An Effect of Prolactin on Prostate Adenylate Cyclase

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It has been shown that many protein hormones mediate their action on their target tissue through stimulation of the enzyme adenylate cyclase (Major & Kilpatrick, 1972). There is evidence that prostatic development can be directly affected by pituitary hormones. Grayhack & Lebowitz (1967) showed that pituitary prolactin stimulates the growth of the lateral lobe of the prostate in hypophysectomized orchidectomized rats (perhaps by enhancing the uptake of testosterone; Farnsworth, 1970). In our experiments the effects of prolactin and other hormones on prostatic adenylate cyclase were examined. Adenylate cyclase activity was assayed by measuring the conversion of [α - 32 P]ATP into cyclic AMP (adenosine 3':5'-cyclic monophosphate) during incubation of homogenates of rat prostatic tissue. The cyclic AMP produced was purified by a batch procedure based on the DEAE-Sephadex procedure of Rosett *et al.* (1970). Final traces of AMP, ADP and ATP were removed from the cyclic AMP fraction by adsorption on nascent BaSO₄ and Zn(OH)₂. Ovine prolactin stimulated adenylate cyclase activity when present in the incubation medium at a concentration of 1.5 μ g/ml, but was ineffective when present at 60 μ g/ml. Testosterone was also ineffective over a wide concentration range, and a synergistic effect was not seen. Human chorionic somatomammotrophin, human luteinizing hormone and human growth hormone all stimulated prostatic adenylate cyclase activity.

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Enzymically Catalysed Binding of Amino Acids to the Protein Cyst Coat of the Ciliate *Colpoda steinii*

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The protein coat that surrounds the encysted form of the ciliate *Colpoda steinii* is probably not simply a protective layer but may play an active part in cell excystment. In an enzymically catalysed reaction D-glucose binds to the coat (Tibbs & Marshall, 1970), and there is some evidence that this glucose is metabolized as the cell excysts (Marshall & Tibbs, 1970). Under suitable conditions a number of amino acids also appear to bind covalently to it. Thus on incubation of the coat with L-[U- 14 C]leucine the latter became attached, and could not be removed by prolonged washing with unlabelled leucine or by dissolving the protein coat (Tibbs, 1966) and dialysing it. Kinetic experiments carried out at the optimum pH 7.4 in 50 mM-tris buffer at 20°C, where there was little loss in activity on standing, and over a 10 min incubation period where uptake was linear with time at all concentrations used, showed K_m about 8 mM. Activity was lost on heating and was inhibited by *p*-hydroxymercuribenzoate in concentrations down to about 20 μ M.

The reaction is optically specific, no competition effects being observed when unlabelled D-leucine was included in the incubation mixtures. Similarly, a number of other L-amino acids showed little competition with L-leucine for the L-leucine-binding site. Even L-norvaline and L-isoleucine were not very effective, with K_i values between 20 and 40 mM. The fact that some, at least, of the amino acids that do not compete with L-leucine will themselves bind to the coat suggests a variety of specific binding sites.

Some evidence has been obtained for the involvement of the amino groups of the attached leucine in the reaction. Coat to which L-[U- 14 C]leucine had been attached was allowed to react with 1-fluoro-2,4-dinitrobenzene, and after removal of excess of the reagent the product was hydrolysed with acid and the hydrolysate extracted with ether. Practically all the radioactivity remained in the aqueous layer, and although other explanations are possible, the most obvious interpretation of these results is that the leucine amino groups had been blocked by bonding to the coat.

It is not unlikely that *in vivo* the accumulation of bound metabolites on the coat is closely connected with the recognition by the cyst of an environment suitable for excystment and subsequent growth.

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