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Short communication

Galantide distinguishes putative subtypes of galanin receptors in mudpuppy parasympathetic neurons

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Abstract

The effect of the chimeric ligand galantide on the galanin-induced activation of membrane K^+ conductance and inhibition of voltage-dependent Ca^{2+} conductance has been studied using voltage-clamped dissociated mudpuppy parasympathetic neurons. Galantide did not activate the K^+ conductance but produced a concentration-dependent antagonism ($IC_{50} = 4$ nM) of the galanin-induced increase in K^+ conductance. Galantide acted like galanin and inhibited the voltage-dependent Ba^{2+} current (I_{Ba}). The inhibition of I_{Ba} also was concentration dependent ($IC_{50} = 16$ nM) and the maximum inhibition produced by galantide was approximately 40%. We also demonstrate that the galanin-(1–16) fragment increased the membrane K^+ conductance and decreased I_{Ba}, suggesting that the NH_2 portion of the galanin molecule is sufficient to mediate both actions. One interpretation of these observations is that different galanin receptors mediate the different effects of galanin on the mudpuppy parasympathetic neurons.

Keywords: Galantide; Galanin; Parasympathetic neuron; K^+ conductance; Ca^{2+} conductance

1. Introduction

Galanin is a ubiquitous neuropeptide that has numerous pharmacological actions in the peripheral and central nervous systems of many species (Bartfai et al., 1993a). Ligand binding studies using galanin fragments have demonstrated that the NH_2-terminal portion of the galanin molecule is essential for peptide-receptor interactions (Fisone et al., 1989; Gregersen et al., 1991). Recently developed chimeric galanin receptor ligands such as galantide (galanin-(1–12)-pro-substance P-(5–11) amide) have been used as potential antagonists to better establish galanin function. In early studies with galantide, it was reported that this ligand is a galanin receptor antagonist within central nervous system nuclei, nuclei within the dorsal horn of the spinal cord and peripheral endocrine structures such as the pancreas (Bartfai et al., 1992). These observations suggest that galantide may be a selective inhibitor of galanin receptors.

Previously, we found that galanin has two distinct actions on mudpuppy parasympathetic neurons: activation of an inwardly rectifying K^+ current and inhibition of voltage-dependent Ca^{2+} currents (Parsons and Merriam, 1992; Merriam and Parsons, 1995). Both actions of galanin in the mudpuppy parasympathetic neurons are sensitive to pretreatment with pertussis toxin suggesting the involvement of a G_i or G_o type GTP-binding protein (Merriam et al., 1994). However, galanin is about 100-fold less effective in activating the K^+ conductance than inhibiting the Ca^{2+} conductance: the EC_{50} for activation of the K^+ is 34 nM and the IC_{50} for the inhibition of the Ca^{2+} conductance is 0.42 nM (Merriam et al., 1994; Merriam and Parsons, 1995). This difference in the effectiveness of galanin on the K^+ and Ca^{2+} conductances was unexpected. Because it has been reported that there may be multiple subtypes of galanin receptors (Bartfai et al., 1993b; Botella et al., 1995; Gu et al., 1995), we considered it possible that these two actions of galanin were mediated through different receptors on these parasympathetic neurons. Consequently, studies were done to compare the effects of galantide on the galanin-induced activation of K^+ conductance and inhibition of Ca^{2+} conductance.

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Using the galanin-(1–16) fragment, experiments also were completed to establish that the NH_2-terminal portion of the galanin molecule mediated both actions of galanin in mudpuppy parasympathetic neurons.

2. Materials and methods

2.1. Neurons and solutions

All experiments were performed in vitro on enzymatically dissociated mudpuppy cardiac parasympathetic neurons using procedures described in detail previously (Merriam and Parsons, 1995). Membrane currents were recorded with the perforated patch method of whole cell recording (Merriam and Parsons, 1995; Mulvaney and Parsons, 1995). For K^+ current recordings, the cells were maintained in a solution which contained (in mM): 100 *n*-methyl-D-glucamine-Cl (NMG-Cl); 12.5 KCl; 3.6 $CaCl_2$; 2 $MgCl_2$; 0.0003 tetrodotoxin; 5 Hepes; pH 7.3 and the pipette solution contained (in mM): 90 K-aspartate; 20 KCl; 8 $MgCl_2$; 10 Hepes; pH 7.15. For recording currents through voltage-dependent Ca^{2+} channels, Ba^{2+} was used as the charge carrier (I_{Ba}) with cells maintained in a solution which contained (in mM): 100 NMG-Cl; 2 $BaCl_2$; 10 tetraethylammonium Cl; 5 glucose; 0.0003 tetrodotoxin; 5 Hepes; pH 7.3 and the pipette solution contained (in mM): 90 Cs-aspartate; 20 CsCl; 5 $MgCl_2$; 10 Hepes; pH 7.15. For all recordings, nystatin (0.33 mg/ml) was included in the pipette solution. During the experiments, fresh solution was continually applied to a 0.5 ml bath chamber using a gravity flow system (1–3 ml/min). Porcine galanin, galanin-(1–16) (both obtained from Sigma Chemical Company, St. Louis, MO, USA) and galantide (Bachem) were dissolved in the bathing solutions.

2.2. Recording protocols

All voltage clamp protocols were generated and currents recorded using an Axopatch 1-C amplifier and pClamp 5.51 acquisition system (Axon Instruments, Foster City, CA, USA) following protocols published previously (Merriam and Parsons, 1995; Mulvaney and Parsons, 1995).

3. Results

3.1. Actions of galantide on the galanin-induced K^+ conductance

In the first series of experiments, we compared the effects of galanin and galantide on K^+ currents. For these experiments, the cells were voltage clamped to −40 mV and stepped for 300 ms every 5 s to −100 mV prior to and during exposure to either galanin or galantide. As found previously, galanin activated an inwardly rectifying membrane K^+ conductance (Parsons and Merriam, 1992). In contrast, galantide, with concentrations to 10^{-6} M, did not activate the K^+ conductance (Fig. 1A). Galantide did, however, antagonize the activation of the K^+ conductance by galanin

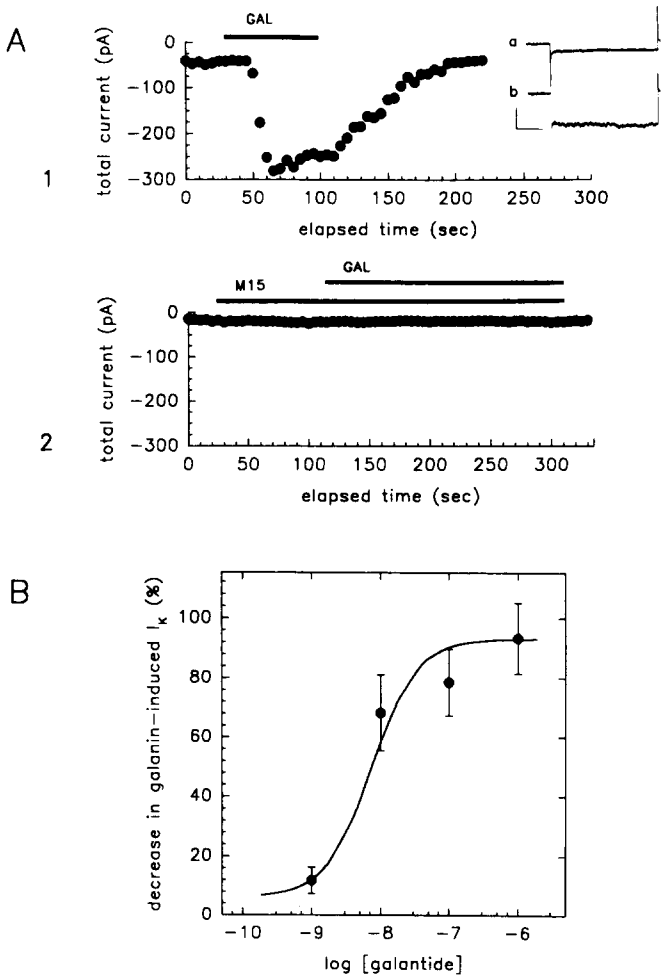

Fig. 1. The galanin-induced K^+ current is antagonized by galantide. A: K^+ current (I_K) amplitudes measured before, during and after application of galanin (GAL) or galantide (M15). A_1: Application of 10^{-6} M galanin (duration of application indicated by solid bar) causes an increase in inward current during 300 ms hyperpolarizing voltage steps to −100 mV. $V_{hold} = -40$ mV. Current amplitude measured as average of 50 consecutive data points (total time 20 ms) near end of voltage step. Representative current traces shown in inset: a: control current, b: total current during application of galanin. Calibration bars: *y*-axis = 100 pA; *x*-axis = 80 ms. A_2: Application of 10^{-6} M galantide causes no change in I_K. Addition of 10^{-6} galanin (GAL) during galantide (M15) application results in no additional current. B: Galantide antagonism of galanin-induced increase in I_K is concentration dependent, with the IC_{50} of galantide = 4 nM. Antagonism of galanin action is expressed as a percentage of total I_K elicited by 10^{-6} M galanin in control cells that were not exposed to galantide. Symbols and error bars represent mean values ± S.E.M. from at least 3 cells for each concentration of galantide.

(Fig. 1B). The IC_{50} for the galantide inhibition of the galanin-induced activation of the K^+ conductance was 4 nM.

3.2. Actions of galantide on Ba^{2+} currents

Next, we compared the effects of galanin and galantide on I_{Ba}. Galanin decreased I_{Ba} as shown previously with the maximum effect at approximately 50% inhibition with concentrations greater than 10^{-8} M (Merriam and Parsons, 1995). Galantide also produced a concentration-dependent inhibition of I_{Ba} ($IC_{50} = 16$ nM) (Fig. 2A and C). However, the maximum extent of inhibition by galantide ($\sim 40\%$ with 10^{-6} M) was less than that produced by galanin. Also, the maximum inhibition of I_{Ba} by galantide and 10^{-7} M galanin together was the same as that produced by 10^{-7} M galanin alone. This suggested that galantide and galanin were acting on the same population of receptors, but galantide may either be only a partial agonist or a much weaker agonist requiring concentrations greater than 10^{-6} M to reach maximum inhibition. The observation that the inhibition produced by galantide and galanin together remained constant over a range of galantide concentrations suggested that galantide is not acting as an antagonist for the receptor mediating the galanin-induced inhibition of I_{Ba} (Fig. 2A). Also, characteristics of the galantide-induced inhibition of I_{Ba} were similar to that produced by galanin (Merriam and Parsons, 1995). The maximum galantide-induced inhibition of I_{Ba} occurred with voltage-clamp steps from holding potential of -80 mV to 0 mV. Significant inhibition also occurred with steps to more positive voltages (Fig. 2B).

Galantide is a chimeric ligand composed of galanin-(1–13) complexed with a substance P moiety (Bartfai et al., 1991). Substance P has been shown to inhibit Ca^{2+} conductances in other amphibian autonomic neurons (Bley and Tsien, 1990). Consequently, we tested whether the inhibition by galantide on I_{Ba} might be related to the substance P portion of the molecule. We found that substance P (10^{-7}–10^{-6} M) had no discernible effect on I_{Ba} (during exposure to substance P, I_{Ba} amplitude decreased by $2.7 \pm 1.1\%$, $n = 6$ cells). In three of these cells, the effect of 10^{-7} M galanin was tested after the substance P application. In all three, galanin decreased I_{Ba} ($33.9 \pm 6.2\%$). Therefore, we conclude the agonist action of galantide cannot be ascribed to a substance P action.

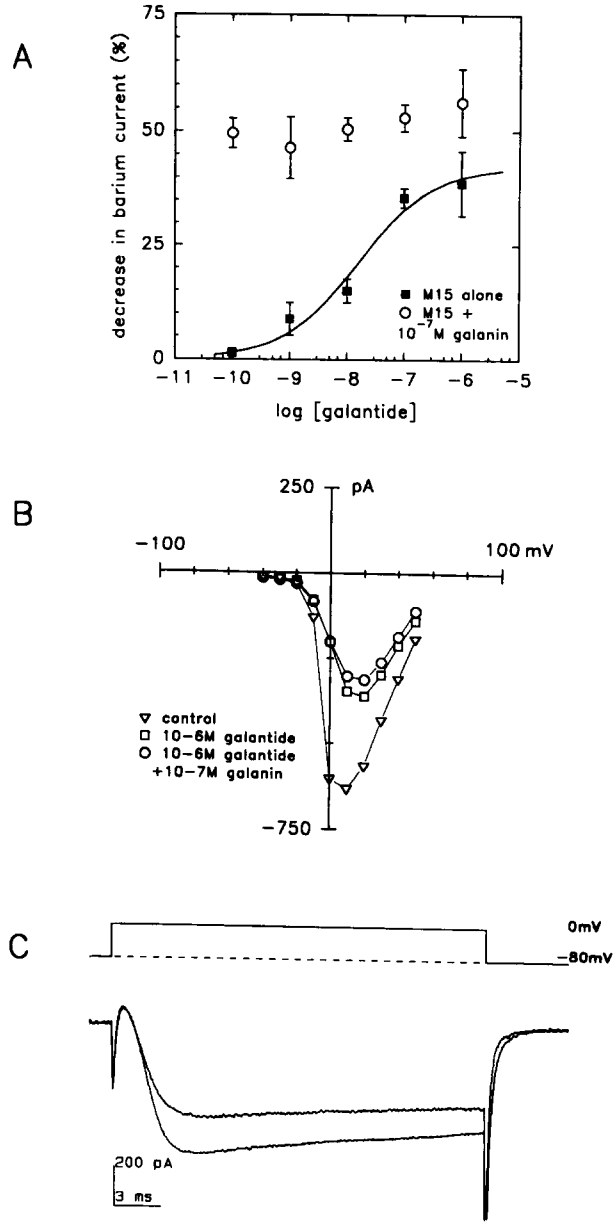

Fig. 2. Galantide causes a concentration-dependent decrease in I_{Ba}. A: Concentration dependence of I_{Ba} inhibition by galantide (M15) compared with various concentrations of galantide (M15) plus 10^{-7} M galanin. IC_{50} for galantide inhibition of I_{Ba} was 16 nM. Peak I_{Ba} was recorded at +10 mV. $V_{hold} = -80$ mV. Symbols and error bars represent mean values ± S.E.M. from at least 4 cells for each concentration of galantide. B: Current-voltage relationship for peak I_{Ba} recorded in control solution, in 10^{-6} M galantide, and in 10^{-6} M galantide plus 10^{-7} M galanin. $V_{hold} = -80$ mV. C: Typical I_{Ba} recorded before (lower trace) and during application of 10^{-6} M galantide (upper trace). Voltage step protocol is shown above current traces.

3.3. Effects of galanin-(1–16)

Because galantide exhibited different effects on the K^+ conductance and Ca^{2+} conductance in the mudpuppy neurons, experiments were completed to determine whether the NH_2-terminal fragment, galanin-(1–16), exerted actions similar to that of galanin. Galanin-(1–16) activated an inwardly rectifying K^+ current (-195.2 ± 102.5 pA with 10^{-7} M, 4 cells) and inhibited peak I_{Ba} ($36.7 \pm 7.2\%$ with 10^{-7} M, 4 cells). These results demonstrated that the first 16 amino acids are sufficient to activate the K^+ conductance and

inhibit the Ca^{2+} conductance in the mudpuppy neurons suggesting that both actions are mediated by the NH_2-terminal portion of the peptide.

4. Discussion

The results of the present study demonstrated in mudpuppy parasympathetic neurons that galantide acts as an antagonist for the galanin-induced increase in K^+ conductance whereas it acts as an agonist for the inhibition of I_{Ba}. Since both effects of galanin have been shown to be inhibited by pertussis toxin pretreatment, it is likely that both actions are mediated by activation of a G_o/G_i G protein (Merriam et al., 1994; Merriam and Parsons, 1995). Further, both actions of galanin were mimicked by galanin-(1–16) supporting the view that it is the binding of the NH_2-terminal portion of galanin which is responsible for the galanin-induced increase in K^+ conductance and inhibition of I_{Ba}. Other investigators have also reported that galantide can exhibit agonist activity (Gu et al., 1993; Heuillet et al., 1994). One interpretation of these various observations is that there are two subtypes of galanin receptors on the mudpuppy neurons; one which regulates a membrane K^+ conductance and another which mediates the inhibition of Ca^{2+} conductance. Other investigators have suggested the presence of different subtypes of galanin receptors in different tissues (Bartfai et al., 1993b; Botella et al., 1995; Gu et al., 1995). It is premature, however, to rule out the possibility that the actions of galanin are mediated by a single receptor which is coupled to different G proteins and/or different intracellular transduction mechanisms. In conclusion, we suggest that mudpuppy parasympathetic postganglionic neurons provide an excellent model system to establish mechanisms by which galanin modulates ionic conductances in neurons.

Acknowledgements

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