J. Pharm. Pharmacol. 1983, 35: 692
Communicated May 16, 1983

DeoxyATP as a substrate for cardiac adenylate cyclase: a radioimmunoassay for cyclic deoxyAMP

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Adenosine can affect adenylate cyclase through either the 'P-site' or the 'R-site (s)', (Londos et al 1981). P-site effects are always inhibitory, and are brought about by μM to mM concentrations of adenosine or adenosine analogues modified in the ribose moiety (Fain et al 1972). R-site effects can be either inhibitory or stimulatory and require nM concentrations of adenosine or analogues modified in the purine moiety (Sattin & Rall 1970). Studies of R-site actions have been complicated by the breakdown of ATP, the normal cyclase substrate, to adenosine by endogenous membrane phosphohydrolases. The presence of this adenosine in the assay medium can obscure the effects of added R-site effectors. A partial solution to this problem has been the inclusion of adenosine deaminase in the assay medium which allows the measurement of the effects of deaminase-resistant analogues, but not of adenosine itself (Sattin & Rall 1970). However, even this approach has been criticized because of the possibiity that the deaminase-resistant analogues may be having their effect by inhibiting adenosine deaminase and raising endogenous adenosine levels. Cooper & Londos (1979) have used dATP as the cyclase substrate in their studies on R-site effects in liver, fat cells and Leydig I-10 tumor cells and have found that 2'deoxyadenosine does not interfere with R-site mediated effects; the cyclic deoxyAMP produced was measured by a modification of the Salomon assay for cAMP.

We have found that cdAMP may also be measured using an existing radioimmunoassay for cAMP. The antigen used was the labelled succinyl tyrosine methyl ester of cAMP (New England Nuclear 125-I RIA Kit). 50% displacement of the labelled antigen from its antibody complex was brought about by 1·86 ± 0·13 pmol/100 μl cAMP compared with 1·88 ± 0·13 pmol/100 μl c dAMP (n = 9). Cross reactivity profiles for dATP, dADP and dAMP were similar to those for ATP, ADP and AMP. At a concentration of 1 nmol/100 μl, none of the deoxynucleotides displaced more than 5% of the labelled antigen. The recovery of cdAMP from the assay medium was 89·5 ± 4·8% (n = 5). A greater sensitivity of the antibody to cAMP could be obtained by the acetylation of cAMP at the 2'-position. However, this is not possible with cdAMP.

Using this method to measure cdAMP, the suitability of dATP as a substrate for rabbit cardiac adenylate cyclase was assessed. Cardiac sarcolemmal membranes were prepared by the method of Mas-Oliva et al (1979) and stored at −80 °C until required. The cyclase assay medium contained (mM) Tris-acetate (pH 7·5) 22, dithiothreitol 0·72, EDTA 0·32 and Mg acetate 12, theophylline 10, Na-ATP or Na-dATP in the range 0·01 to 0·5, creatine phosphate 5, and 2 mg ml^{-1} creatine kinase. Sarcolemmal protein concentration was 0·1 to 0·2 mg ml^{-1}. After 20 min incubation at 30 °C, 50 μl aliquots were removed into 500 μl of 0·05 mM Na acetate buffer at pH 4·0. This was buffered to pH 6·2 with 1 M Tris and 2 × 100 μl aliquots taken for measurement.

The K_m of the adenylate cyclase was 34 ± 4 μM for ATP and 45 ± 8 μM for dATP (n = 3). Using 0·5 mM substrate, the basal activity was 70·1 ± 7·2 pmol cAMP min^{-1} mg^{-1} protein (n = 27) and 69·6 ± 6·5 pmol c dAMP min^{-1} mg^{-1} protein (n = 16). These findings are similar to those in He La cells and from the Ac- variant of the S49 Lymphoma line where dATP and ATP were equally good substrates (Londos et al 1981) but contrast with the cyclases from liver, fat cells and Leydig I-10 tumor cells where the basal activity using dATP was 3 to 5 fold greater than that with ATP (Cooper & Londos 1979). We conclude that dATP can be used as a substrate for the cardiac adenylate cyclase and that this radioimmunoassay is a suitable technique for the measurement of the cdAMP produced.

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