Abstract

Melatonin entrains the biological clock (the suprachiasmatic nucleus, SCN) by activation of specific melatonin receptors. In mammals, two types (MT_1 and MT_2) of melatonin receptors have been identified by molecular cloning. MT_1 is expressed in the pars tuberalis and in the SCN, i.e., in the presumed sites of the reproductive and circadian actions of melatonin, whereas MT_2 is mainly expressed in the retina. In addition, the distinct peripheral distribution and the numerous physiological processes in which melatonin appears to be involved such as sleep, as well as other circadian, immunological, oncological, cardiovascular, reproductive and anti-oxidant functions, imply that melatonin may signal through a variety of precisely-regulated pathways. The currently available *in vitro* models to study pharmacological and functional properties of human melatonin receptors are based on rodent cells (CHO, NIH3T3), raising the possibility of species-specific effects that could interfere with the interpretation of date obtained. Therefore, in order to study reliably specific properties of the two human melatonin receptors in a homologous context, new cell lines, derived from human cells (SK-UT-1B uterine tumor cells) by stable transfection with either the human MT_1 or MT_2 receptor were developed. Binding studies and the inhibition of forskolin-stimulated adenosine 3'-5'-cyclic monophosphate levels by melatonin confirmed the functional expression of high-affinity melatonin receptors. MT_2-transfected cells modulated cGMP levels in a dose-dependent manner. In contrast, MT_1 receptors had no effect on cellular cGMP levels.

To investigate whether melatonin can alter gene expression and to identify whether these effects are due specifically to either MT_1 or MT_2 receptor activation, microarray analyses from both transfected and untransfected cells following overnight exposure to melatonin were performed. More than twenty independent genes, implicated in different physiological processes such as apoptosis, cell cycle regulation and cell growth, showed a greater than 2-fold change in expression depending on the melatonin receptor subtype involved.

Among the apparently regulated genes by melatonin, two (ERK1/2 and cyclin G2) were examined in more detail. The ERK1/2 pathway is thought to play a role in cell proliferation and differentiation, whereas cyclin G2 is an unconventional cyclin that is highly expressed in cells undergoing apoptosis and has been reported to play a role in cell cycle arrest. Melatonin-induced effects on the expression of these genes were shown in cells expressing the MT_2 melatonin receptor, and 4P-PDOT, a specific antagonist for the MT_2 melatonin receptor, could block these effects. As these actions could be expected to reduce cell proliferation and

in view of the fact that melatonin has already been shown to inhibit the growth of various tumors *in vivo* and *in vitro*, the influence of melatonin on cell proliferation was tested.
The results support the idea that melatonin, especially via the MT_2 receptor, can suppress cell proliferation by regulation of various gene products, including cyclin G2 and MAPK activity.