K^+-ATPase/antagonists and inhibitors
Parietal cell
Gastric/drug effects
Selectivity
Mechanism of action
Fast onset

ABSTRACT

AZD0865 is a member of a drug class that inhibits gastric H^+,K^+-ATPase by K^+-competitive binding. The objective of these experiments was to characterize the mechanism of action, selectivity and inhibitory potency of AZD0865 in vitro. In porcine ion-leaky vesicles at pH 7.4, AZD0865 concentration-dependently inhibited K^+-stimulated H^+,K^+-ATPase activity (IC_{50} 1.0 ± 0.2 μM) but was more potent at pH 6.4 (IC_{50} 0.13 ± 0.01 μM). The IC_{50} values for a permanent cation analogue, AR-H070091, were 11 ± 1.2 μM at pH 7.4 and 16 ± 1.8 μM at pH 6.4. These results suggest that the protonated form of AZD0865 inhibits H^+,K^+-ATPase. In ion-tight vesicles, AZD0865 inhibited H^+,K^+-ATPase more potently (IC_{50} 6.9 ± 0.4 nM) than in ion-leaky vesicles, suggesting a luminal site of action. AZD0865 inhibited acid formation in histamine- or dibutyryl-cAMP-stimulated rabbit gastric glands (IC_{50} 0.28 ± 0.01 and 0.26 ± 0.003 μM, respectively). In ion-leaky vesicles at pH 7.4, AZD0865 (3 μM) immediately inhibited H^+,K^+-ATPase activity by $88 \pm 1\%$. Immediately after a 10-fold dilution H^+,K^+-ATPase inhibition was 41%, indicating reversible binding of AZD0865 to gastric H^+,K^+-ATPase. In contrast to omeprazole, AZD0865 inhibited H^+,K^+-ATPase activity in a K^+-competitive manner (K_i 46 ± 3 nM). AZD0865 inhibited the process of cation occlusion concentration-dependently (IC_{50} 1.7 ± 0.06 μM). At 100 μM, AZD0865 reduced porcine renal Na^+,K^+-ATPase activity by $9 \pm 2\%$, demonstrating a high selectivity for H^+,K^+-ATPase. Thus, AZD0865 potently, K^+-competitively, and selectively inhibits gastric H^+,K^+-ATPase activity and acid formation in vitro, with a fast onset of effect.

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1. Introduction

The gastric H^+,K^+-ATPase, which is responsible for the production of gastric acid, belongs to a class of ion-translocating ATPases that are characterized by the formation of a covalently phosphorylated enzyme intermediate as part of their catalytic cycle [1]. Other enzymes in this class include Na^+,K^+-ATPase and Ca^+-ATPases. The catalytic cycle of gastric H^+,K^+-ATPase involves moving between the conformational states E_1, in which the cation-binding site faces the parietal cell cytoplasm with low affinity for K^+, and E_2, with the cation-binding site facing the canaliculus with high affinity for K^+ [2]. K^+ plays a vital part in this catalytic cycle as it is required for the dephosphorylation of the H^+,K^+-ATPase and the subsequent conformational changes.

The final step of gastric acid secretion can be inhibited by agents that are competitive with respect to K^+ binding to the parietal cell gastric H^+,K^+-ATPase [3–6]. The identity of the binding site (or sites) for these agents has not been ascertained fully, but recent mutational studies have demonstrated that the binding site is distinct from the K^+ binding site and that amino acid residues at or near the K^+ binding site are involved

* *Corresponding author*. Tel.: +46 31 776 1036; fax: +46 31 776 3761.
E-mail address: karin.gedda@astrazeneca.com (K. Gedda).

Abbreviations: PPI, proton pump inhibitor; DMSO, dimethylsulphoxide; MESG, 2-amino-6-mercapto-7-methyl-purine riboside; PNP, purine nucleoside phosphorylase

0006-2952/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2006.09.020

in the binding. It is known that these agents have a luminal site of action [5–7], binding preferentially to the E_2 conformation. Recent evidence suggests the presence of a binding site in the cavity formed by the M1, −4, −5, −6, and −8 transmembrane segments and the extracellular loops formed by M5/M6, M7/M8 and M9/M10, present only in the E_2 conformation of the enzyme [8–13]. By reversibly binding to this cavity in the gastric H^+,K^+-ATPase and preventing K^+ from occupying its binding site, these agents may prevent the conformational changes necessary for ion transport, thereby inhibiting the H^+,K^+-ATPase and blocking acid secretion.

AZD0865 (8-[(2,6-dimethylbenzyl)amino]-*N*-[2-hydroxyethyl]-2,3-dimethylimidazo[1,2-*a*]pyridine-6-carboxamide) is a novel member of the class of compounds that inhibit gastric H^+,K^+-ATPase by K^+-competitive binding (Fig. 1A) [14]. It is a lipophilic weak base, with a pK_a value of 6.1 and a log K_d value of 4.2. Here, we report on the mechanism of action, inhibitory potency, and selectivity of AZD0865 in vitro. H^+,K^+-ATPase containing gastric membrane vesicles from pig and glands from rabbit gastric mucosa have been used as test systems to evaluate the inhibitory mechanism of the compound. The inhibitory potency of AZD0865 was evaluated at different pH values and the effect was compared to the effect of its permanent cation analogue AR-H070091 (Fig. 1B). A method to continuously measure ATPase activity was used to study the onset of inhibitory activity and the restoration of enzyme activity following washout. To study the effect of AZD0865 on the cation occlusion step, thallium was used as a K^+ cognate [15,16]. The selectivity of AZD0865 was studied using pig renal Na^+,K^+-ATPase. Finally, the effect of an active metabolite (Fig. 1C), generated at a low level in vivo, was determined in ion-leaky vesicles (pH 7.4) and in the gastric gland model.

2. Materials and methods

2.1. Materials

2.1.1. Preparation of H^+,K^+-ATPase

Ion-tight and ion-leaky membrane vesicles enriched in gastric H^+,K^+-ATPase were derived from pig stomach according to the method of Saccomani [17] with some modifications. Briefly, tissue was homogenized and a microsomal fraction was obtained by differential centrifugation. The pelleted material was separated on a discontinuous density gradient and the fraction at the interface between the 0.25 M sucrose and 0.25 M sucrose plus 7.5% Ficoll layers (i.e., the ion-tight membrane vesicle fraction) was collected, mixed with an equal volume of 60% sucrose and stored in aliquots at −70 °C. To obtain the ion-leaky membrane vesicles, the ion-tight fraction was diluted with 1 mM Pipes/Tris (pH 7.4) to give a 1% sucrose concentration, homogenized and centrifuged at 100,000 × *g* for 2 h. The

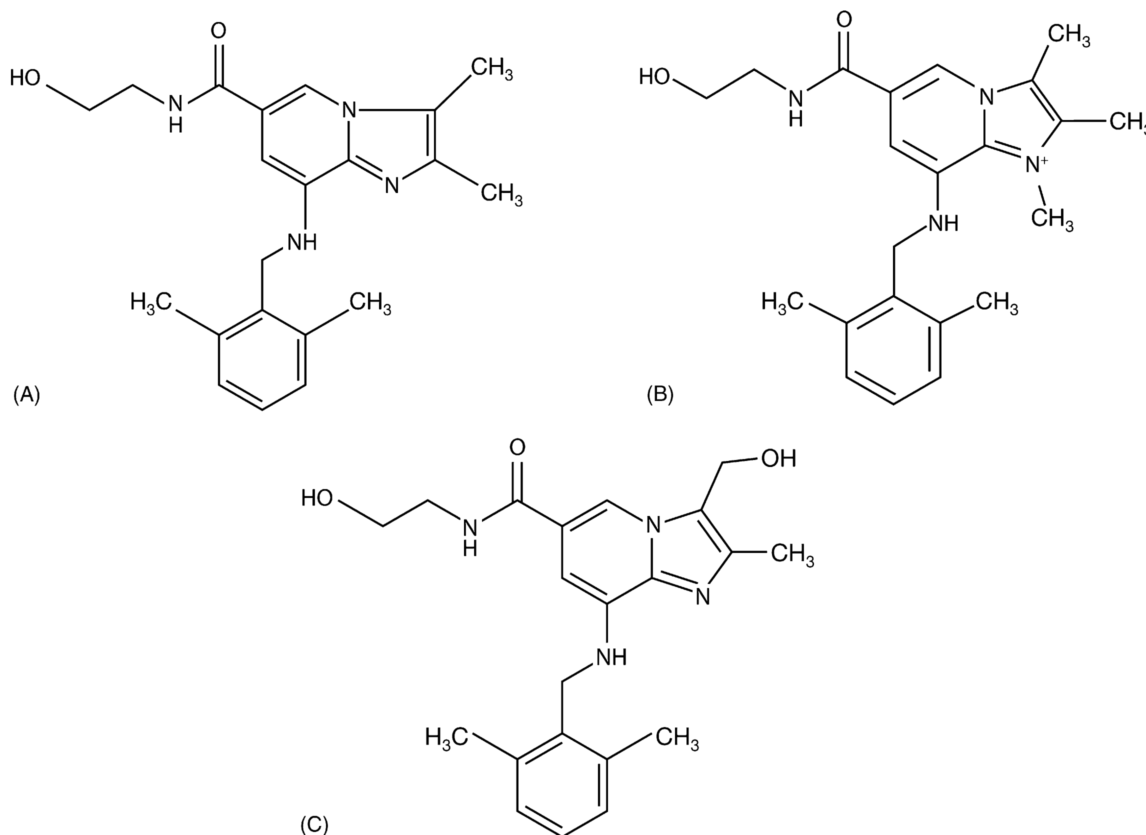

Fig. 1 – Chemical structure of AZD0865 ((8-[(2,6-dimethylbenzyl)amino]-*N*-(2-hydroxyethyl)-2,3-dimethylimidazo[1,2-*a*]pyridine-6-carboxamide (A), the AZD0865 permanent cation analogue AR-H070091 (8-[(2,6-dimethylbenzyl)amino]-6-{[(2-hydroxyethyl)amino]carbonyl}-1,2,3-trimethylimidazo[1,2-*a*]pyridin-1-ium) (B), and the AZD0865 metabolite AR-H044881 (8-[(2,6-dimethylbenzyl)amino]-N-(2-hydroxyethyl)-3-(hydroxymethyl)-2-methylimidazo[1,2-*a*]pyridine-6-carboxamide (C).

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