Circadian production of melatonin in cartilage modifies rhythmic gene expression.

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Melatonin is mainly synthesized at night in the pineal gland in response to neutral signal, and a chronologic molecular regulator of circadian rhythm. The aim of this study is to explore the effects of melatonin on metabolism and rhythmic gene expression in chondrocytes. Using mouse epiphyseal cartilage and primary cultured chondrocytes, expression of melatonin receptors MT1 and MT2 as well as melatonin-synthesizing enzyme AANAT mRNA was analysed. Addition of melatonin to cultured chondrocytes enhanced cell proliferation and increased expression of Col2a1, Acan and Sox9, but inhibited Col10a1 expression. Addition of melatonin rapidly upregulated Aanat, Mt1, and Mt2 mRNA expression. Expression of the master clock gene Bmal1 was induced, while Per1 was downregulated, indicating that exogenous melatonin affects the rhythms of clock gene expression in chondrocytes. Chronobiological analysis of chondrocytes showed addition of melatonin induced cyclic expression of Aanat and adjusted the cyclic rhythm of Bmal1 close to 24h period. Rhythmic expression of Mt1 and Mt2 was also modified by melatonin with different period from Bmal1 and Aanat, indicating the existence of different regulatory genes. In addition, in mouse cartilage, melatonin stimulates chondrocyte proliferation but inhibits its maturation through Mt1 and Mt2. Chondrocytes could produce melatonin with their own rhythm and exogenous melatonin could adjust it to the central rhythm.

To further explore the effect of melatonin on human cartilage, human articular chondrocytes were prepared from 7 to 82 years old individuals. Negative correlation between age and the expression level of Aanat and Bmal1 was confirmed. Expression of Aanat and Bmal1 mRNA was rapidly enhanced by the addition of melatonin in human articular chondrocytes from relatively younger donors. Chronobiological analysis of gene expression using RNA collected every 4 hours for approximately 2 days confirmed that BMAL1, PER1 showed rhythmic gene expression in all the samples of articular chondrocytes, but melatonin did not affect period of the rhythmic cycle. However, period of the rhythmic expression of AANAT was modified, and appeared to close to 24 hours by the addition of melatonin in all ages examined.

Our results indicate that in mouse chondrocyte, exogenous and endogenous melatonin may work together to regulate melatonin synthesis. Melatonin may affect Aanat rhythmic expression pattern through regulation of Bmal1 rhythmic expression. In human chondrocytes, melatonin adjusts expression of BMAL1 and AANAT expression in relatively younger samples within 3 hours. Unlike mice, the clock gene rhythmic pattern does not seem to be regulated by melatonin, while the rhythmic pattern of AANAT expression can be adjusted to the central one by melatonin, which means not Bmal1, but some other

transcriptional factors that are induced by melatonin regulates circadian melatonin synthesis in human chondrocytes.

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