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Blockade of Ca^{2+}-activated K^+ channels by galantamine can also contribute to the potentiation of catecholamine secretion from chromaffin cells

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Abstract

Galantamine is a drug in clinical use for the treatment of Alzheimer's disease, but its mechanism(s) of action remains controversial. Here we addressed the question whether galantamine could potentiate neurotransmitter release by inhibiting small conductance Ca^{2+}-activated K^+ channels ($K_{Ca}2$). Galantamine potentiated catecholamine secretory responses induced by 10 s pulses of acetylcholine and high $[K^+]_o$ applied to fast-superfused bovine adrenal chromaffin cell populations. Catecholamine release was significantly enhanced by galantamine although we did not find concentration dependence in the range 0.1–1 μM. The $K_{Ca}2$ channel blocker apamin (0.3 μM) occluded the potentiating effects of galantamine on acetylcholine-evoked secretion. Like apamin, galantamine also modified the firing of action potentials, but to a lesser extent. In addition, 1 μM galantamine reduced by 41% the $K_{Ca}2$ current without modifying the voltage-dependent Ca^{2+} currents. These results constitute the first direct evidence that galantamine can potentiate neurotransmitter release by blocking $K_{Ca}2$ channels, in addition to its already demonstrated capacity to mildly block acetylcholinesterase or potentiate allosterically nicotinic receptors.

Keywords: Galantamine; $K_{Ca}2$ channels; Catecholamines; Chromaffin cell; Apamin

1. Introduction

Alzheimer's disease is a progressive, degenerative disease with a constellation of symptoms that clearly suggest a complex etiology involving abnormal function and morphology in several neurotransmitter systems. For years, a strategy to improve cognitive function in Alzheimer patients has been the enhancement of cholinergic neurotransmission in the brain by inhibition of acetylcholinesterase. However, the therapeutic success of these compounds has been limited. Galantamine (an alkaloid extracted originally from the daffodil bulb *galanthus nivalis*) is an acetylcholinesterase inhibitor used in the clinic that, in addition to blocking acetylcholinesterase with a mild potency (Thomsen et al., 1991; Woodruff-Pak et al., 2002) behaves as an allosteric potentiating ligand at nicotinic receptors (Schrattenholz et al., 1996; Albuquerque et al., 1997; Samochocki et al., 2003). Galantamine has been shown to potentiate glutamatergic and GABA-ergic (*G*amma-*a*mino*b*utyric *a*cid) neurotransmission between Schaffer collaterals and CA1 neurons (Santos et al., 2002) and also to potentiate nicotine-evoked increases in intracelullar Ca^{2+} and [^{3}H]noradrenaline release in SH-SY5Y cells (Dajas-Bailador et al., 2003). The current hypothesis by which galantamine enhances synaptic transmission is by acting on nicotinic receptors as an allosteric potentiating ligand.

However, we had very preliminary results from our laboratory showing that galantamine could also enhance K^+ induced catecholamine secretion; this suggested to us that another mechanism could be involved in galantamine's mechanism of action, besides allosteric modulation of nicotinic receptors.

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Since we had previously demonstrated that blockade of small conductance Ca^{2+}-dependent K^+ channels by apamin could increase neurotransmitter release (Uceda et al., 1992; Montiel et al., 1995; Lara et al., 1995) and that data from the literature showed that blockade of $K_{Ca}2$ channels can improve memory and learning (Ikonen et al., 1998; Messier et al., 1991; Behnisch and Reymann, 1998), we decided to study if galantamine could also be interfering with these channels.

We used adrenal medullary chromaffin cells as a model in this study because they share with neurons a common embriological origin and they have similar functional and morphological features, including excitability and the ability to store chemical mediators (Kondo et al., 1986). These cells express various subtypes of nicotinic receptor subunits i.e. α3, α5, α7, β4 (Campos-Caro et al., 1997) and various types of K^+ channels, including *ether-à-go-go related gene* (ERG) potassium channels (Gullo et al., 2003), small-conductance Ca^{2+}-activated K^+ channels ($K_{Ca}2$) (Artalejo et al., 1993; Park, 1994; Lara et al., 1995) and big-conductance Ca^{2+}-activated K^+ channels (Marty and Neher, 1985; Lovell et al., 2000). In spite of their high density in chromaffin cells (Prakriya and Lingle, 1999, 2000), big-conductance Ca^{2+}-activated K^+ channels do not contribute much to the regulation of catecholamine release (Montiel et al., 1995). In contrast, selective inhibition of $K_{Ca}2$ channels causes a sharp increase of catecholamine release evoked electrically, by acetylcholine or K^+ application (Montiel et al., 1995; Uceda et al., 1992; Lara et al., 1995). Therefore, chromaffin cells are an adequate model system to study the effects of galantamine on $K_{Ca}2$ channels. In the present study, we have measured the effects of galantamine on the firing properties and the stimulus-evoked exocytosis of catecholamine release using electrophysiological and electrochemical detection techniques (Chow et al., 1994). We report here an additional mechanism for galantamine, besides its acetylcholinesterase blocking activity and allosteric potentiating ligand action; it can partially block small conductance Ca^{2+}-activated K^+ channels, increase the firing of action potentials and enhance the neurotransmitter release.

2. Materials and methods

2.1. Cell preparation and solutions

Primary cultures of rat chromaffin cells was prepared as described by Gullo et al. (2003). We used adult male Sprague Dawley rats (275–325 g) from a colony of our animal quarters; they were sacrificed following the rules of the ethical committee for the care and use of animals in research, of our medical school. Bovine adrenal medulla chromaffin cells were isolated following standard methods (Livett, 1984) with some modifications (Moro et al., 1990). Cells were suspended in DMEM supplemented with 5% foetal calf serum, 50 I.U./ml penicillin and 50 g/ml streptomycin. For secretion experiments 5×10^6 cells were plated in 5 cm diameter Petri dishes. Cells were kept in an incubator at 37 °C, in a 5% CO_2 and 95% air atmosphere, and used 1–5 days thereafter.

Galantamine was obtained from Janssen, Beerse, Belgium. Apamin was purchased from Sigma (A-1289). Concentrated solutions of galantamine (10^{-3} M) and apamin (10^{-4} M) were prepared and stored at −20 °C. Aliquots of galantamine and apamin were unfrozen before use.

2.2. On-line measurement of catecholamine release from bovine chromaffin cells

Cells attached to the bottom of the culture dish were collected by gentle rubbing, centrifuged and resuspended in 200 μl Krebs-HEPES (composition in mM: NaCl, 144; KCl, 5.9; $MgCl_2$, 1.2, $CaCl_2$, 2; HEPES 10, and Glucose, 11) pH 7.4 at 37±2 °C, and trapped in a microchamber with glass wool. Then, the cells were continuously superfused with Krebs-HEPES at a flow rate of 2 ml/min; the liquid flowing from the perfusion chamber reached an electrochemical detector model Metrohn 641 VA, that monitored on-line the oxidation current (nA) that was proportional to the amount of catecholamines secreted (Borges et al., 1986). Catecholamine secretion was stimulated by perfusion during 10 s for 5 min, a Krebs-HEPES solution containing 10 μM acetylcholine, or 35 mM K^+; exchange of the solutions was performed by means of electrovalves. The experimental protocols used were designed to study the rate and extent of potentiation of the secretory response induced by galantamine and apamin. Secretion was quantified by measuring the areas and the amplitudes of the amperometric peaks in *n*C and *n*A, respectively.

2.3. Electrophysiological recordings

Voltage-clamp and current-clamp electrophysiological data were collected using a List EPC-10 patch-clamp amplifier and standard clamp protocols were designed with the Pulse software (Heka ElektroniK, Lambretch, Germany). Data were acquired at 1 kHz for voltage-clamp and at 10 kHz for current-clamp traces. For perforated patch, pipettes (3–5 MΩ) were immersed briefly in the internal solution (mM): K^+-Aspartate 140, NaCl 10, $MgCl_2$ 2,HEPES-KOH 10, pH=7.3; they were backfilled with the same solution containing amphotericin B (150 μg/ml) made from a stock solution (20 mg/ml in dimethylsulphoxide (DMSO), prepared before each experiment) and kept at 4 °C. All experiments were performed at room temperature (22 °C). High resistance seals (of 3–6 GΩ) were achieved and recordings were made when the access resistance had dropped to 50 MΩ. For some cells, a small holding current was used to maintain cell resting potentials between − 65 and − 75 mV. For excitability measurements, a series of 5-s current pulses of increasing strength was used to elicit repetitive firing.

For small conductance Ca^{2+}-activated K^+ ($K_{Ca}2$) current recordings (according to Park, 1994), cells were used starting from day 1 after dissociation. Voltage-clamp experiments were done using the perforated patch configuration and the recording was started as soon as the access resistance reached stable values below 10–15 MΩ; cells showing unstable currents were discarded. The pipette solution was (in mM): K^+ aspartate 140, NaCl 10, $MgCl_2$ 2 and HEPES 10, pH 7.3. A high K^+ external solution (NaCl 83 mM, KCl 40 mM, $CaCl_2$ 10 mM, $MgCl_2$ 2 mM, HEPES 10 mM and Glucose 5 mM, pH 7.3) was used to