Interaction of adenosine with adenylate cyclase in rat fat cell membranes

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Recently the interaction sites of adenosine (ADO) with adenylate cyclase in a variety of tissues were differentiated into a so called P and R-site on the basis of the activities of a large series of adenosine analogues (Londos & Wolff, 1977). The P (purine) site mediates inhibition and accepts ADO analogues modified in the ribose ring. The R (ribose) site mediates activation and accepts ADO analogues modified in the purine moiety. In addition it was proposed that only the action of R-site active compounds can be blocked by theophylline (THEO) and that in some cases the presence of GTP is required (Londos *et al.*, 1978).

We studied the effects of ADO and N^6-phenylisopropyladenosine (PIA) on adenylate cyclase activity in rat fat cell membranes. Adenylate cyclase was measured according to Salomon, Londos & Rodbell (1974) in a medium containing Tris-HCl (50 mM), ascorbic acid (2 mM), ATP (1 mM), cAMP (2 mM), creatine phosphate (20 mM), creatine phosphokinase (10 u), BSA (0.1%), $MgCl_2$ (5 mM) *or* $MnCl_2$ (1 mM) at pH 7.5 and 37°C.

Basal adenylate cyclase activity was the same when $MgCl_2$ (5 mM) was substituted by $MnCl_2$ (1 mM). The dose-dependent inhibition by ADO of basal and NaF stimulated adenylate cyclase was stronger in the presence of Mn^{2+} than with Mg^{2+}. In contrast, the inhibition of isoprenaline (ISO) stimulated adenylate cyclase was not affected by changing the divalent cation. In no case could the inhibition of adenylate cyclase by ADO be blocked by THEO (1 mM). PIA inhibited ISO and NaF stimulated adenylate cyclase only in the presence of Mg^{2+}. When Mn^{2+} was present, a potentiation of NaF stimulated activity by PIA was observed. This potentiation is also seen with varying concentrations of ATP and/or $MnCl_2$, or in the presence of GTP. The effects of PIA on adenylate cyclase in the presence of Mg^{2+} or Mn^{2+} could not be antagonized by THEO, whether GTP was present or not. We conclude that (1) there are possibly two ADO sensitive sites at the fat cell hormone receptor adenylate cyclase complex, the expression of at least one site being regulated by the divalent cation present and (2) the antagonism by THEO of the inhibitory actions of ADO on hormone stimulated cAMP production and stimulated lipolysis in isolated fat cells are not mediated via a common interaction site on adenylate cyclase.

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C.76

Effect of oxytocin on ^{45}Ca movements and contractile responses in the rat isolated aorta

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Previous studies have shown that oxytocin depressed the contractile responses of the vas deferens of the rat induced by different agonists and by electrical stimulation (Beneit, Hidalgo & Tamargo, 1979). They suggested that oxytocin depressed the contractile responses at least partly by reducing the availability of calcium from an extracellular source. In the present study we have examined in rat thoracic aortic strips the influence of oxytocin on ^{45}Ca movements and on vascular smooth muscle contractions induced by different agonists.

Helically cut strips were suspended in baths containing 10 ml of Tris-buffered solution bubbled with 5% CO_2 in oxygen at 34°C and placed under 1 g tension. Control and experimental contractions were