Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 558 (2007) 133 – 143
www.elsevier.com/locate/ejphar

Functional effects of the late sodium current inhibition by AZD7009 and lidocaine in rabbit isolated atrial and ventricular tissue and Purkinje fibre

Frida Persson *, Birgit Andersson, Göran Duker, Ingemar Jacobson, Leif Carlsson

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

Received 25 September 2006; received in revised form 17 November 2006; accepted 27 November 2006
Available online 1 December 2006

Abstract

AZD7009 (*tert*-Butyl-2-(7-[(2S)-3-(4-cyanophenoxy)-2-hydroxypropyl]-9-oxa-3,7-diazabicyclo[3.3.1]non-3-yl)ethylcarbamate) is an antiarrhythmic agent that increases atrial refractoriness, shows high antiarrhythmic efficacy and has low proarrhythmic potential. This study was primarily undertaken to determine the effects of AZD7009 on the late sodium current and to examine the impact of late sodium current inhibition on action potential duration in various myocardial cells. AZD7009 inhibited the late sodium current in Chinese Hamster Ovary K1 (CHO K1) cells expressing $hNa_v1.5$ with an IC_{50} of 11 ± 2 μM. The late sodium current in isolated rabbit atrial and ventricular myocytes was also concentration dependently inhibited by AZD7009. Action potentials were recorded during exposure to 5 μM E-4031 (1-[2-(6-methyl-2pyridyl)ethyl]-4-(4-methylsulfonyl aminobenzoyl)piperidine), a compound that selectively inhibits the rapid delayed rectifier potassium current (I_{Kr}), and to E-4031 in combination with AZD7009 or lidocaine in rabbit atrial and ventricular tissue and Purkinje fibres. In Purkinje fibres, but not in ventricular tissue, AZD7009 and lidocaine attenuated the E-4031-induced action potential duration prolongation. In atrial cells, AZD7009, but not lidocaine, further prolonged the E-4031-induced action potential duration. E-4031 induced early afterdepolarisations (EADs) in Purkinje fibres, EADs that were totally suppressed by AZD7009 or lidocaine. In conclusion, excessive action potential duration prolongation induced by E-4031 was attenuated by AZD7009 and lidocaine in rabbit Purkinje fibre, but not in atrial or ventricular tissue, most likely by inhibiting the late sodium current. Furthermore, the opposite effect by AZD7009 on action potential duration in atrial tissue suggests that AZD7009, in addition to inhibiting I_{Kr}, also inhibits other repolarising currents in the atria.
Published by Elsevier B.V.

Keywords: Atrial fibrillation; Antiarrhythmic drug; Action potential; Voltage-clamp; Late sodium current; Nav1.5; Atrium; Ventricle; Purkinje fibre

1. Introduction

AZD7009 (*tert*-Butyl-2-(7-[(2S)-3-(4-cyanophenoxy)-2-hydroxypropyl]-9-oxa-3,7-diazabicyclo[3.3.1]non-3-yl)ethylcarbamate) is an antiarrhythmic agent that demonstrates marked effects on atrial electrophysiology with smaller influence on the ventricle (Carlsson et al., 2006, 2004; Edvardsson et al., 2005; Goldstein et al., 2004). Furthermore, AZD7009 has been shown to be highly efficacious in terminating atrial fibrillation in various animal models as well as in man (Crijns et al., 2006; Duker et al., 2005; Goldstein et al., 2004; Löfberg et al., 2006) and in experimental models of Torsades de Pointes, AZD7009 showed low proarrhythmic activity (Carlsson et al., 2004; Wu et al., 2005). Ion channel studies have revealed that AZD7009 exerts its effect by a combined inhibition of repolarising potassium currents primarily the human ether-a-go-go related gene (hERG), $K_v1.5$ and $K_v4.3$ and the cardiac sodium current. The 50% inhibitory concentrations (IC_{50}) previously reported for block of hERG, $hK_v1.5$, $hK_v4.3$/hKChIP2.2, hK_vLQT1/minK and $hNa_v1.5$ expressed in Chinese Hamster Ovary (CHO) cells are 0.6, 27, 24, 193 and 8 μM, respectively (Persson et al., 2005a,b).

The α subunit of the cardiac sodium current ($hNa_v1.5$) is encoded by the gene SCN5A and is expressed in both atria and ventricles of the human heart (Gellens et al., 1992). The sodium

* Corresponding author. Tel.: +46 31 776 28 36; fax: +46 31 776 37 66.
E-mail address: frida.persson@astrazeneca.com (F. Persson).

0014-2999/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.ejphar.2006.11.040

current can be separated into two functional components, the peak sodium current, which activates and inactivates rapidly, and the late sodium current, which persists throughout the plateau of the action potential. The peak sodium current is responsible for the action potential initiation and propagation in the heart whereas the late sodium current is involved in determining the shape and duration of the action potential plateau. Quantitatively, the late sodium current is much smaller than the peak sodium current and is thought to be mediated by sodium ions passing through a few sodium channels that fail to inactivate completely (Maltsev et al., 1998).

We have previously hypothesized that the low proarrhythmic activity of AZD7009 may be explained by inhibition of the late sodium current in midmyocardial cells and Purkinje cells (Persson et al., 2005a). Hence, the aim of the present study was to assess the effects of AZD7009 on the late sodium current in CHO K1 cells expressing $hNa_v1.5$ and in acutely isolated rabbit ventricular and atrial myocytes. Furthermore, we examined the impact of this late sodium current inhibition on the action potential duration in rabbit atrial and ventricular tissue and Purkinje fibre in general and in order to seek a mechanistic explanation for the low proarrhythmic potential of AZD7009.

2. Materials and methods

This study was approved by the ethical committee for animal research at the University of Göteborg, Sweden and was conducted in accordance with Swedish animal care guidelines.

2.1. Cell culturing and isolation of atrial and ventricular myocytes

Generation of the CHO K1 cell line stably expressing $hNa_v1.5$ has been described elsewhere (Persson et al., 2005a). Briefly, CHO K1 cells were transfected with $hNa_v1.5$ cDNA (GenBank accession no. AY148488 with the following deviation: T559A) using Lipofectamine™ 2000 (Invitrogen). The cells were cultured in HAM/nut mix F12 with Glutamax-1 (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 1.1 mg/ml geneticin (Sigma-Aldrich). Cells used for voltage clamp experiments were plated out in petri dishes 1 to 3 days before the experiment. On the day of the experiment the cells were washed with Tyrode's solution, detached with a cell scraper and stored at room temperature for a maximum of 6 h before use.

Atrial and ventricular myocytes were isolated from male New Zealand White rabbits (body weight 2.4 to 3.3 kg). Heparin 1000 IE/kg was given before anaesthesia (pentobarbital sodium, 60 mg/kg i.v.). The heart was excised, mounted on a Langendorff apparatus and perfused retrogradely through the aorta with warmed (37 °C) Tyrode's solution, Ca^{2+}-free Tyrode's solution and Ca^{2+}-free Tyrode's solution containing collagenase Type 2 140 U/ml (Worthington Biochemical Corporation) and albumin 0.2 mg/ml (Sigma-Aldrich). $CaCl_2$ was added three times to a final concentration of 0.25, 0.5 and 1 mM. The perfusion time with collagenase Type 2 was varied between 9 and 20 min and the addition of Ca^{2+} were made every 2–5 min after that, in total 15 to 40 min of enzyme perfusion. Some of the atrial myocytes were isolated using a K^+-enriched Ca^{2+}-free Tyrode's solution instead of the normal Ca^{2+}-free Tyrode's solution and without any addition of Ca^{2+} during the perfusion. The right atria or part of the right ventricle (papillary muscles and some underlying tissue) was cut into small pieces and placed in 1.5 ml of fresh enzyme containing Tyrode's solution in a water bath (37 °C) with magnetic stirring. The collagenase containing solution was replaced every 2 to 3 min and the suspension was centrifuged during 30 s at 200 g and washed with Tyrode's solution containing albumin 5 mg/ml. The isolated myocytes were kept in Tyrode's solution at room temperature under 100% O_2 and cells were used within 12 h.

2.2. Voltage clamp recordings in CHO K1 $hNa_v1.5$ cells and isolated myocytes

Voltage clamp recordings were made with an EPC9 amplifier (HEKA Elektronik, Lambrecht, Germany) using the perforated-patch (myocytes) or the whole-cell (CHO cells) configuration of the patch-clamp technique. Electrodes were fabricated from thick-walled, filamented, borosilicate glass capillaries (Harvard apparatus Ltd, Edenbridge, Kent, UK) with an inner diameter of 0.86 mm and outer diameter of 1.5 mm. The glass capillaries were pulled with a micropipette puller, model P-2000 (Sutter Instruments, Novato, CA, USA) and had a resistance of 2 to 4 MΩ when filled with electrode solution. The cells were placed in the recording bath of a Dynaflow 8-channel (myocytes) or 16-channel (CHO cells) chip (Dynaflow™, DF-8 Pro II or DF-16, Cellectricon AB, Göteborg, Sweden) mounted on an inverted microscope. The perforated patch recordings in isolated myocytes were made using Amphotericin B (Sigma-Aldrich) diluted in pipette solution to a final concentration of 200 μg/ml. Gentle suction was applied to achieve GigaΩ or near GigaΩ seal formation, whole-cell access was established within 20 min and whole-cell capacitance was compensated for. The series resistance was 20.5±1.6 MΩ before and 18.2±1.6 MΩ after drug application in the ventricular myocytes (n=13) and 28.7±5.9 MΩ before and 28.1±5.6 MΩ after drug application in the atrial myocytes (n=9). The series resistance was compensated for by 50%. In CHO K1 $hNa_v1.5$ cells, currents were recorded using the standard whole-cell configuration; the series resistance was kept under 15 MΩ and compensated for by 80–85%. Currents were sampled at 20 kHz after filtering (2.9 kHz, EPC9 internal 4-pole Bessel filter) using Pulse software v8.63 (HEKA Elektronik, Lambrecht, Germany). All experiments were carried out at room temperature (22 °C) and the holding potential was −80 mV. In isolated myocytes, potassium currents were eliminated by use of Cs^+ instead of K^+ in the extracellular and electrode solutions. Ca^{2+}-currents were eliminated by use of 5 μM nifedipine in the extracellular solution. Anemonia sulcata toxin (ATX-II, Alomone labs) 10 nM was used to increase the amplitude of the late sodium current in both CHO K1/$hNa_v1.5$ cells and isolated myocytes. To avoid binding of ATX-II to the Dynaflow superfusion system, 1 mg/ml albumin (Sigma-Aldrich) was added to all dilutions of ATX-II. Sodium currents were activated