Comparison of the phosphodiesterases in the submaxillary gland, brain and liver

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Although the role of cyclic adenosine 3′,5′-monophosphate (cAMP) as a regulator of intracellular events is well established, evidence for cAMP modulating secretory activity in exocrine glands has been based, until recently, on experiments using theophylline and dibutyryl cAMP (Sutherland, 1971). Since cAMP levels might also be controlled through induction of phosphodiesterases (PDE), the cAMP PDE activity in the submaxillary gland was determined and compared with that in the brain and liver (Sutherland & Rall, 1958). The presence of phosphodiesterases (exonucleases) capable of hydrolyzing 3′- and 5′-nucleotide bonds was also examined using specific chromogenic substrates.

Guinea-pig submaxillary gland, brain and liver were homogenized in Tris-HCl buffer (50 mM ; pH 7·0) and centrifuged at $3{\cdot}5 \times 10^5$ *g* ; the pellets were treated with Triton X-100 and recentrifuged. Enzyme activity was measured in the homogenate, the initial supernatant, the supernatant and residue of the Triton X-100 treated pellets ; the specific activity of each enzyme is expressed as nmoles substrate hydrolized/min for each mg of tissue protein and is shown in brackets below. The cAMP PDE activity was assayed using tritiated cAMP (Poch, 1971) ; the 5′- PDE I and 3′-PDE II phosphodiesterases were measured using the p-nitrophenol esters of 5′- and 3′-deoxythymidine nucleotide.

The specific activity of cAMP PDE in homogenates of submaxillary glands (0·53) differed from that in the brain (2·79) and liver (0·16). Although such a variation was also found in the exonucleases in the submaxillary gland (PDE I, 5·41 ; PDE II, 1·78), brain (PDE I, 0·49 ; PDE II, 8·9) and liver (PDE I, 1·39 ; PDE II, 3·6) no correlation was observed between the differences in the tissue concentration of the three enzymes. The submaxillary gland activity was analysed further by extending the enzyme measurements to subcellular fractions. cAMP PDE activity was almost completely recovered from the cytosol, whereas the 5′-exonuclease was mainly associated with particulate fractions, particularly the microsomes. Although the 3′-exonuclease activity was less selectively distributed in sucrose density-gradient experiments, the highest activity was found in the secretory granule fraction.

Cyclic AMP PDE activity was strongly inhibited by theophylline (8 mM), caffeine (10 mM) and papaverine (0·2 mM) whereas the 5′- and 3′-exonucleases were unaffected. In addition to cAMP PDE both the exonucleases were significantly affected by EDTA and EGTA.

The present experiments indicate the differences in the specific activity and intracellular location of cAMP PDE and the 3′- and 5′-exonucleases. Although cAMP is believed to be implicated in the cellular processes which mediate enzyme secretion cAMP PDE activity has not previously been characterized in the submaxillary gland.

*M. J. C. Lemon is an M.R.C. Scholar. K. D. Bhoola thanks the Royal Society and the Medical Research Council for grants.

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