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Research Report

Melatonin signaling in mouse cerebellar granule cells with variable native MT1 and MT2 melatonin receptors

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ARTICLE INFO

Article history:
Accepted 13 June 2008
Availale online 28 June 2008

Keywords:
Melatonin
Cerebellar
MT1
MT2
ERK
Akt

ABSTRACT

Although G protein-coupled MT1 and MT2 melatonin receptors are expressed in neurons of the mammalian brain including in humans, relatively little is known about the influence of native MT1 and MT2 melatonin receptors on neuronal melatonin signaling. Whereas human cerebellar granule cells (CGC) express only MT1 receptors, mouse CGC express both MT1 and MT2. To study the effects of altered neuronal MT1/MT2 receptors, we used CGC cultures prepared from immature cerebella of wild-type mice (MT1/MT2 CGC) and MT1- and MT2-knockout mice (MT2 and MT1 CGC, respectively). Here we report that in MT1/MT2 cultures, physiological (low nanomolar) concentrations of melatonin decrease the activity (phosphorylation) of extracellular-signal-regulated kinase (ERK) whereas a micromolar concentration was ineffective. Both MT1 and MT2 deficiencies transformed the melatonin inhibition of ERK into melatonin-induced ERK activation. In MT1/MT2 CGC, 1 nM melatonin inhibited serine/threonine kinase Akt, whereas in MT1 and MT2 CGC, this concentration was ineffective. Under these conditions, both MT1 and MT2 deficiencies prevented melatonin from inhibiting forskolin-stimulated cAMP levels and cFos immunoreactivity. We demonstrated that selective removal of native neuronal MT1 and MT2 receptors has a profound effect on the intracellular actions of low/physiological concentrations of melatonin. Since the expression of MT1 and MT2 receptors is cell-type-specific and species-dependent, we postulate that the pattern of expression of neuronal melatonin receptor types in different brain areas and cells could determine the capabilities of endogenous melatonin in regulating neuronal functioning.

1. Introduction

Since the discovery of the in-vitro (Giusti et al., 1995) and in-vivo (Uz et al., 1996) neuroprotective actions of melatonin, studies have focused on the neuronal effects of supraphysiological, i.e., micromolar, concentrations of this hormone. On the other hand, the endogenous levels of serum and cerebrospinal fluid melatonin are in a low nanomolar range (Rousseau et al., 1999). These levels show dramatic diurnal variability (Pääkkönen et al., 2006). The nanomolar concentrations of melatonin act on the G protein-coupled melatonin receptors MT1 and MT2 (formerly known as Mel1a and Mel1b) (Dubocovich et al., 2003; Witt-Enderby et al., 2006). These receptors, which typically are linked to the inhibition of cAMP-mediated signaling, are expressed in various types of mammalian neurons including in the human brain (Brunner et al., 2006; Jimenez-Jorge et al., 2007; Savaskan et al., 2002, 2005; Thomas et al., 2002; Uz et al., 2005; Wu et al., 2006, 2007). The

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doi:10.1016/j.brainres.2008.06.082

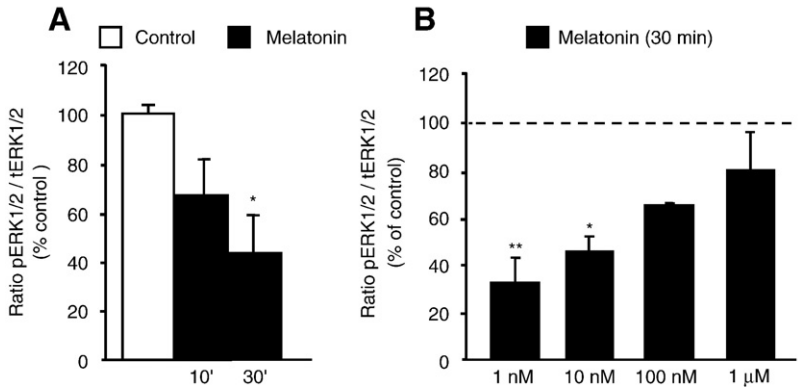

Fig. 1 – Effects of melatonin on ERK activity, i.e., phosphorylated forms of ERK (pERK1/2) in MT1/MT2 CGC. The signals for phosphorylated proteins were normalized by measuring the immunoreactivity of their respective total ERK in the same blot. Results (mean ± SEM; $n = 4–6$ different culture preparations) are presented as a percentage of the corresponding vehicle-treated control (open bar). (A) 1 nM melatonin (closed bars) decreases ERK phosphorylation in 10 min, and this becomes significant by 30 min. $^{*}p < 0.05$ vs. the corresponding control; ANOVA followed by the Dunnett's test. (B) Increasing melatonin concentration up to 1 μM (30 min incubation) failed to activate ERK (presented as a % of the 100% control indicated by a dotted line; $^{*}p < 0.05$; $^{**}p < 0.01$ compared to control).

pattern of MT1 and MT2 expression appears to be cell-type-specific and species-dependent. For example, whereas mouse cerebellar granule cells (CGC) express both MT1 and MT2 receptors, human CGC express MT1 but not MT2 mRNA (Al-Ghoul et al., 1998).

MT1 and MT2 receptors are operative as monomers and homo- and heterodimers (Ayoub et al., 2002, 2004). Typically, studies of melatonin-receptor-type-specific effects employ cells transfected with MT1/MT2 receptors (Ayoub et al., 2002, 2004; Chan et al., 2002), and cells that endogenously express only one type of melatonin receptors, e.g., MT1 (Bordt et al., 2001; Chan et al., 2002). Alternatively, selective MT1 and MT2 knockouts have been established in mice and these animals have been used in studies of central nervous system functioning (Larson et al., 2006; Sumaya et al., 2005).

In cells expressing only MT1 receptors, melatonin increases the activity (i.e., phosphorylation) of extracellular-signal-regulated kinase (ERK), a member of the mitogen-activated protein kinases (MAPKs) (Bordt et al., 2001; Chan et al., 2002), whereas in cells expressing both MT1 and MT2 receptors, melatonin inhibited ERK phosphorylation (Cui et al., 2008). In these latter conditions, melatonin also inhibited the phosphorylation (i.e., activity) of serine/threonine kinase Akt (also known as protein kinase B) (Cui et al., 2008).

Although experimental models based on transfecting cells that do not express native melatonin receptors with exogenous MT1 and MT2 receptors are useful in characterizing the signaling mediated by these receptors (i.e., by individual receptor types vs. their combinations), the limitations of these constructs include an unnatural cellular environment. For example, data obtained from transfected non-neuronal cells are not directly applicable to neuronal conditions, suggesting the need for prudent use of the heterologous cell transfection technique (Gabellini et al., 1994).

To study the effects of altered MT1/MT2 receptors on neuronal melatonin signaling, we used a model of primary cultures of mouse CGC. These cultures are advantageous because they comprise a uniform population of neurons. In addition, we took advantage of the availability of MT1- and MT2-knockout mice. Hence, in this work, CGC cultures were prepared from the cerebella of wild-type mice (MT1/MT2 CGC) and MT1- and MT2-knockout mice (MT2 and MT1 CGC, respectively). Furthermore, we focused these studies on the effects of the receptor-relevant low nanomolar concentrations of melatonin.

2. Results

2.1. *Effects of melatonin on ERK phosphorylation*

The initial qualitative assay of the effects of 1 nM melatonin on ERK phosphorylation revealed a major difference in the

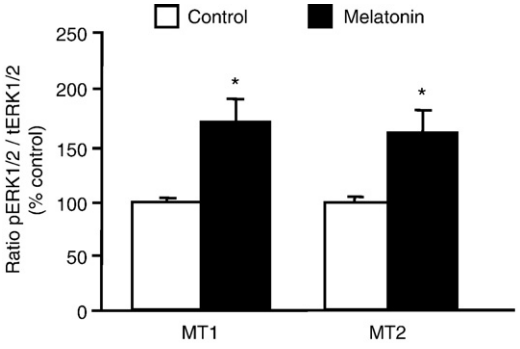

Fig. 2 – Effects of 1 nM melatonin on ERK activity, i.e., phosphorylated forms of ERK (pERK1/2) in MT1 CGC and MT2 CGC. The signals for phosphorylated proteins were normalized by measuring the immunoreactivity of their respective total ERK in the same blot. Results (mean ± SEM; $n = 4–6$ different culture preparations) are presented as a percentage of the corresponding vehicle-treated control (open bars). After 10 min incubation, melatonin (closed bars) increased the pERK content in both MT1 CGC and MT2 CGC. $^{*}p < 0.05$ vs. the corresponding control (t-test).