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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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

always obtained from the same muscle and the strips were incubated with oxytocin for 5 min prior to the exposure to the agonists. The ^{45}Ca uptake of a fraction not removable by La^{3+} (2 mM) and ^{45}influx were measured as described by Van Breemen, Farinas, Gerba & McNaughton (1972) in a model SL-3000 Intertechnique liquid scintillation spectrometer.

The inhibitory effects of oxytocin (200–1000 μm/ml) on the contractile responses elicited by submaximal equipotent concentrations of noradrenaline (1 μM), serotonin (20 μM), potassium (80 mM) and barium (12 mM) were measured in isolated aortic strips. In all cases, prior exposure to oxytocin inhibited in a dose-dependent manner the contractile response to each of these agents. The inhibitory effect of oxytocin was readily reversed after replacement of the media with drug-free solution. In another experiment, aortic strips were suspended in Ca^{2+}-free solution containing EDTA (0.1 mM) for 2 hours. Then the solution was replaced with Ca^{2+}-free high-K^+ (80 mM) solution for 5 min and then Ca^{2+} (1–5 mM) was added in stepwise fashion over 45 minutes. Oxytocin (500 μm/ml) shifted the dose-response curve to Ca^{2+} downward and to the right and the maximum contractile response induced by addition of Ca^{2+} (5 mM) was significantly reduced ($P < 0.001$). Addition of oxytocin (500 and 1000 μm/ml) reduced the ^{45}Ca uptake and the ^{45}Ca influx in a sustained manner in rat aortic strips at all incubation time intervals (5, 10, 15 and 30 minutes).

Thus, it appears that oxytocin acts on: (a) the membrane to inhibit the influx of extracellular Ca^{2+}. The experiments performed on Ca^{2+}-free high-K^+ solution provided additional evidence for a membrane to inhibit the influx of extracellularCa^{2+}. Ca^{2+}, since oxytocin inhibits the noradrenaline-induced contractile responses.

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Diuretic and antidiuretic responses to oxytocin administration in the rat

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Although the renal saluretic response is well established, the effect of oxytocin on urine flow in rats has been variously described as diuretic or antidiuretic. Chan (1976) has related these effects of the hormone to its ability to interact with the vasopressin receptor. Accordingly the influence of oxytocin on urine flow rate in normal rats, and in rats congenitally lacking endogenous vasopressin (Brattleboro strain) has been investigated.

Male rats (300 g) anaesthetized with 5-ethyl-5(1, methylphenyl)-2-thiobarbiturate (0.11 g/kg, Inactin) were placed on a continuous jugular infusion of 0.45% NaCl at 150 μl/minute. Following a 3.5 h equilibration period 5 min urine collections were made for volume and osmolality measurements during both control periods and 20 min periods of oxytocin administration at 0.15 or 1.5 mu/minute. Values (means ± s.e. mean) are compared by paired *t* test.

In normal rats ($n = 7$) urine flow (μl/min) increased from 150 ± 12 to 175 ± 15 ($P < 0.02$) during administration of oxytocin at 0.15 mu/min, though urine osmolality was unchanged. Administration of oxytocin at 1.5 mu/min increased the peak flow rate further to 232 ± 17 μl/min ($P < 0.001$). Maximal urine flow was, however, delayed until 5–10 min after the end of oxytocin administration. Urinary osmolality (mosmol/kg) showed a diphasic change rising from 283 ± 11 to 357 ± 15 ($P < 0.01$) during oxytocin administration but falling to 195 ± 10 ($P < 0.01$) coincident with the delayed peak in urine flow. If the period of oxytocin administration was extended to 40 min the peak of urine flow was still delayed until 5–10 min after the period of administration.

In contrast the Brattleboro rat showed no significant change in urine flow when oxytocin was given at 0.15 mu/minute. However, a consistent antidiuresis, maximal in the first 10 min of hormone administration was observed when oxytocin was given at 1.5 mu/minute. Urine flow rate (μl/min) fell from 200 ± 15 ($n = 6$) to 151 ± 14 ($P < 0.02$) coincident with a rise in urine osmolality (mosmol/kg) from 175 ± 14 to 258 ± 21 ($P < 0.001$). A similar antidiuretic response to 1.5 mu/min oxytocin was produced in normal rats when the rate of 0.45% NaCl infusion was doubled to 300 μl/minute. In these rats in which endogenous vasopressin release might be expected to be largely suppressed urine flow rate (μl/min) decreased from 312 ± 44 ($n = 5$) to 244 ± 46 ($P < 0.01$) when oxytocin was given.