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Galantamine inhibits slowly inactivating K^+ currents with a dual dose–response relationship in differentiated N1E-115 cells and in CA1 neurones

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ABSTRACT

Galantamine, one of the major drugs used in Alzheimer's disease therapy, is a relatively weak acetylcholinesterase inhibitor and an allosteric potentiating ligand of nicotinic acetylcholine receptors. However, a role in the control of excitability has also been attributed to galantamine via modulation of K^+ currents in central neurones. To further investigate the effect of galantamine on voltage-activated K^+ currents, we performed whole-cell voltage-clamp recordings in differentiated neuroblastoma N1E-115 cells and in dissociated rat CA1 neurones. In both cell models, one can identify two main voltage-activated K^+ current components: a relatively fast inactivating component (*I*fast; time constant ≈ hundred milliseconds) and a slowly inactivating one (*I*slow; time constant ≈ 1 s). We show that galantamine (1 pM–300 μM) inhibits selectively *I*slow, exhibiting a dual dose–response relationship, in both differentiated N1E-115 cells and CA1 neurones. We also demonstrate that, in contrast with what was previously reported, galantamine-induced inhibition is not due to a shift on the steady-state inactivation and activation curves. Additionally, we characterized a methodological artefact that affects voltage-dependence as a function of time in whole-cell configuration, observed in both cell models. By resolving an inhibitory role on K^+ currents in a non-central neuronal system and in hippocampal neurones, we are attributing a widespread role of galantamine on the modulation of cell excitability. The present results are relevant in the clinical context, since the effects at low dosages suggest that galantamine-induced K^+ current inhibition may contribute to the efficiency of galantamine in the treatment of Alzheimer's disease.

1. Introduction

It has long been demonstrated that the degeneration of cholinergic neurones in the basal forebrain may be partially responsible for the learning and cognitive deficits observed in patients with Alzheimer's disease (Bartus et al., 1982; Francis et al., 1999). The recognition of cholinergic dysfunction in Alzheimer's disease led to the development of drugs that enhance cholinergic function, mainly by inhibition of the catabolic enzyme, acetylcholinesterase (AChE). One such compound is galantamine, a selective competitive, reversible and relatively weak AChE inhibitor (Lilienfeld, 2002; Thomsen et al., 1991; Thomsen and Kewitz, 1990), that has also been described as an allosteric potentiating ligand of nicotinic receptors (Schrattenholz et al., 1996; Maelicke et al., 1997; Samochocki et al., 2000). As a result of the allosteric potentiation of nicotinic acetylcholine receptors, galantamine has been shown to enhance GABA and glutamate release in hippocampal slices (Santos et al., 2002). Additionally, galantamine has been proven to potentiate the NMDA receptor whole-cell current in cortical neurones (Moriguchi et al., 2004), to stimulate dopamine release in striatal slices (Zhang et al., 2004b) and to enhance dopaminergic (Schilstrom et al., 2007) and purinergic neurotransmission (Caricati-Neto et al., 2004).

An alternative hypothesis to explain synaptic facilitation by galantamine has been linked to galantamine-induced changes in neuronal excitability. There is evidence that galantamine can increase excitability by inhibiting postburst afterhyperpolarization (AHP) and accommodation of CA1 hippocampal pyramidal neurones, via modulation of muscarinic transmission (Oh et al., 2006). Moreover, galantamine reduces delayed rectifier K^+ current ($I_{K(DR)}$) in acutely dissociated rat hippocampal pyramidal neurones (1–100 μM galantamine; (Pan et al., 2003b) and in cloned Kv2.1 channel current (0.1–100 μM; (Zhang et al., 2004a). In both studies, hyperpolarizing shifts on the activation and steady-state inactivation curves were reported under 10 μM galantamine; such shifts are not coherent with galantamine-induced current reduction. The inhibition of central outward K^+ currents by other AChE inhibitors currently used in Alzheimer's disease therapy—tacrine (Li and Hu, 2002), rivastigmine (Pan et al., 2003a) and donepezil (Yu and Hu, 2005)—has also been reported. Altogether, these data suggest that the reduction of K^+

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currents in the brain may provide a mean of enhancing cognitive function in Alzheimer's disease. In fact, it was demonstrated that by modulating some K^+ channels, one can tune long term potentiation (LTP; Ramakers and Storm, 2002; Chen et al., 2006)—the experimental paradigm of memory (reviewed in Lisman, 2003), or improve cognitive deficits when memory is impaired (Inan et al., 2000). Indeed, treatment of aged rats with galantamine has been shown to extend LTP decay (Barnes et al., 2000).

It has been reported that mean galantamine plasma concentrations of Alzheimer's disease patients, when sampled within 10 h of administration, ranged from 82 μg/l to 126 μg/l (223–342 nM) (Raskind et al., 2000; Wilcock et al., 2000; Farlow, 2003). However, according to the studies described before concerning galantamine effects on K^+ conductances (Oh et al., 2006; Pan et al., 2003b; Zhang et al., 2004a), with such range of concentrations (223–342 nM), only marginal effects were observed.

Therefore, in the present report we aimed to study the effect of galantamine on voltage-activated K^+ currents, using a wider range of concentrations, with doses of galantamine ranging from subnanomolar to micromolar. Two different cell models were used: differentiated neuroblastoma N1E-115 cells—a cell line from the sympathetic nervous system—and acutely dissociated rat hippocampal pyramidal neurones from the CA1 region. It is presently shown that galantamine (1 pM–300 μM) selectively inhibits a slowly inactivating K^+ current in both cell models, exhibiting dual dose–response relationships. We also show that this current inhibition is not due to a shift in the voltage-dependence of steady-state inactivation and activation. The present work extends our understanding about how galantamine acts at the nerve cell.

2. Materials and methods

2.1. Neuroblastoma cell-culture and isolation of CA1 neurones

Mouse neuroblastoma cells of the clone N1E-115 (ECACC, 88112303) were routinely grown in Dulbbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 0.1% antibiotic (10 000 U/ml penicillin and 10 000 μg/ml streptomycin). The cultures were maintained at 37 °C in a humidified atmosphere containing 5%CO_2. Cells were split weekly when 70–80% confluent and were plated in 35 mm plastic Petri dishes (Nunc) using medium containing reduced serum content—2.5% FBS—and 1.5% dimethyl sulphoxide (DMSO), for induction of neuronal differentiation. Cells at 6–14 days after induction of differentiation, showing clear neuritic processes, were selected for recording.

Hippocampal pyramidal cells from the mid-third CA1 region of P21-30 Wistar rats were isolated as described before (Costa et al., 1994). Sub-slices of the CA1 region were incubated at 32 °C in an oxygen saturated solution under moderate stirring; the composition of the incubating solution was as follows (in mM): NaCl 120, KCl 5, CaCl2 1, MgCl2 1, 1,4-Piperazinediethanesulfonic acid (PIPES) 20, D-Glucose 25, adjusted to pH 7 with 1 mM NaOH. Trypsin (0.6 mg/ml) was added to this solution shortly after the preparation of the sub-slices; incubation period was 30–50 min, depending on rat's age. Sub-slices were transferred to an oxygen saturated enzyme-free solution after a brief wash with this solution and kept at room temperature at moderate stirring. A trypsin inhibitor (0.5 mg/ml) was added to the enzyme-free solution; the preparation remained viable for about 5–6 h. Cells from the CA1 layer were isolated by gentle trituration of the sub-slices using fire polished Pasteur pipettes (1.5–2 mm bore).

2.2. Electrophysiological recordings

The 35 mm plastic Petri dishes were used as recording chambers for either cultured or acutely isolated cells. Voltage-clamp recordings were performed in the whole-cell patch clamp configuration. Patch pipettes (1.5–3 MΩ), pulled from borosilicate glass (Science Products GmbH, GB150T-8P), were filled with pipette solution (N1E-115-cells) containing (in mM): $KMeSO_4$ 140, $Na_{1/2}$HEPES 10, $CaCl_2$ 1, $MgCl_2$ 1, EGTA 10, Na_2ATP 2, NaGTP 0.4 and adjusted to pH 7.2–7.3 with 1 mM NaOH (calculated free $[Ca^{2+}] = 60$ nM, by Webmaxclite 1.15, MaxChelator). In the case of recordings in CA1 neurones, modifications of the above solution were used, as follows: $KMeSO_4$ was either 132 mM or replaced by KF 140 mM (osmolarity was kept within the same range).

The recording chamber was perfused by gravity (2–3 ml/min) with the following solution (in mM): NaCl 135, KCl 5.4, $Na_{1/2}$HEPES 10, $CaCl_2$ 2, $MgCl_2$ 1.5, D-Glucose 25 and adjusted to pH 7.4 with 1 mM KOH (calculated equilibrium potential for K^+ was −82 mV). Tetrodotoxin (TTX) (50 nM) was added to the external solution in most of the experiments. During recording, cells were kept near the surface and remained under continuous bath perfusion. The estimated junction potential for the filling and bath solution combinations mentioned above is, respectively, −9.8 mV, −9.4 and −9.2 mV (calculated with JPCalc 2.00, School of Physiology and Pharmacology University of New South Wales). Data were not corrected for the junction potential.

Currents were recorded with an Axopatch 200B electrometer (Axon Instruments) and stored using a DigiData 1200 interface (Axon Instruments) and pCLAMP 6.0.3 software (Axon Instruments) Signals were filtered at 2 kHz (−3 dB, four pole Bessel) with a 5 kHz sampling rate. Series resistance was compensated to about 70%. Electrode and cell membrane capacitances were compensated. Leak subtraction was applied to raw data during data processing (see below). Experiments were carried out at room temperature (about 20 °C).

2.3. Experimental design (voltage protocols)

In experiments with N1E-115 cells, current amplitude and clamp conditions were monitored throughout the experiment with a set of two 5.6 s in duration depolarizing pulses to 0 mV and +40 mV, every 60 s. For the study of the voltage-dependence of activation a set of 6.4 s command pulses from −60 mV to +50 mV (in 10 mV steps) was applied. For the subsequent estimate of leak current, a set of pulses (−75 mV to −53 mV, 2 mV increments, 100 ms) was applied prior (60 ms) to the command pulses. This allowed calculating and subtracting leak current at each voltage. For the study of steady-state inactivation, currents were evoked by a 4.8 s in duration depolarizing command pulse to a fixed voltage (+40 mV) preceded by 6.4 s prepulses ranging from −60 mV to +50 mV (in 10 mV steps). The holding potential was −70 mV.

In experiments with CA1 neurones, the voltage-clamp protocols were equivalent to those described above, differing as follows. A hyperpolarizing pre-pulse to −120 mV, 200 ms in duration, was used. Such pre-pulse aimed the removal of inactivation of channels underlying the fast current component (previously named A–D type). Current amplitude and clamp conditions were monitored throughout the experiment with a set of two 2.1 s in duration depolarizing pulses to −30 mV and +40 mV, every 60 s. To study the fast current component in isolation, a different voltage protocol was used: the same depolarizing pulse to +40 mV (2 s, holding potential −50 mV) was preceded either by a pre-pulse to −30 mV or −120 mV, with a duration (30–200 ms) that was adjusted to each cell/current; the subsequent current traces were subsequently subtracted and, thus, the fast current was then isolated (Lima et al., 2008; see inset in Fig. 2B).

For the study of the voltage-dependence of activation a set of 1 s command pulses from −70 mV to +50 mV (in 10 mV steps) was applied; command pulses were preceded by a set of 160 ms prepulses from −70 mV to −52 mV (2 mV increments), to subsequently estimate and subtract leak current. For the study of steady-state inactivation, currents were evoked by a 4 s in duration depolarizing command pulse to a fixed voltage (+40 mV) preceded by 640 ms