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Galantamine (Reminyl®) delays cardiac ventricular repolarization and prolongs the QT interval by blocking the HERG current

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ABSTRACT

Galantamine is a reversible inhibitor of acetylcholinesterase and an allosteric-potentiating ligand of the nicotinic acetylcholine receptors. It is used for treating mild-to-moderate Alzheimer's disease. Interestingly, QT interval prolongation on the electrocardiogram (ECG), malignant ventricular arrhythmias and syncope have been reported with galantamine. Our objective was to evaluate the effects of galantamine on cardiac ventricular repolarization. Three sets of experiments were undertaken: 1) Whole cell patch-clamp experiments: HERG- or KCNQ1 + KCNE1-transfected cells were exposed to galantamine 0.1–1000 μmol/l (n = 25 cells, total) to assess drug effect on HERG and KCNQ1 + KCNE1 currents. 2) Langendorff perfusion experiments: Isolated hearts from male Hartley guinea pigs (n = 9) were exposed to galantamine 1 μmol/l to assess drug-induced prolongation of monophasic action potential duration measured at 90% repolarization ($MAPD_{90}$). 3) Cardiac telemetry experiments: Guinea pigs (n = 7) implanted with wireless transmitters were injected a single intraperitoneal (i.p.) dose of galantamine 3 mg/kg and 24 h ECG recordings were made. 1) The estimated IC_{50} for galantamine on HERG current was 760.2 μmol/l. Moreover, galantamine 10 μmol/l had a small inhibiting effect on KCNQ1 + KCNE1 current (12.17 ± 2.19% inhibition, n = 10 cells). 2) While pacing at cycle lengths of 150, 200 or 250 ms, galantamine 1 μmol/l prolonged $MAPD_{90}$ by respectively 5.1 ± 1.6 ms, 9.4 ± 1.9 ms and 12.1 ± 2.1 ms. 3) Galantamine 3 mg/kg i.p. caused a maximal 11.9 ± 2.7 ms prolongation of the corrected QT (QTc). Galantamine is a weak HERG blocker. This contributes to its mild QT-prolonging effect. Patients could be at risk of cardiac proarrhythmia during drug overdosage or interactions involving cytochrome 2D6 drug-metabolizing enzyme.

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1. Introduction

Alzheimer's disease is the most common form of dementia (Farlow, 2003). This neurodegenerative disease is characterized by progressive cognitive and functional deficits. Although the etiology is unknown, degeneration of the acetylcholine-producing neurons has been reported in patients with Alzheimer's disease. The first-line treatment for Alzheimer remains symptomatic. Galantamine (Reminyl®) is a reversible competitive inhibitor of acetylcholinesterase (AChIs) and an allosteric-potentiating ligand of the nicotinic acetylcholine receptors widely used for managing cognitive and noncognitive outcomes of mild-to-moderate Alzheimer's disease (Galantamine monograph, 2008). This treatment has been shown to be effective and well tolerated in patients with cognitive impairment (Burns et al., 2009; Zhao et al., 2002). However, as with other AChIs such as donepezil and rivastigmine, QT interval prolongation, arrhythmias and syncope have been reported with galantamine (Fisher and Davis, 2008; Nelson and Buchanan, 2006; Robert and Spivey, 2007).

The objectives of the present study were therefore to elucidate the effects of galantamine on cardiac ventricular repolarization. Three sets of experiments at three different levels (*in vitro*, *ex vivo* and *in vivo*) were therefore undertaken, using respectively whole cell patch-clamp technique, Langendorff perfusion and cardiac wireless telemetry in conscious and unrestrained guinea pigs.

2. Materials and methods

Experiments were performed in accordance with our institutional guidelines on animal use in research and conform to the guiding principal of the Declaration of Helsinki. The protocol was reviewed and approved by the Laval University Animal Protection Committee.

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Animals were housed and maintained in compliance with the *Guide to the Care and Use of Experimental Animals* of the Canadian Council on Animal Care.

2.1. Patch-clamp experiments

2.1.1. Cell culture, transfection and whole cell voltage-clamp recordings

Experiments were performed on either human *ether-a-go-go*-related gene (HERG) stably transfected in human embryonic kidney cells (HEK293) to recapitulate I_{Kr} current, or in Chinese hamster ovary (CHO) cells transiently transfected with 2 μg (each) of KCNQ1+KCNE1 cDNAs to recapitulate I_{Ks} current. The CHO cells were transfected using the calcium-phosphate method. Green fluorescent protein (GFP) was coexpressed to assess transfection efficiency and to identify expressing CHO cells. HEK293 cells were maintained in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS, Invitrogen, Burlington, ON, Canada), 1% penicillin–streptomycin (Invitrogen), 1% L-alanyl-L-glutamine (GlutaMAX™, Invitrogen), 1% non-essential amino acids solution for MEM and 1% sodium pyruvate. CHO cells were maintained in F12-Kaighn's medium supplemented with 10% fetal bovine serum (FBS, Invitrogen), 1% penicillin–streptomycin (Invitrogen) and 2% L-alanyl-L-glutamine (GlutaMAX™, Invitrogen). Both cell lines were incubated at 37 °C in a 5% CO_2 humid atmosphere incubator.

Recordings were performed from either HEK293 or CHO cells in 35 mm Petri dishes mounted on stage of an inverted microscope (Olympus IX51). HERG currents were recorded in the whole cell configuration of the patch-clamp technique using an Axopatch 200A amplifier (Molecular Devices-Axon Instruments, Union City, CA). Voltage clamp was controlled by the pCLAMP software package (version 9.0, Molecular Devices-Axon Instruments). Cells were superfused with the bath solution containing (mmol/l): 145 NaCl, 4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 HEPES (pH 7.35, adjusted with NaOH). Micropipettes were pulled from borosilicate glass capillaries (Warner Instruments, Hamden, CT) on a horizontal patch electrode puller and heat-polished to obtain a tip resistance between 1 and 3 MΩ when filled with the following intracellular solution containing (mmol/l): 110 KCl, 1 $MgCl_2$, 5 BAPTA-K4, 5 K_2ATP, and 10 HEPES (pH 7.2, adjusted with KOH). The liquid junction potential between the patch pipette and the bath solution was corrected by −5 mV. The recordings were made 10 min after obtaining the whole cell configuration to allow the currents to stabilize and the contents of the patch electrode to diffuse adequately. HERG currents were generated from a holding potential of −80 mV, using 1-sec depolarizing steps from −40 to +60 mV in 10 mV increments, and tail currents were measured at −40 mV. The series resistance was compensated >80% to improve whole cell voltage-clamp measurements. Currents were filtered at 5 kHz using a 4-pole Bessel filter (−3 dB/octave) and sampled at 2 kHz. Experiments were performed at room temperature (22–23 °C). Rundown is a well-known problem for studies of KCNQ1+KCNE1 currents in the whole cell patch-clamp configuration. As we wanted to evaluate the potential 'I_{Ks}-blocking' effect of galantamine, having the capacity to discriminate rundown of current from a potential drug effect was mandatory. Interestingly, Dong et al. showed that rundown of KCNQ1+KCNE1 current was eliminated by using the perforated-patch configuration with 200 μg/ml amphotericin B in the pipette solution (Dong et al., 2006). We therefore decided to conduct our KCNQ1–KCNE1 perforated-patch experiments as described by Dong et al. The recordings were made 10 min after the patch was perforated to allow the currents to stabilize and the contents of the patch electrode to diffuse adequately. KCNQ1+KCNE1 currents were generated from a holding potential of −80 mV, using 5-sec depolarizing steps from −40 to +60 mV in 10 mV increments, and tail currents were measured at −40 mV.

Required amounts of galantamine hydrobromide (Sigma-Aldrich, St. Louis, MO) were dissolved in dimethylsulfoxide (DMSO ≤0.01%) in order to obtain final concentrations of 0.1 to 1000 μmol/l when added to the Tyrode solution perfusing the cells. Galantamine's IC_{50} on HERG current was estimated using tail current maximal amplitude measured at −40 mV after a voltage step to +20 mV (n=25 cells, total), normalized to baseline, plotted as a function of galantamine concentration, and fitted to the Hill equation.

2.2. Langendorff perfusion experiments

As we described in the past (Drolet et al., 1998), male Hartley guinea pigs (Charles River Laboratories, Montréal, QC, Canada) weighing 250–350 g were anticoagulated by intraperitoneal injection of heparin sodium (400 IU). Thirty minutes later, animals were killed by cervical dislocation, and the hearts were rapidly extirpated and immersed in cold (4 °C) Krebs–Henseleit buffer containing (in mmol/l): glucose 5, KCl 4.7, $CaCl_2$ 1.2, $NaHCO_3$ 25, NaCl 118.5, $MgSO_4$ 2.5 and KH_2PO_4 1.2. This solution was continuously gassed with 95% oxygen plus 5% carbon dioxide (pH 7.4, 37 °C).

Each heart was cannulated and retrogradely perfused via the aorta with the buffer at a constant pressure equivalent to 70 mm Hg, using a custom-made Isolated Heart IH-SR double warming coil heart perfusion system from Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany. Hearts were electrically stimulated with a small coaxial stimulation electrode connected to a programmable stimulator module. Monophasic action potential (MAP-tip) recording electrodes were securely positioned on the surface (epicardium) of each ventricle to obtain visually adequate signals (amplitude >25 mV, stable phase 4). Both MAP signals were continuously recorded (digital sampling rate, 1 kHz), along with perfusion pressure, and stored on hard disk for analysis. These values were averaged by the use of a routine designed specifically for this purpose and incorporated in the ClampFit 9.2 software package from Molecular Devices-Axon Instruments. At least 12 complexes were used for each measurement. MAP signals were recorded at a basic pacing cycle length (BCL) of 250 ms. Then, to assess rate dependency, BCL was changed to either 200 ms or 150 ms, and the heart was paced for 1 min before the MAP was recorded. Thereafter, perfusion was performed during 15 min with buffer containing galantamine 1 μmol/l (dissolved in 200 μl of dimethylsulfoxide: final DMSO in buffer: 0.04% v/v) for a period of 15 min at a BCL of 250 ms. To assess rate-dependent effects of the drug, MAP signals were recorded again at BCL of 200 ms and 150 ms. Perfusion with buffer containing no drug was then restarted during a 15-minute washout period to assess reversibility of drug effects.

2.3. Wireless cardiac telemetry experiments

Male Hartley guinea pigs (Charles River Laboratories, Montréal, QC, Canada) weighing 250–350 g (n=7 animals) were surgically implanted with wireless cardiac telemetry radio transmitters (Model TAIICTA-F40, Data Sciences International; DSI, St. Paul, MN). Aseptic surgery was performed to implant a telemeter in the peritoneal cavity of each animal, following the general procedures recommended by the manufacturer (DSI). Animals were anesthetized by isoflurane inhalation (4 l/min of isoflurane 3% to induce anesthesia, and 1 l/min to maintain it). A midline abdominal incision was made in the skin and muscle layers. The body of the telemeter was laid gently onto the intestines and attached to the overlying muscle wall with non-absorbable sutures. The muscle wall was then closed with absorbable sutures. The telemeter is equipped with two leads for sensing the heart electrical activity; these leads ran through small punctures made in the muscle wall to exteriorize them from the peritoneal cavity. A small trochar was slid between the skin and muscle of the upper abdomen and chest to form two narrow subdermal tracks for the leads to lie in. The final position of the telemeter leads was with the negative lead tip near the right shoulder and the positive lead tip below the left axilla, on the 5th left rib; this simulates a conventional lead II ECG. The abdominal skin was then closed with

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