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Short communication

Melatonin inhibits calcitonin gene-related peptide-induced vasodilation and increase in cAMP in rat middle cerebral arteries

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Abstract

The action of melatonin to alter calcitonin gene-related peptide (CGRP)-mediated vasodilation and stimulation of adenylate cyclase activity in middle cerebral arteries of rats was investigated. Concentration-dependent dilation of the rat middle cerebral artery produced by CGRP (EC_{50} of 9.4×10^{-10} M) was significantly inhibited in the presence of 10^{-8} M melatonin (EC_{50} of 3.4×10^{-9} M). In addition, CGRP (10^{-7} M)-mediated increase in adenylate cyclase activity was also significantly attenuated by the receptor mediated action of melatonin. These results indicate that melatonin may interact with CGRP to regulate cerebral arterial tone.

Keywords: CGRP (calcitonin gene-related peptide); Melatonin; Cerebral artery; Adenylate cyclase; Vascular tone; Cerebral circulation

1. Introduction

Melatonin is a pineal hormone that is believed to act as a neuroendocrine transducer of photoperiod in vertebrates (Reiter, 1991). High-affinity receptors for melatonin have been localized and characterized in brain structures as well as in peripheral tissues (Morgan et al., 1994). Previous studies in the rat have described the selective expression of melatonin receptors in the tail artery and arteries forming the circle of Willis at the base of the brain (Capsoni et al., 1994; Viswanathan et al., 1990). Melatonin has been demonstrated to increase the tone of cerebral arteries in vitro (Geary et al., 1997; Viswanathan et al., 1997), as well as in vivo (Regrigny et al., 1999), an effect that is believed to be mediated by the inhibition of large conductance potassium channels (Geary et al., 1997; Regrigny et al., 1999).

Recent evidence suggests that in rats, melatonin decreases the lower limit of cerebral blood flow autoregulation during hemorrhagic hypotension (Regrigny et al., 1998). Since cerebral blood flow autoregulates effectively via vasodilatation during brief periods of acute hypotension (Kontos et al., 1978), the mechanism by which melatonin, a vasoconstrictor, influences cerebral blood flow autoregulation during hypotension is not clear. Vasodilatation of rat pial arteries in response to transient hemorrhagic hypotension has been demonstrated to be mediated in part by calcitonin gene-related peptide (CGRP) released from perivascular sensory fibers (Hong et al., 1994). These findings raise the possibility of the existence of an interaction between melatonin and CGRP in the regulation of cerebral blood flow autoregulation.

The vasodilatory action of CGRP is mediated primarily through activation of adenylate cyclase and the accumulation of 3′, 5′-adenosine monophosphate (cAMP) (Edwards et al., 1991). Based on this finding (Edwards et al., 1991), and that melatonin receptors in cerebral arteries of rats inhibit forskolin-stimulated cAMP formation (Capsoni et al., 1994), we tested the hypothesis that melatonin modulates cerebral arterial dilatation by inhibition of CGRP-induced stimulation of adenylate cyclase.

2. Materials and methods

Male Sprague–Dawley rats (7–8 weeks old; Harlan, Indianapolis, IN) were killed by decapitation, and their brains were quickly removed and kept in ice-cold physio-

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logical salt solution (PSS) containing (in mM) NaCl 119, KCl 4.7, $MgSO_4$ 1.17, $NaHCO_3$ 22, $CaCl_2$ 1.6, (*N*-(2-Hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)) 8, KH_2PO_4 1.18, glucose 5 (gently bubbled with 5% CO_2/95% oxygen; pH 7.4). Segments (1–2 mm long) of middle cerebral artery (153 ± 11 μm diameter) were dissected, kept in ice-cold PSS, cleaned of excess connective tissue, and transferred to a vessel chamber (Living Systems Instrumentation, Burlington, VT) filled with cold PSS. Both ends of the arteries were mounted on glass cannulae and secured using fine nylon filaments. During dissections and mountings, arteries were handled carefully so as not to damage the endothelium. The PSS in the chamber was warmed to 37°C, cannulas at the proximal and distal ends connected to pressure transducers, and peristaltic pumps were connected to the cannula at the proximal end of the artery. The artery was perfused with PSS at a rate of 75 μl/min, using an electronic pressure servo system (Living Systems), and the intraluminal pressure was maintained at 60 mm Hg. The lumen diameter of the artery was constantly monitored and measured using a video camera connected to an inverted microscope and a video dimension analyzer (Living Systems).

Arteries were allowed to stabilize for 1 h at a transluminal pressure of 60 mm Hg, and those which failed to develop myogenic tone were discarded. Increasing concentrations of CGRP (from 10^{-12} to 5×10^{-6} M) were cumulatively added to the superfusion medium every 3 min, and the lumen diameter was recorded continuously. In separate experiments, the effect of cumulative concentrations of CGRP was tested in the presence of 10^{-8} M melatonin after 10 min of pretreatment. This concentration of melatonin was chosen based on EC_{50} values of 7×10^{-9} M obtained from melatonin concentration response curves obtained under identical conditions in preliminary experiments. The diameter of the artery after the addition of melatonin was taken as the baseline to calculate the effect of CGRP.

Adenylate cyclase activity was measured in 2-mm long segments of middle cerebral artery using a previously described method for rat cerebral arterioles (Edwards et al., 1991). Middle cerebral arteries were isolated and dissected into 2-mm long pieces, and each piece was transferred to an individual round bottom well of a 96-well polypropylene plate (Costar, Corning, NY) containing 5 μl of hypotonic medium consisting of (in mM); Tris 8, $MgCl_2$ 1, EDTA 0.25, and 0.1% protease-free bovine serum albumin, pH 7.4. The samples were frozen and thawed twice on dry ice and the incubation was started by the addition of 5 μl of incubation buffer (pH 7.4) consisting of (in mM); Tris 100, $MgCl_2$ 4, EDTA 0.25, 3-isobutyl-1-methylxanthine 1, ATP 0.3, GTP 0.1, phosphocreatine 20, creatine kinase 200 U/ml and appropriate agonists or antagonist. Samples were incubated for 30 min and the incubation was stopped by the addition of 10 μl of 0.2 N HCl and rapid freezing of the samples on dry ice. This was followed by the addition of 10 μl of 0.2 M NaOH and 170 mM sodium acetate buffer, pH 6.2. cAMP was measured using a commercially available enzyme immunoassay kit (Amersham Pharmacia Biotech, Piscataway, NJ) on acetylated samples. Adenylate cyclase activity was expressed as fmol cAMP/mm artery/30 min.

CGRP (rat) was from Peninsula Laboratories (San Carlos, CA), melatonin was from Research Biochemicals International (Natick, MA), and luzindole was from Tocris Cookson (Ballwin, MO). All other chemicals were from Sigma (St. Louis, MO).

Vessel diameters at each drug concentration were determined as a percentage of control vessel lumen diameter. Magnitude of change in diameter is expressed as the percent change in diameter from control vessel diameter. Values are expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by a Bonferroni procedure for post-hoc comparison. A value of $P < 0.05$ was considered significant.

3. Results

CGRP produced concentration-dependent dilation of the rat middle cerebral artery. Maximal diameter was obtained at a concentration of 10^{-7} M with an EC_{50} of 9.4×10^{-10} M (Fig. 1). When vessels were pretreated with 10^{-8} M melatonin, significant contraction was obtained, as seen before (Viswanathan et al., 1997). The percentage of contraction caused by 10^{-8} M melatonin was $11.9 \pm 2.6\%$ (data not shown). In the presence of melatonin, vasodilation mediated by CGRP was significantly inhibited (Fig. 1). The concentration–response curve obtained with increasing concentrations of CGRP shifted to the right, with an EC_{50} of 3.4×10^{-9} M. In addition, presence of melatonin significantly reduced ($P < 0.05$; Student's unpaired

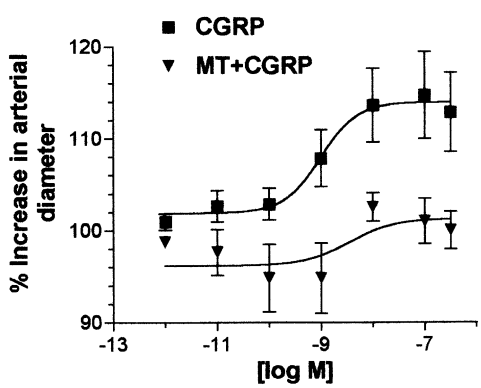

Fig. 1. Effect of increasing concentrations of calcitonin gene-related peptide (CGRP), in the absence and presence of melatonin (10^{-8} M; MT), on dilation of the rat middle cerebral artery. Data shown are means ± SEM ($n = 6$), and the concentration response curve was fitted by non-linear regression (GraphPad Prizm software).

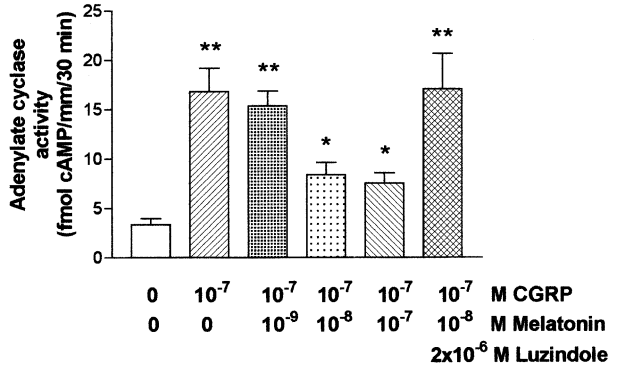

Fig. 2. Effects of calcitonin gene-related peptide (CGRP) on adenylate cyclase activity in rat middle cerebral artery in the presence and absence of melatonin (10^{-9}, 10^{-8} and 10^{-7} M), and the effect of luzindole (2×10^{-6} M) on antagonizing the action of melatonin (10^{-8} M). $^{**}P <$ 0.001 when comparing adenylate cyclase activity in the arteries, without and with the addition of CGRP (10^{-7} M), and $^{*}P < 0.05$ when comparing the effect of melatonin on CGRP-induced stimulation of adenylate cyclase activity, as determined by analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. Bars are means ± SEM ($n = 12$).

t-test) the maximum dilation to 101 ± 2.5% from 115 ± 5.2% in the absence of melatonin.

In order to test whether the inhibition of CGRP-mediated vasodilation by melatonin was accompanied by parallel changes in adenylate cyclase activity, cAMP generation by CGRP in middle cerebral artery samples was examined in the presence of increasing concentrations of melatonin. While melatonin at a concentration of 10^{-9} M did not cause significant inhibition of adenylate cyclase activity, concentrations of 10^{-8} M and 10^{-7} M brought about significant inhibition (Fig. 2). Melatonin receptor antagonist, luzindole (2×10^{-6} M) significantly suppressed the inhibitory action of 10^{-8} M melatonin on adenylate cyclase activity (Fig. 2). Luzindole (2×10^{-6} M) has been previously demonstrated to significantly inhibit melatonin-mediated contraction of rat middle cerebral arteries up to a concentration of 10^{-7} M (Geary et al., 1997).

4. Discussion

The major finding in this study is that cerebral arterial dilation mediated by one of the most powerful endogenous vasodilator peptides CGRP, is markedly inhibited by melatonin. Inhibition of CGRP-induced increase in adenylate cyclase appears to be one of the mechanisms by which melatonin causes this action. These observations, for the first time, raise the possibility that melatonin may act as a physiological modulator of the action of CGRP during autoregulatory vasodilation of rat pial arteries in response to conditions like hemorrhagic hypotension (Hong et al., 1994).

CGRP, a 37-amino acid neuropeptide expressed predominantly in the nervous system and particularly abundant in perivascular nerves, is the most potent endogenous vasodilatory peptide (Wimalawansa, 1996). The dilatory action of CGRP in rat cerebral arterioles is believed to be mediated by the stimulation of adenylate cyclase and the resultant increase in cAMP (Edwards et al., 1991). We have previously reported that melatonin receptors in cerebral arteries, which form the circle of Willis of rats, inhibit forskolin-stimulated cAMP formation (Capsoni et al., 1994). These findings prompted us to test the hypothesis that the action of CGRP on enhancing adenylate cyclase activity in rat cerebral arteries is inhibited by melatonin. Our findings indicate that melatonin, in a concentration dependent manner, is capable of inhibiting CGRP-stimulated activity of adenylate cyclase. Luzindole, a melatonin receptor antagonist (Krause and Dubocovich, 1991), at a concentration of 2×10^{-6} M, has been shown to be effective in completely inhibiting the contractile action of melatonin in rat middle cerebral arteries (Geary et al., 1997). We demonstrated that this concentration of luzindole was able to prevent the inhibitory action of melatonin on CGRP-stimulated activity of adenylate cyclase. Our findings indicate that CGRP and melatonin may share an active role in the maintenance of arterial tone in cerebral vasculature.

Several studies have demonstrated that melatonin causes constriction of rat cerebral arteries (Geary et al., 1997; Regrigny et al., 1999; Viswanathan et al., 1997). The vasoconstrictor effect is believed to be mediated by inhibition of Ca^{2+}-activated K^{+} channels, and not through the action of melatonin on the nitric oxide pathway (Geary et al., 1997; Regrigny et al., 1999). The physiological importance of a vasoconstrictive effect of melatonin was recently revealed in a study of the cerebral blood flow autoregulation in rats following hemorrhagic hypotension (Regrigny et al., 1998). This study found that acute administration of melatonin to rats shifted the lower limit of cerebral blood flow autoregulation to a significantly lower pressure level, and that this action of melatonin was concentration-dependent (Regrigny et al., 1998).

It is conceivable that in conditions such as hemorrhagic hypotension, the function of melatonin may be to modulate the action of endogenous regulatory agents that are activated to maintain cerebral blood flow autoregulation. Autoregulatory vasodilation of rat pial arteries in response to hypotension is believed to be mediated, in part, by CGRP released from perivascular sensory nerve fibers (Hong et al., 1994). Therefore, maintenance of arterial tone may be maintained by the combined action of CGRP and melatonin. Although a report of increased levels of melatonin in the circulation of mice during hemorrhagic shock (Wichmann et al., 1996) lends support to our hypothesis that melatonin may be an endogenous factor involved in the regulation of cerebrovascular tone through its interaction

with CGRP, further studies are needed to ascertain such an interaction in vivo.

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