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Melatonin modulates cholinergic transmission by blocking nicotinic channels in the guinea-pig submucous plexus

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Abstract

Melatonin, a hormone produced and released by the pineal gland, is also synthesized by cells of the gastrointestinal wall, where it might be a local regulator of gut functions. In this study, we investigated the possible role of melatonin as a modulator of the enteric nervous system. Intracellular recordings were made in neurons of the submucosal plexus from the guinea-pig ileum to measure the melatonin effects on their electrophysiological properties. Melatonin did not alter the membrane potential, the membrane resistance and the noradrenergic inhibitory postsynaptic potentials. However, melatonin (30–3000 μM) reversibly decreased the amplitude of nicotinic excitatory postynaptic potentials (EPSPs) in a concentration-dependent manner (IC_{50} = 247 μM). These actions of melatonin were not modified by the presence of idazoxan and atropine indicating that they are not mediated by endogenous release of acetylcholine, noradrenaline, or by direct activation of α_2-adrenoceptors or muscarinic receptors. The superfusion of melatonin also blocked the nicotinic depolarizations induced by locally applied acetylcholine, indicating that at least part of its effects are postsynaptic. In voltage-clamp experiments, using the whole-cell configuration, melatonin also inhibited the nicotinic inward currents induced by acetylcholine (I_{ACh}) in a concentration-dependent manner (IC_{50} = 257 μM). Melatonin decreased the maximal I_{ACh} but did not affect the potency of acetylcholine to induce this current, indicating a noncompetitive antagonism. This effect was voltage-dependent. Our observations indicate that melatonin inhibits the fast EPSPs by directly and specifically blocking the nicotinic channels. The relative high concentrations of melatonin required to produce such an effect rules this out as one of its humoral actions. Such an effect, however, might be of physiological significance close to the cells that release melatonin in the gastrointestinal wall or in other organs.

Keywords: Melatonin; Nicotinic channel; Cholinergic transmission; Synaptic modulation; Submucosal neuron; Enteric neuron; Nerve terminal

1. Introduction

Melatonin is well known as a hormone of the pineal gland from where it is secreted with a circadian rhythm. This hormone is an indole, synthesized by acetylation of serotonin to *N*-acetylserotonin by arylalkylamine *N*-acetyltransferase and subsequently *O*-methylated through the action of hydroxyindole-*O*-methyltransferase (Reiter, 1991). Several extrapineal tissues have also been reported to synthesize and secrete melatonin, which include the retina, the harderian gland, the lacrimal gland and the gut (Reiter, 1991; Pang et al., 1993). In the gastrointestinal tract melatonin is known to be contained in enterochromaffin cells of the mucosa, which also contain serotonin and the enzyme hydroxyindole-*O*-methyltransferase (Raikhlin et al., 1975; Raikhlin and Kvetnoy, 1976; Bubenik et al., 1977; Bubenik, 1980).

This indole was first isolated in 1959 by Lerner (Lerner, 1959) and since then, many physiological and pharmacological effects of melatonin have been described; e.g. melatonin is known to modulate reproductive and locomotor activities in several species (Cassone, 1990; Reiter, 1991), and to regulate the pattern of sleep in healthy humans (Dollins et al., 1994) or in patients with sleep disorders (Jan et al., 1994). In the gut, no function has yet

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been identified for melatonin but it has been proposed that it might work as a local regulator of gastrointestinal functions (Lee and Pang, 1993; Bubenik and Pang, 1994). In support of this hypothesis, the intraperitoneal injection of melatonin into mice decreased the food transit time and blocked the serotonin-induced decrease in intestinal food transit time (Bubenik and Dhanvantari, 1989). In addition, melatonin also reduced the serotonin-induced contractions of the intestinal smooth muscle (Quastel and Rahamimoff, 1965; Bubenik, 1986). The objective of this study was to investigate the possible role of melatonin as a modulator of submucosal neurons.

2. Materials and methods

2.1. Tissue preparation

Young guinea-pigs (150–300 g), either male or female, were stunned and immediately exsanguinated by severing major neck blood vessels. A segment of distal small intestine (about 3–5 cm in length) was removed, placed in modified Krebs solution and opened longitudinally. The mucosa was removed and the submucosal layer (submucosal preparation) was dissected from the underlying layers of smooth muscle.

2.2. Intracellular recordings

The submucosal preparation was pinned to the Sylgard base of a recording chamber (capacity about 0.5–1 ml). An inverted microscope (×400; TMD, Nikon Canada Instruments, Mississauga, Ontario, Canada) was used to visualize the submucosal ganglia. Intracellular recordings were made with glass microelectrodes filled with 2–3 M KCl (resistance 60–90 MΩ). Membrane potential was measured using an Axoclamp-2A preamplifier (Axon Instruments, Foster City, CA, USA). Membrane input resistance was measured as previously described (Barajas-López, 1993). The output of this preamplifier was displayed on an oscilloscope (Beckman 9020, Tucker Electronics Co., Garland, TX, USA) and recorded with a PC and Axotape software (Axon Instruments). Classification of individual neurons was made according to previous criteria: S-type neurons have fast nicotinic excitatory postsynaptic potentials and AH-type neurons show a long-lasting after hyperpolarization following a single action potential and no fast nicotinic excitatory postsynaptic potentials (Barajas-López et al., 1994; Barajas-López et al., 1995). Synaptic potentials were evoked as previously described (Barajas-López et al., 1995).

Submucosal preparations were continuously superfused with heated (35–37°C) physiological saline solution at 2–5 ml/min. This solution had the following composition (mM): NaCl 126, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, KCl 5, $NaHCO_3$ 25, glucose 11; gassed with 95% O_2 and 5% CO_2. In intracellular experiments, all drugs were applied by superfusion, changing the solution to one that differed only in its content of the drug. Acetylcholine was, in some experiments, applied by pressure ejection to induce fast depolarizations. In this case, one pipette (tip diameter 3–6 μm) was filled with a solution of acetylcholine (2 mM) and pressure was used (typically 140 kPa for 10–250 ms) to eject a few nanoliters of this solution on the ganglia containing the recorded cell. To avoid unwanted effects due to solution leakage from the pipette, the tip of the pipette was always placed about 500 μm from the ejection site and was advanced up to the desired site, just before applying the solution.

2.3. Whole-cell recordings

Whole-cell currents were recorded from short-term (4–40 h) primary cultures of submucosal neurons from the guinea-pig ileum. Methods for dissociating and culturing myenteric neurons have been described elsewhere (Barajas-López et al., 1994, 1996). Briefly, the neurons were dissociated using sequential enzymatic treatments, first with a papain solution (0.1 ml/ml; activated with 0.4 mg/ml of L-cysteine) and latter with a collagenase (1 mg/ml) plus dispase (4 mg/ml) solution. After washing out these enzymes, the cells were plated on rounded coverslips coated with sterile rat tail collagen. Culture medium was minimum essential medium 97.5%, containing 2.5% guinea pig serum, L-glutamine 2 mM, penicillin 10 U/ml, streptomycin 10 μg/ml and glucose 15 mM.

Membrane currents were recorded using an Axopatch 1D amplifier. Patch pipettes were made, as previously reported (Barajas-López et al., 1994) and had resistances between 1–4 MΩ. With a typical pipette of 2 MΩ resistance and for maximal currents (usually no more than 2–4 nA), the voltage error due to series resistance would be lower than 10 mV. Therefore, the series resistance was usually not compensated for. Unless otherwise stated, the holding potential was −70 mV. Experiments were performed using the following solution inside the pipette (in mM): Cs-glutamate 160; EGTA 10; Hepes 5; NaCl 10, ATPMg 3 and GTP 0.1; adjusted to pH 7.4 with CsOH. The external solution had the following composition (in mM): NaCl 160, CaCl 2.5, glucose 11, Hepes 5 and CsCl 3; the pH was adjusted to 7.3–7.4 with NaOH. With such solutions, the input resistance of the neurons ranged from 1 to 10 GΩ. Whole-cell currents were recorded with a PC using axotape software (Axon Instruments). Membrane potentials were corrected for the liquid junction potentials (pipette 11 mV negative).

During the whole-cell experiments, acetylcholine and melatonin were applied by abruptly changing the tube in front of the recorded cell, which delivered the external solution. Rapid changes in the external solution were made by using an eight-barrelled device. The multibarrelled device was moved under microscope visual field using a

Narishige micromanipulator (WR-88 Narishige Sci. Inst. Lab., Tokyo, Japan). These experiments were performed at room temperature (~ 23°C).

2.4. Drugs

The following drugs were used: acetylcholine (Sigma Chemical, St. Louis, MO, USA), idazoxan (Research Biochemicals, Natick, MA, USA) and atropine (Research Biochemicals). Stock solutions of melatonin were made in dimethylsulfoxide at a concentration of 1 M. Stock solution of atropine and acetylcholine were in water (1–100 mM). All solutions were always kept at −4°C.

2.5. Data analysis

Results were expressed as means ± S.E.M. and the number of used cells as n. The paired Student's t-test was used to evaluate differences between mean values coming from the same cells; two-tailed P-values of 0.05 or less were considered statistically significant. Data of Fig. 1 and Fig. 2 and Fig. 5 were fitted to a two- or three-parameter logistic function to calculate the Hill slope value and the concentration required to produce half-maximal inhibition (IC_{50}) or activation (EC_{50}), as previously described (Fieber and Adams, 1991; Kenakin, 1993).

3. Results

3.1. Intracellular experiments

Results reported here are from intracellular recordings made in 39 S-type submucosal neurons from the guinea-pig ileum. Electrophysiological properties of these neurons were as previously published (Barajas-López, 1994; Barajas-López et al., 1994)

Melatonin (0.5–1 mM) did not alter the membrane potential, the input membrane resistance ($n = 6$) and the noradrenergic inhibitory postsynaptic potentials (IPSPs; $n = 4$). However, superfusion of melatonin (30–3000 μM) reversibly decreased the amplitude of nicotinic EPSPs in all submucosal neurons tested ($n = 33$; Fig. 1A). No evidence of desensitization was observed because this inhibitory action persisted for as long as melatonin was present (up to 15 min) and similar inhibitory effects were obtained by cumulative and noncumulative applications. The melatonin effect was concentration-dependent (Fig. 1B) and the IC_{50} and Hill slope values were 257 ± 20 μM and 1.01 ± 0.07, the latter was not different than unity. Maximal concentrations of melatonin (> 2 mM) almost completely inhibited the fast EPSPs. These effects were observed within the first 30 s after starting the melatonin perfusion, were maximal 2–3 min after, and were completely reverted by discontinuing application of the drug, usually within the following 5 min (Fig. 2A,B).

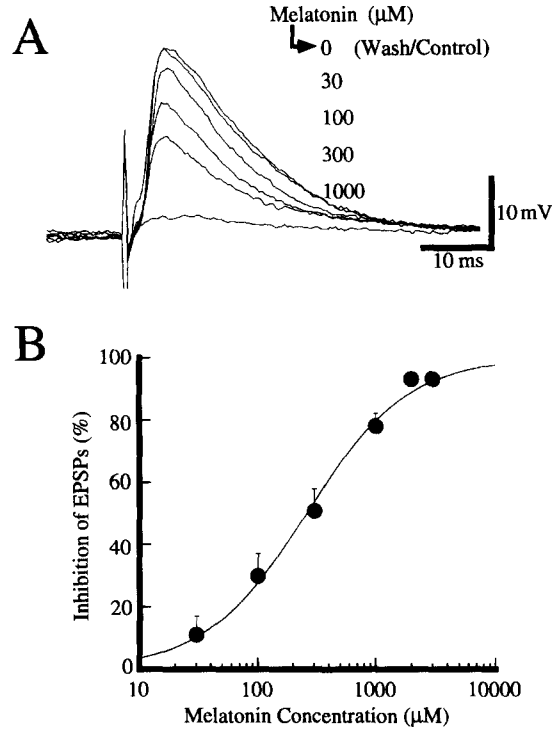

Fig. 1. Melatonin inhibited nicotinic excitatory postsynaptic potentials (EPSPs) in a concentration-dependent manner in guinea-pig submucosal neurons. (A) EPSPs recorded before (Control), in several concentrations of melatonin and after the removal of this indole from the external solution. These EPSPs are from the same submucosal neuron. (B) Concentration-response curve for melatonin effects on EPSPs. Squares are means ± S.E.M. of 4–18 submucosal neurons. The sigmoidal line was fitted with a two-parameter logistic function, assuming a maximal inhibition equal to 100%.

These actions of melatonin were not modified by the presence of 1 μM idazoxan (α_2-adrenoceptor antagonist) and 1 μM atropine (muscarinic receptor antagonist); 1 mM melatonin inhibited the fast EPSPs by 75 ± 5% before and 76 ± 5% in the presence of these agonists ($n = 4$). This would indicate that the melatonin effect is not mediated by endogenous release of noradrenaline or acetylcholine acting on presynaptic receptors or by direct activation of presynaptic α_2-adrenoceptors or muscarinic receptors with melatonin. The superfusion of melatonin (1 mM) also blocked the nicotinic depolarizations induced by locally applied acetylcholine; control depolarizations had an average amplitude of 37 ± 4 mV and in the presence of melatonin this was 15 ± 4 mV ($n = 5$; $P < 0.01$; Fig. 3). Such findings would indicate that at least part of the melatonin effect on fast EPSPs is postsynaptic. The solvent dimethylsulfoxide did not affect the fast EPSPs at a concentration of 0.1% (v/v); EPSP amplitude was 51 ± 8 and 50 ± 8 mV before and in the presence of this solvent, respectively ($n = 5$). We further studied this postsynaptic action by investigating the actions of melatonin on the acetylcholine-induced nicotinic currents, which were recorded using the whole-cell configuration of the patch clamp technique.

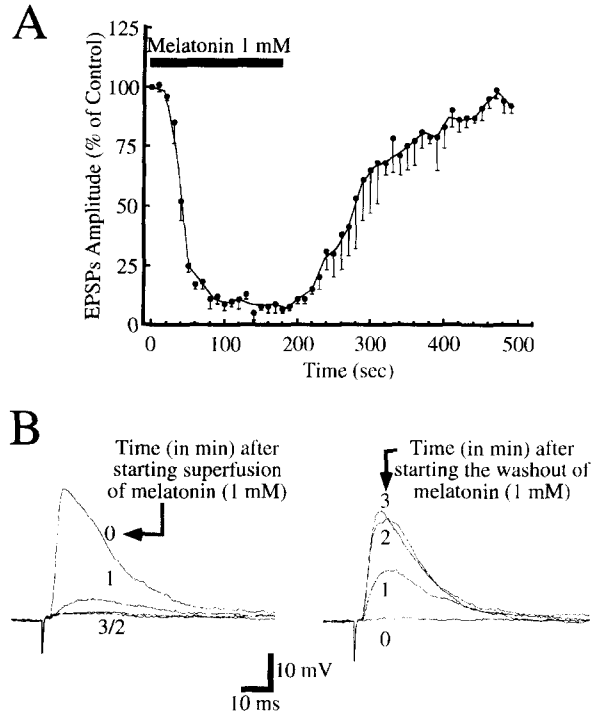

Fig. 2. Temporal course of melatonin actions on nicotinic excitatory postsynaptic potentials (EPSPs). (A) Melatonin was applied as indicated by the bar. Circles are means ± S.E.M. of 4 submucosal neurons. (B) EPSPs recorded during that onset and offset of melatonin actions, from one submucosal neuron.

3.2. Melatonin effects on the nicotinic currents activated by acetylcholine

Acetylcholine (10–6000 μM) application activated a rapidly desensitizing current (I_{ACh}) in 35 out of 40 submucosal neurons (Fig. 4A). This I_{ACh} was inward at negative membrane potentials, its amplitude was increased with hyperpolarization and became outward at potentials around +5 mV (see Fig. 6). The amplitude of the I_{ACh} was also increased by raising the acetylcholine concentration (Fig. 5); the EC_{50} and Hill slope values were 52 ± 3 μM and 2.4 ± 0.34, the latter was significantly different from unity ($P < 0.01$).

Melatonin (30–3000 μM) reversibly inhibited the I_{ACh} in all neurons tested ($n = 35$). This effect was observed when melatonin was applied 1–3 min before (Fig. 4A, *upper panel*) or during acetylcholine stimulation (Fig. 4, *lower panel*). When applied during acetylcholine stimulation, this effect appeared to reach the maximum and inactivated within 2–3 s after adding or washing the melatonin. Such an inhibitory effect was concentration-dependent (Fig. 4B); IC_{50} and Hill slope values were 247 ± 67 μM and 1.03 ± 0.24, the latter was not different than unity. This inhibitory effect of melatonin was not surmounted by increasing acetylcholine concentration, indicating a physiological antagonism (Fig. 5). In agreement with this interpretation, 0.5 mM melatonin did not affect the potency of acetylcholine to induce the nicotinic inward current (EC_{50} was 52 ± 3 and 48 ± 8 μM before and during the presence of melatonin, respectively) but decreased the maximal effects (3067 ± 68 to 1173 ± 80 pA; $P < 0.01$). Melatonin effects appear to be voltage-dependent because the blockage of the I_{ACh} was larger for inward than for outward currents (Fig. 6). Indeed, the inhibitory effect of melatonin on outward currents appeared to decrease in proportion to the depolarization (Fig. 6A). Whereas there was not any difference on the inhibition of inward currents at the different membrane potentials tested (Fig. 6A). The voltage dependency of this inhibitory effect might indicate that melatonin binds close enough to the mouth of the pore to sense the membrane potential and to interfere with the ion influx through the nicotinic channels (Hille, 1992).

We also investigated the effects of melatonin on serotonin- and ATP-gated ion channels, which have been

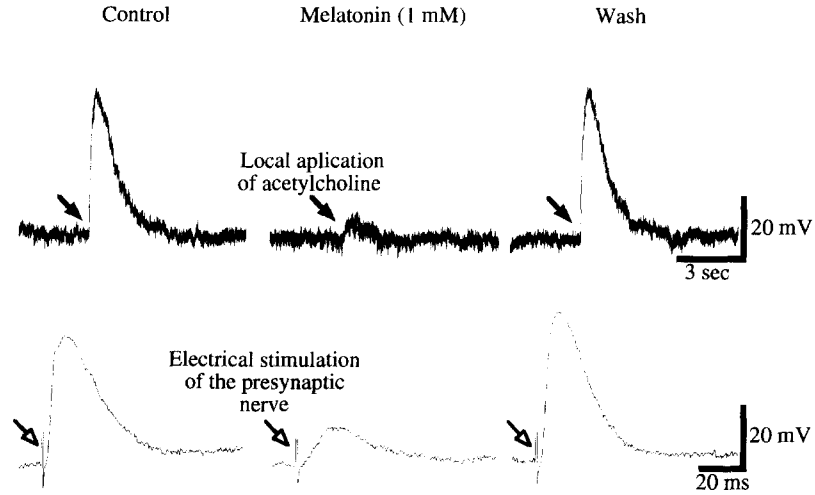

Fig. 3. Melatonin also inhibited fast depolarizations induced by external application of acetylcholine. Depolarizations induced by local application of acetylcholine (*upper panel*) and by stimulation of presynaptic nerve (*lower panel*; fast excitatory postsynaptic potentials) recorded from the same submucosal neuron, before (Control), during (Melatonin) and after (Wash) application of melatonin. This indole was applied by pressure ejection.

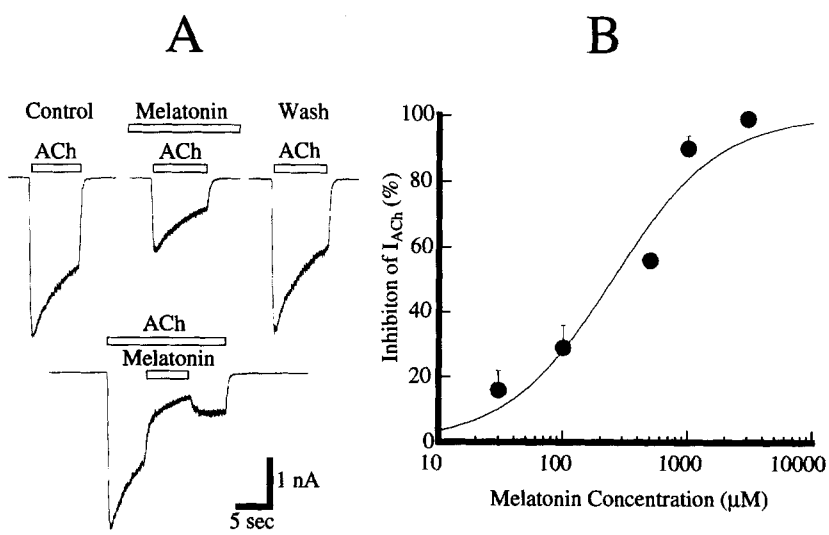

Fig. 4. Melatonin also blocked acetylcholine-activated inward currents (I_{ACh}) in a concentration-dependent manner. (A) *Upper panel.* Whole-cell currents induced by 100 μM acetylcholine (ACh) and recorded before (Control), in presence (Melatonin), and after (Wash) melatonin (0.5 mM). *Lower panel.* Melatonin was also effective when added during acetylcholine (100 μM) stimulation. All these recordings were taken from the same submucosal neuron when holded at −70 mV. (B) Concentration-response curve for melatonin effects on the acetylcholine-evoked current. Circles are means ± S.E.M. of 5–7 submucosal neurons. The sigmoidal line was fitted with a two-parameter logistic function, assuming a maximal inhibition equal to 100%.

shown to be expressed in submucosal neurons (Derkach et al., 1989; Barajas-López et al., 1994). This was of particular interest because it has been suggested that melatonin acts as a modulator for serotonin action on the gastrointestinal tract (Cho et al., 1989). Fig. 7 shows the results of these experiments. Melatonin (0.5 mM) inhibited only 11 ± 14 and 12 ± 4% of the whole-cell currents induced by ATP (30 μM; $n = 5$) and serotonin (30 μM; $n = 7$), respectively; whereas in four of these cells the same concentration of melatonin inhibited 60 ± 2% of the cur-

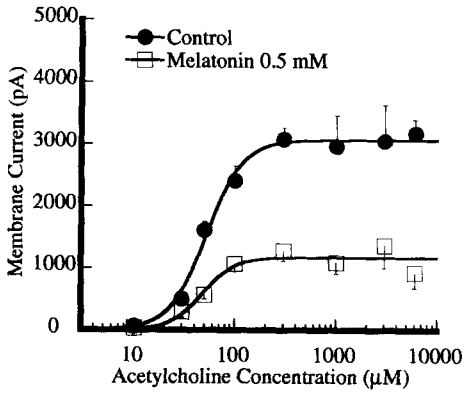

Fig. 5. Melatonin blocked acetylcholine-activated inward currents in a noncompetitive manner. Average values of inward currents (pA) induced by different concentrations of ACh, before (Control) and then in the presence of 0.5 mM melatonin. Notice that melatonin did not change the acetylcholine-concentration required to produce the half-maximal response but decreased the maximal response to acetylcholine. Symbols are means ± S.E.M. of 3–6 submucosal neurons. The sigmoidal line was fitted with a three-parameter logistic function.

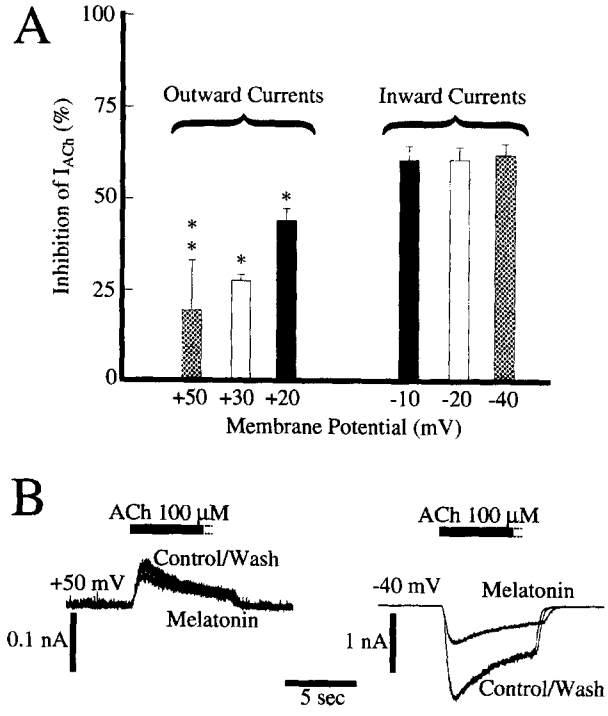

Fig. 6. The inhibition of acetylcholine-activated currents (I_{ACh}) induced by melatonin was voltage-dependent. (A) Melatonin-induced inhibition of acetylcholine currents was determined at equidistant membrane potentials, above and below the average reversal potential for this current (+5 mV). Differences between mean values taken at these two membrane potentials (bars with the same filling) were evaluated. One and two asterisks indicate a P value of less than 0.05 and 0.01, respectively. Data are from 4 submucosal neurons and the line on top represents the S.E.M. B, currents induced by acetylcholine (ACh) before (Control), during (Melatonin) and after (Wash) 0.5 mM melatonin. Current traces are from the same submucosal neuron and were recorded at the indicated membrane potential (mV).

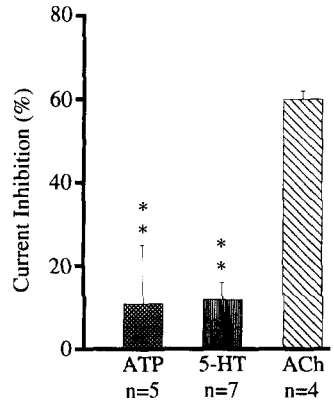

Fig. 7. The inhibitory effect of melatonin is relatively specific for acetylcholine-activated channels. Inhibition induced by 0.5 mM melatonin of whole-cell currents activated by 30 μM ATP, serotonin (5-HT) or acetylcholine (ACh). The inhibition of the ATP- and serotonin-activated currents was significantly lower than the obtained for acetylcholine. Bars are means and the line on top represents the S.E.M. ** $P < 0.01$ (unpaired Student's t-test).

rents activated by 30 μM acetylcholine. This indicates that melatonin has certain specificity for nicotinic channels.

4. Discussion

The main findings of this study were the demonstration that melatonin inhibits the fast EPSPs and the ion currents activated by acetylcholine in the guinea-pig submucosal neurons. We speculate that in the gastrointestinal tract, melatonin might be a local modulator of acetylcholine actions on nicotinic receptors.

Two findings indicate that melatonin inhibits EPSPs mainly, if not only, by a postsynaptic action. First, melatonin inhibited the fast depolarizations induced by external application of acetylcholine. Second, the concentration-response curves for melatonin to inhibit the I_{ACh} and fast EPSPs were very similar. In addition, the fast onset and offset of the melatonin actions and the voltage dependency of these effects suggest that melatonin inhibits EPSPs by blocking the nicotinic channels. This is the first report on the melatonin-induced inhibition of cholinergic fast EPSPs and nicotinic currents.

These effects were seen at concentrations (≥ 30 μM) far higher than those (< 10 nM) found in the serum of mammals (Vaughan et al., 1986; Huether, 1994) indicating that they are unlikely to occur as the result of a hormonal action of melatonin. Micromolar concentrations, however, might be reachable in the vicinity of cells that produce this indole and therefore, the blockage of nicotinic channels could be one of the postulated paracrine effects of melatonin. Cholinergic terminals are known to express nicotinic receptors (Buckley and Caulfield, 1992). The function of nicotinic receptors at nerve terminals is unknown but it has been proposed that they might modulate transmitter release (Javed and Cooke, 1992). In the gut, cholinergic fibers are known to innervate the mucosa (Javed and Cooke, 1992) and some of these might run close enough to enterochromaffin cells so as to be affected by their secretory products, e.g. melatonin, serotonin. A similar paracrine effect of melatonin might also be postulated in the retina and in the pineal gland where nicotinic receptors are known to be expressed by several cells, including pinealocytes (Stankov et al., 1993) and ganglionic neurons of the retina (Britto et al., 1994). In the pineal gland, activation of nicotinic receptors, though having no effect per se on the synthesis and release of melatonin, significantly diminished the norepinephrine-stimulated melatonin accumulation. The effect of melatonin on nicotinic channels of submucosal neurons is similar to that reported for hexamethonium in enteric (Nishi and North, 1973; Hirst and McKirdy, 1975) and other peripheral neurons (Buckley and Caulfield, 1992). It would be interesting to investigate also the effect of melatonin on nicotinic fast EPSPs of other peripheral ganglia and on synaptic transmission mediated by other ligand-gated ion channels; e.g. those activated by glutamate and γ-aminobutyric acid. Altogether, these observations indicate that the effect of melatonin on nicotinic channels might be involved in a paracrine action of this indole and that melatonin might be a useful tool to pharmacologically manipulate the function of nicotinic channels.

In conclusion, melatonin inhibits the fast EPSPs in the submucosal plexus. This effect is postsynaptic and likely due to the direct blockage of acetylcholine-activated ion channels. Our results indicate that melatonin might be a local modulator of nicotinic channels in the gastrointestinal tract.

Acknowledgements

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References

Barajas-López, C., 1993, Adenosine reduces the potassium conductance of guinea-pig submucosal plexus neurons by activating protein kinase A, Pflügers Arch. 424, 410.

Barajas-López, C., 1994, Interactions between inhibitory and excitatory modulatory signals in single submucosal neurons, Am. J. Physiol. 267, C1359.

Barajas-López, C., R. Espinosa-Luna and V. Gerzanich, 1994, ATP closes a potassium and opens a cationic conductance through different receptors in neurons of guinea-pig submucous plexus, J. Pharmacol. Exp. Ther. 268, 1396.

Barajas-López, C., M.J. Muller, B. Prieto-Gómez and R. Espinosa-Luna, 1995, ATP inhibits the synaptic release of acetylcholine in submucosal neurons, J. Pharmacol. Exp. Ther. 274, 1238.

Barajas-López, C., A.L. Peres and R. Espinosa-Luna, 1996, Cellular mechanisms underlying adenosine actions on cholinergic transmission in enteric neurons, Am. J. Physiol. 271, (in press).

Britto, L.R., S.W. Rogers, D.E. Hamassaki-Britto and R.M. Duvoisin, 1994, Nicotinic acetylcholine receptors in the ground squirrel retina: localization of the beta 4 subunit by immunohistochemistry and in situ hybridization, Visual. Neurosci. 11, 569.

Bubenik, G.A., 1980, Localization of melatonin in the digestive tract of the rat. Effect of maturation, diurnal variation, melatonin treatment and pinealectomy, Hormone Res. 12, 313.

Bubenik, G.A., 1986, The effect of serotonin, *N*-acetylserotonin, and melatonin on spontaneous contractions of isolated rat intestine, J. Pineal Res. 3, 41.

Bubenik, G.A. and S. Dhanvantari, 1989, Influence of serotonin and melatonin on some parameters of gastrointestinal activity, J. Pineal Res. 7, 333.

Bubenik, G.A. and S.F. Pang, 1994, The role of serotonin and melatonin in gastrointestinal physiology: ontogeny, regulation of food intake, and mutual serotonin-melatonin feedback, J. Pineal Res. 16, 91.

Bubenik, G.A., G.M. Brown and L.J. Grota, 1977, Immunohistological localization of melatonin in the rat digestive system, Experientia 33, 662.

Buckley, N.J. and M. Caulfield, 1992, Transmission: Acetylcholine, in: Autonomic Neuroeffector Mechanisms, eds. G. Burnstock and C.H.V. Hoyle (Hardwood Academic Publishers, Chur, Switzerland) p. 277.

Cassone, V.M., 1990, Effects of melatonin on vertebrate circadian systems [Review], Trends Neurosci. 13, 457.

Cho, C.H., S.F. Pang, B.W. Chen and C.J. Pfeiffer, 1989, Modulating action of melatonin on serotonin-induced aggravation of ethanol ulceration and changes of mucosal blood flow in rat stomachs, J. Pineal Res. 6, 89.

Derkach, V., A. Surprenant and R.A. North, 1989, 5-HT_3 receptors are membrane ion channels, Nature 339, 706.

Dollins, A.B., I.V. Zhdanova, R.J. Wurtman, H.J. Lynch and M.H. Deng, 1994, Effect of inducing nocturnal serum melatonin concentrations in daytime on sleep, mood, body temperature, and performance, Proc. Natl. Acad. Sci. USA 91, 1824.

Fieber, L.A. and D.J. Adams, 1991, Acetylcholine-evoked currents in cultured neurones dissociated from rat parasympathetic cardiac ganglia, J. Physiol. (London) 434, 215.

Hille, B., 1992, Ionic channels of excitable membranes (Sinauer Associates Inc., Sunderland, MA).

Hirst, G.D. and H.C. McKirdy, 1975, Synaptic potentials recorded from neurones of the submucous plexus of guinea-pig small intestine, J. Physiol. (London) 249, 369.

Huether, G., 1994, Melatonin synthesis in the gastrointestinal tract and the impact of nutritional factors on circulating melatonin [Review], Ann. NY Ac. Sci. 719, 146.

Jan, J.E., H. Espezel and R.E. Appleton, 1994, The treatment of sleep disorders with melatonin, Dev. Med. Child Neurol. 36, 97.

Javed, N.H. and H.J. Cooke, 1992, Acetylcholine release from colonic submucous neurons associated with chloride secretion in the guinea pig, Am. J. Physiol. 262, G131.

Kenakin R.,, 1993, Pharmacologic Analysis of Drug-receptor Interaction (Raven Press, Ltd., New York, NY) p. 176.

Lee, P.P. and S.F. Pang, 1993, Melatonin and its receptors in the gastrointestinal tract [Review], Biol. Signals 2, 181.

Lerner, A.B., 1959, Structure of melatonin, J. Am. Chem. Soc. 81, 6084.

Nishi, S. and R.A. North, 1973, Intracellular recording from the myenteric plexus of the guinea-pig ileum, J. Physiol. (London) 231, 471.

Pang, S.F., M.L. Dubocovich and G.M. Brown, 1993, Melatonin receptors in peripheral tissues: a new area of melatonin research [Review], Biol. Signals 2, 177.

Quastel, M.R. and R. Rahamimoff, 1965, Effects of melatonin on spontaneous contractions and response to 5-hydroxytryptamine of rat isolated duodenum, Br. J. Pharmacol. 24, 455.

Raikhlin, N.T., I.M. Kvetnoy and V.N. Tolkachev, 1975, Melatonin may be synthesised in enterochromaffin cells, Nature 255, 344.

Raikhlin, N.T. and I.M. Kvetnoy, 1976, Melatonin and enterochromaffine cells, Acta Histochem. 55, 19.

Reiter, R.J., 1991, Pineal melatonin: cell biology of its synthesis and of its physiological interactions [Review], Endocrine Rev. 12, 151.

Stankov, B., M. Cimino, P. Marini, V. Lucini, F. Fraschini and F. Clementi, 1993, Identification and functional significance of nicotinic cholinergic receptors in the rat pineal gland, Neurosci. Lett. 156, 131.

Vaughan, G.M., A.D. Mason, Jr. and R.J. Reiter, 1986, Serum melatonin after a single aqueous subcutaneous injection in Syrian hamsters, Neuroendocrinology 42, 124.