

SYNTHESIS AND SECRETION OF RAT PINEAL PROTEINS



IN-VITRO

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Abstract

This thesis reassesses pineal proteins. The highly sensitive technique of two dimensional gel electrophoresis [2D-SDS/PAGE] was established, evaluated and applied to answer questions about the nature and patterns of pineal proteins synthesized and secreted in-vitro and factors influencing their production. In conjunction with radiolabelling, 2D-SDS/PAGE demonstrated a large number of proteins synthesized and secreted from individual rat pineal glands in-vitro. Major factors found to influence these protein secretions were the photoperiod and melatonin. The photoperiodic influences indicated two peaks of incorporation of ^{35}S -methionine into secreted proteins during the 24 hour diurnal cycle - a major peak at the midlight phase [ML] and a second less intense but significant peak at the middark phase [MD]. The diurnal fluctuations in total protein content of the pineal glands followed a similar bimodal distribution. Protein patterns as determined by 1 and 2D-SDS/PAGE were significantly different [$p < 0.01$] at these two times in all samples examined. Melatonin at physiological doses was able to inhibit protein synthesis and secretion and at a dose of 10^{-7}M mimicked the MD pattern very closely. Further assessment of the possible mechanisms of action of melatonin on pineal protein synthesis demonstrated that its mode of action is via transcription. Melatonin inhibition of protein synthesis was reversible and dose related between 10^{-7}M and 10^{-4}M .

These results were the highlight of the current study and although raising further questions, clearly add to our understanding and reassessment of the importance of pineal bioactive secretions [both indoles and proteins]. In particular, the data further emphasizes the need to review the potential autocrine and/or paracrine mechanisms of action of melatonin. It is possible that the photoperiodic influences on pineal protein synthesis and secretion are mediated via melatonin. The techniques established in these studies provide a valuable tool for providing insights into a hitherto neglected arm of the functions of this very important gland.