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Investigating the role of vitamin A in melatonin production in the pineal gland

A. J. Ashton, P. N. Stoney and P. J. McCaffery

Institute of Medical Sciences, School of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, Scotland, UK

The pineal gland is situated at the midline of the brain and produces melatonin, the hormone responsible for signalling darkness to the body and entraining daily physiological rhythms. The nightly production of melatonin is induced by upregulation of arylalkylamine *N*-acetyltransferase (AANAT) gene expression and activity, the rate-limiting enzyme for melatonin synthesis. A reduction in melatonin is associated with metabolic dysfunction, increased body weight and type 2 diabetes[1,2], it is therefore important to understand how melatonin synthesis is regulated. Studies have shown there are high levels of retinol (vitamin A) and retinol binding proteins in the mammalian pineal gland[3,4] and that vitamin A deficiency causes a reduction in AANAT and melatonin levels[5,6]. The effects of retinol are thought to be mediated by the active metabolite retinoic acid (RA), a potent regulator of gene transcription. This study aims to confirm whether RA signalling components are present in the pineal gland and determine whether RA can regulate melatonin production.

Male Sprague Dawley rat pineal glands were collected at six hour intervals throughout the light/dark cycle and analysed by qPCR to identify RA signalling components and determine whether they exhibit a circadian rhythm in expression ($n \geqslant 7$ pineal glands per timepoint). Organotypic culture of ex vivo rat pineal glands and qPCR analysis were then used to study the effect of RA on *Aanat* gene expression. Pineal glands were treated for four hours with RA alone, RA with cyclic AMP (the physiological mediator of *Aanat* upregulation), cyclic AMP alone or vehicle control ($n \geqslant 5$).

RA receptors and key synthetic enzymes were found in the rodent pineal gland, some of which exhibit diurnal (day/night) changes in expression. Expression of the RA synthetic enzyme retinol dehydrogenase 10 (*Rdh10*) and the RA receptor subtype gamma (*Rarg*) increased at night compared to day ($P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively), suggesting rhythms in RA signalling in this gland. Treatment of cultured pineal glands with RA and cyclic AMP was found to significantly increase *Aanat* expression compared to cyclic AMP alone ($P < 0{\cdot}01$). *Aanat* expression in pineal glands treated with RA alone and vehicle control was comparable. The results demonstrate that the components required for RA signalling are present in the pineal gland and suggest that RA has a role in the regulation of melatonin synthesis.

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