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Short communication

Melatonin mediates two distinct responses in vascular smooth muscle

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Abstract

The pineal hormone melatonin was found to produce two distinct contractile responses in vascular smooth muscle. In isolated rat caudal artery segments, denuded of endothelium, melatonin (10^{-10}–10^{-7} M) potentiated phenylephrine-induced contractions in a concentration-dependent manner. At higher melatonin concentrations (10^{-7}–10^{-5} M), however, the potentiating effect was attenuated. In the presence of the melatonin MT_2 receptor antagonist, 4-phenyl-2-acetamidotetraline (4P-ADOT), the attenuated constrictor responses were selectively enhanced. These results are consistent with the hypothesis that melatonin activates two receptor subtypes in vascular smooth muscle; MT_2 receptors may induce relaxation, while a second receptor subtype mediates vasoconstriction. © 1998 Elsevier Science B.V.

Keywords: Melatonin receptor subtype; Pineal hormone; Artery; Caudal; Rat

1. Introduction

The pineal hormone melatonin is secreted during the hours of darkness, thus providing cues regarding day length and season. The effects of melatonin on target tissues, however, are not well characterized. Recently, melatonin has been shown to act on certain vascular beds. In the rat, melatonin constricts the middle cerebral artery (Geary et al., 1997) and potentiates contractile responses to adrenergic stimulation in the caudal artery (Krause et al., 1995). The contractile effects appear to be mediated by $G_{i/o}$-protein coupled melatonin receptors, although the identity of these receptors has not been established.

Antagonists are now available that can discriminate between the two mammalian, G-protein coupled, melatonin receptors that have been cloned, mt_1 and mt_2 (previously termed Mel_{1a} and Mel_{1b}) (Dubocovich, 1995; Dubocovich et al., 1997, 1998). We have tested one of these antagonists, 4-phenyl-2-acetamidotetraline (4P-ADOT), in the rat caudal artery. The affinity of 4P-ADOT for the mt_2 receptor is 330 times higher than that for the mt_1 subtype as determined using recombinant human melatonin receptors (Dubocovich et al., 1997). Here, we report that, using this selective antagonist, the contractile effects of melatonin on vascular smooth muscle can be separated into two components, potentiation of contraction and relaxation, which may be mediated by two distinct melatonin receptor subtypes.

2. Materials and methods

Four-month-old Fischer 344 male rats were sacrificed, and caudal arteries removed. Artery segments (3 mm in length) were denuded of endothelium by intimal rubbing and then mounted on platinum wires in oxygenated, 37°C Krebs' solution (in mM): NaCl, 122; KCl, 5.2; $CaCl_2$, 1.6; KH_2PO_4, 1.2; $NaHCO_3$, 25.5; $MgSO_4$, 1.2; disodium EDTA, 0.027; and glucose, 11.5. Isometric contractions were recorded using Fort 10 force transducers and MacLab analog to digital converter systems (World Precision Instruments, New Haven, CT). After 1 h, tissues were stretched to 1 g resting tension. Maximum contraction in each tissue was determined by exposure to 10^{-4} M phenylephrine. Then, arterial segments were precontracted to approximately 10% of the maximum contraction using 10^{-7}–10^{-6} M phenylephrine, and melatonin (10^{-10}–10^{-5} M) was added cumulatively. Responses were measured as g contraction above control response to phenylephrine. Some tissues were incubated with 4P-ADOT (3×10^{-6} M; 4-phenyl-2-acetamidotetraline) for 10 min prior to and

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during exposure to melatonin. The absence of functional endothelium was demonstrated by the lack of relaxation to acetylcholine (10^{-6} M) following precontraction with 7×10^{-7} M norepinephrine (average of all arterial segments was $15 \pm 2\%$ relaxation).

3. Results

Melatonin had no direct contractile effect on rat caudal artery segments, but it consistently potentiated contractile responses to adrenergic stimulation as we have found previously (Krause et al., 1995). In control artery segments, increasing concentrations of melatonin caused increasing levels of potentiation up to a maximum seen at 10^{-7} M melatonin (Fig. 1A and Fig. 2). At higher concentrations of melatonin ($> 10^{-7}$ M), potentiation was still present, but reduced compared to that produced by 10^{-7} M melatonin. Addition of 4P-ADOT (3×10^{-6} M) produced no contractile effect by itself, nor in the presence of phenylephrine. 4P-ADOT had a small but non-significant inhibitory effect on constrictor responses produced by lower concentrations of melatonin (Fig. 1B and Fig. 2). In contrast, 4P-ADOT substantially enhanced the constriction observed with higher melatonin concentrations (Fig. 1B and Fig. 2). For example, 10^{-5} M melatonin increased contractile force by 0.18 ± 0.03 g in the presence of 4P-ADOT as compared to an increase of only 0.09 ± 0.03 g in the absence of the antagonist ($P < 0.05$).

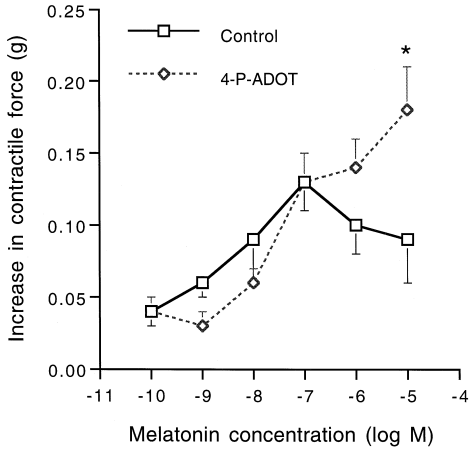

Fig. 2. Effect of 4P-ADOT on the concentration–response curve for melatonin in rat caudal artery segments. Melatonin potentiation of phenylephrine-induced contraction was measured in the absence (□) or presence (◇) of 4P-ADOT (3×10^{-6} M). Responses are expressed as the increase in force (g) produced above the level of precontraction with phenylephrine (10^{-7}–10^{-6} M). Values are means ± S.E.M. ($n = 7$ animals). * $p < 0.05$.

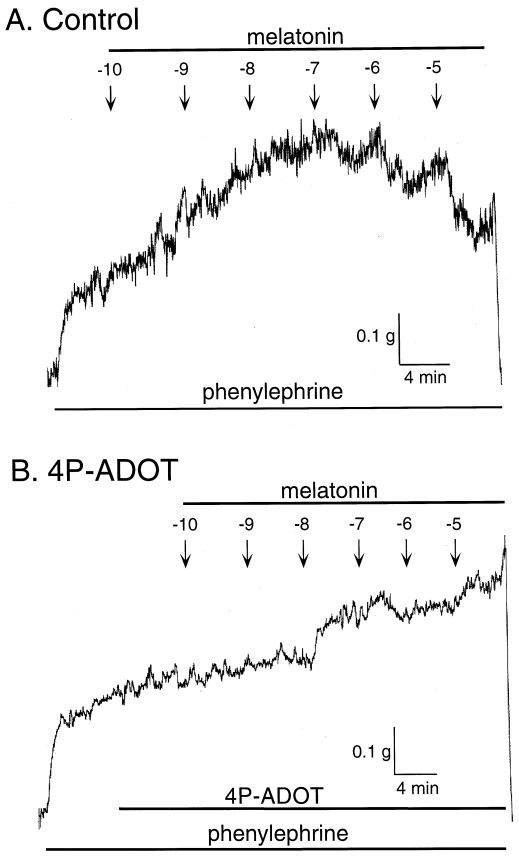

Fig. 1. Effect of melatonin on contractile responses to phenylephrine in the absence (A) or presence (B) of 4P-ADOT (3×10^{-6} M). Representative tracings from isolated rat caudal artery segments illustrate the initial contraction elicited by addition of phenylephrine (6×10^{-7} M) and the subsequent effects of cumulative addition of increasing concentrations of melatonin (expressed in log M), as indicated by arrows. 4P-ADOT (B) was added prior to melatonin and was present throughout the melatonin exposure.

4. Discussion

The simplest explanation of these data is that there are two types of response to melatonin in the rat caudal artery: potentiation of contraction and relaxation. It is not uncommon for a natural ligand to act on heterogeneous populations of receptors in a single tissue. Often, these receptors mediate antagonistic responses. For example, norepinephrine has both constrictor and dilator effects (Cohen and Wiley, 1977).

In the caudal artery, it appears that the predominant receptor for melatonin enhances vasoconstriction. Nanomolar concentrations of melatonin potentiated arterial contraction to phenylephrine, but this response was not significantly affected by 4P-ADOT, a melatonin receptor antagonist with selectivity for the MT_2 subtype (Dubocovich et al., 1997). Using the reported affinities (K_i values) of 4P-ADOT for the recombinant human mt_1 (429 nM) and mt_2 (1.3 nM) subtypes, it would be expected that the concentration of 4P-ADOT we used (3×10^{-6} M) would shift the melatonin concentration–response curve to the right by less than 10-fold if mediated by an MT_1 receptor

subtype and by over 2300-fold if mediated by MT_2 receptors. Thus, the current results are most consistent with potentiation of contraction being produced by an MT_1 receptor; however, confirmation of this hypothesis must await the development of selective MT_1 receptor antagonists.

The biphasic nature of the melatonin concentration–response curve is suggestive of the presence of more than one receptor subtype. Desensitization is unlikely to explain this observation because similar results are observed whether melatonin is added cumulatively or tested using a single concentration per artery segment (Doolen, Krause and Duckles, unpublished observations). The decrease in potentiation of vasoconstriction that was seen with higher melatonin concentrations was selectively affected by the MT_2 receptor antagonist, 4P-ADOT. As a result, potentiation of constriction by melatonin was significantly increased. Thus, in the rat caudal artery, the net response to melatonin may be made up of both contractile and relaxant components.

It is proposed that MT_2 receptors mediate relaxation that opposes the constrictor effect of melatonin, possibly induced via MT_1 receptors. In a different vascular tissue, pressurized segments of rat middle cerebral artery, melatonin causes direct constriction (Geary et al., 1997); however, we have recently observed that melatonin can also directly dilate small branches of this artery (Doolen et al., 1997). Both types of responses are inhibited by the melatonin receptor antagonist luzindole (Geary et al., 1997; Doolen et al., 1997), which does not distinguish between the mt_1 and mt_2 receptor subtypes (Dubocovich, 1995; Dubocovich et al., 1997).

The vascular endothelium was removed from the caudal arteries by intimal rubbing; thus, melatonin most likely acted on smooth muscle cells to mediate both contractile and relaxant effects. Potentiation of contractile effects by melatonin appears more efficacious than relaxation in this tissue. The affinities of melatonin for the two known receptor subtypes are similar (Dubocovich et al., 1997); thus if MT_2 receptors mediate relaxation in the caudal artery, they may be present in relatively lower numbers or with weaker coupling mechanisms as compared to the contractile receptor. The mechanism underlying the relaxant effect observed at higher melatonin concentrations may also explain previous reports that relatively high concentrations of melatonin produce vasodilation in other arteries (Satake et al., 1991). Further experiments utilizing selective melatonin receptor antagonists are needed to define the consequences of possible melatonin receptor heterogeneity in vascular tissue.

Acknowledgements

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