Galanin Causes Presynaptic Inhibition of Cholinergic Transmission in Rabbit Parasympathetic Ganglia

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Galanin, a peptide with 29 amino acid residues, has been considered to be a neuropeptide, since its actions and immunoreactivity are reported to occur in both the central and peripheral nervous systems (Bauer et al. 1986; Crawley, 1990; Bartfai et al. 1992). In the autonomic nervous system, galanin induces a depression of cholinergic transmission (Tamura et al. 1987), a membrane hyperpolarization due to the activation of potassium channels (Tamura et al. 1988; Konopka et al. 1989) and a membrane depolarization (Konopka and Parsons, 1989). Galanin inhibits neuromuscular transmission in the human sacral parasympathetic pathway to the urinary bladder (Maggi et al. 1987). Galanin-like immunoreactivity is distributed in nerve fibers of the rat and human urinary bladder (Bauer et al. 1986). However an action of galanin in pelvic parasympathetic ganglia innervating the urinary bladder has not been reported. The aim of this study is to elucidate the effects of galanin on cholinergic transmission in rabbit vesical parasympathetic ganglia.

Male white rabbits were anesthetized with sodium pentobarbital (40-50 mg/kg, iv). The isolation of the vesical parasympathetic ganglia (VPG) on the surface of the urinary bladder has been described previously (Nishimura et al. 1988). Connective tissue surrounding the ganglia was removed as much as possible by a pin and surgical scissors. The ganglia with the pelvic nerve were then pinned onto a Sylgard block in a recording chamber (0.5 ml in volume) and continuously superfused with a Krebs solution (1 ml/min) having the following composition (in mM): NaCl, 117; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.2; NaH₂PO₄, 1.2; NaHCO₃, 25; and glucose 11. The solutions were aerated with 95% O₂, 5% CO₂ and preheated to 35-37 °C at the recording site. The membrane potential was recorded by conventional intracellular recording techniques. Glass microelectrodes were filled with 3M KCl and the tip resistances ranged from 30 to 50 MΩ. An active bridge circuit was used for current injection into neurons through the recording microelectrode. Fast exci-

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tatory postsynaptic potentials (EPSPs) were evoked by electrical stimulation (5-30 V for 0.01-0.3 ms) of the pelvic nerve at intervals of 5-30 s, utilizing a suction electrode (Nishimura and Akasu, 1989). Signals from the microelectrode were continuously monitored with a pen-writing chart recorder. The individual fast EPSPs were displayed on an oscilloscope with digitized memory and recorded on a pen-writing chart recorder.

Drugs were dissolved in Krebs solution and applied by changing the bath solutions with a three-way stopcock. Alternatively, acetylcholine (ACh) was applied by pressure ejection (20 psi for 10-300 ms) from a glass pipette containing 300-600 μ M ACh. All drugs were purchased from Sigma. Data are expressed as mean $\pm$ SEM.

Bath application of porcine galanin (1 μ M for 5 min) depressed the ampli-

Fig. 1. A: effects of galanin on fast EPSPs. The upward deflections indicate the fast EPSPs. The horizontal bar indicates the time period for the application of galanin (1 μ M). The lower traces are expanded records of fast EPSPs at the times indicated by the respective letters in the upper trace. Three consecutive fast EPSPs are superimposed. The recovery record was obtained 15 min after washing out the peptide. B: effects of galanin on the ACh potentials in the presence of atropine (1 μ M). The downward deflections are electrotonic potentials evoked by anodal current injections (0.2 nA for 500 ms). The right trace was recorded 10 min after washing out the peptide.

tude of the fast EPSPs (Fig. 1A). In this neuron the mean amplitudes of the fast EPSPs were 11.5 ± 0.4 mV ($n=30$) and 5.5 ± 0.2 mV ($n=30$) in the absence and presence of galanin, respectively. The depression of the fast EPSP was reversed within 15 min after washing out the peptide. The fast EPSP was reversibly abolished by the application of $10 \mu\text{M}$ (+)-tubocurarine or $100\text{--}200 \mu\text{M}$ hexamethonium in all neurons ($n=10$). The inhibitory effect of galanin was concentration-dependent. At concentrations of 100 nM , 300 nM and $1 \mu\text{M}$, galanin depressed the amplitude of the fast EPSPs by $17 \pm 5\%$ ($n=3$), $39 \pm 8\%$ ($n=3$) and $65 \pm 7\%$ ($n=3$), respectively. The galanin-induced inhibition of the fast EPSP did not display tachyphylaxis during prolonged application of the peptide (up to 10 min; not shown). Galanin did not affect the resting potential or the input membrane resistance (Fig. 1A, B).

To determine the site of action for the galanin-induced inhibition of the fast EPSP, we next examined the effects of the peptide on nicotinic ACh potentials evoked by pressure application of ACh in the presence of atropine ($1 \mu\text{M}$). Bath application of galanin ($1 \mu\text{M}$, 3 min) did not affect the amplitude or time course of the ACh potential (Fig. 1B). The ACh potential was reversibly abolished by $10 \mu\text{M}$ (+)-tubocurarine or $100 \mu\text{M}$ hexamethonium (not shown). These data indicate that galanin did not alter the sensitivity of the nicotinic receptors in the postganglionic neurons.

The present study demonstrates that galanin is a potent inhibitor of nicotinic transmission in rabbit vesical parasympathetic ganglia. The galanin receptors

that suppress the fast EPSP must be located at the presynaptic nerve terminals, since the postsynaptic sensitivity to nicotinic agonists was not altered by galanin. This means that the activation of presynaptic galanin receptors depresses the amount of ACh liberated by action potentials in the preganglionic nerve terminals. Although the mechanism for the presynaptic effect of galanin is not known, galanin may reduce the calcium influx through voltage-gated calcium channels that are activated during the action potential (Tamura et al. 1988; Bartfai et al. 1992). Similar presynaptic inhibitory actions of galanin on cholinergic transmission have been reported in the guinea-pig myenteric plexus (Tamura et al. 1987) and hippocampus (see Bartfai et al. 1992). Also galanin modulates the resting potential in guinea-pig myenteric neurons (Tamura et al. 1988) and mudpuppy cardiac neurons (Konopka and Parsons, 1989; Konopka et al. 1989). However galanin did not affect the resting potential of neurons in the rabbit VPG. There may be a different expression of these receptors between species and/or ganglia. The physiological role of the presynaptic galanin receptors remains to be clarified. The galanin-induced depression of cholinergic transmission may contribute to the neural inhibition of the sacral parasympathetic pathway to the urinary bladder, thereby inhibiting the micturition reflex and causing a retention of urine.

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