Cyclic AMP production in rat mammary gland: influence of β-adrenergic agents on acini in vitro

IAN MULLANEY and ROGER A. CLEGG
Hannah Research Institute, Ayr KA6 5HL, Scotland, U. K.

Glucagon and adrenaline are agents which raise intracellular concentrations of cyclic AMP in hepatocytes and adipocytes and thereby exert their effects on lipogenesis via phosphorylation and inhibition of acetyl-CoA carboxylase (Holland et al., 1984, 1985). Lipogenesis in preparations of mammary acini is, however, unaffected by either glucagon or adrenaline (Williamson et al., 1984; Robson et al., 1984; N. A. Robson, R. A. Clegg, & V. A. Zammit, unpublished work), despite the reported inhibition of lipogenesis by high concentrations of methyloxanthines and by added dibutyl cyclic AMP in acini (Robson et al., 1984) and in rat mammary tissue explants (Cameron & Rillema, 1983).

In the case of glucagon, it has been established that mammary acini lack receptors for this hormone (Robson et al., 1984). The work described here, and complemented by studies on adenylate cyclase in mammary acini membranes (Ladhia et al., 1985), was undertaken to assess whether adrenergic receptors are present on mammary epithelial cells and if so to examine their influence, via adenylate cyclase, on the intracellular concentration of cyclic AMP in rat mammary acini.

The cyclic AMP content of mammary acini (prepared by the method of Robson et al., 1984) was 215 ± 17 (17 determinations) pmol/g wet wt. This value is comparable with that found by Sapag-Hagar & Greenbaum (1974) for milk-free mammary tissue. Incubation in vitro at 37°C resulted in a slow decline in acini cyclic AMP content over a 45 min period. Addition of the adrenergic agents isoproterenol, adrenaline, or noradrenaline resulted in a rapid and persistent increase of up to 20-fold in the intracellular cyclic AMP of acini in vitro when the phosphodiesterase inhibitor Ro 7-2956 was also present (Table 1). Adrenergic agents or phosphodiesterase inhibitors alone were without effect on acini cyclic AMP levels, an unusual observation for adrenergic agents, although not for phosphodiesterase inhibitors (see e.g. Schechter, 1984). The ranking of EC50 values (where EC50 defines the concentration of agent which induces a response equivalent to half of that maximally attainable with the same agent) for isoproterenol, adrenaline, and noradrenaline in the stimulation of cyclic AMP accumulation within Ro 7-2956-treated acini (0.2 μM, 0.7 μM and >1 μM respectively), suggested the involvement of β2-adrenoceptors (Ladhia et al., 1983). The effects of selective β1- and β2-antagonists reinforced this suggestion (Table 1).

Specific binding to mammary acini membranes of the β-selective radioligand [3H]dihydroalprenolol amounted to 397 fmol/mg of protein. Quantitatively similar binding to adipocyte membranes has been reported (Malbon et al., 1978). Competitive displacement experiments with the labelled β1- and β2-selective antagonists (atenolol and ICI 118551 respectively) demonstrated that the predominant mammary cell β-receptor subtype was β2. Thus, 50% displacement of [3H]dihydroalprenolol was achieved with 0.1 μM-ICl 118551, whereas the highest concentration tested of atenolol (100 μM) displaced only 30%. Similar [3H]dihydroalprenolol-binding properties (Ulfhake & Iversen, 1983) were found in membranes prepared from highly purified mammary epithelial cells (isolated by the method of Soloff et al., 1980), thus confirming that the receptors characterized in acini membranes were those of secretory epithelial cells and not of myo-epithelial or other minor cell types found in the acini preparation.

These results have demonstrated that β2-adrenergic receptors are present on mammary cell membranes. Although they are functionally coupled, in a stimulatory mode, to the adenylate cyclase of these membranes (Ladhia et al., 1985), their occupation by competent agonists results in little or no change in the cyclic AMP content of acini unless inhibitors of phosphodiesterase are also present.

Table 1. Effects of adrenergic agents on the cyclic AMP content of rat mammary acini in vitro

<table>
<thead>
<tr>
<th>Agents(s) present</th>
<th>Mode of action</th>
<th>Cyclic AMP content of acini (pmol/g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>653 ± 31</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>α &lt; β-agonist</td>
<td>1352 ± 631*</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>β &lt; α-agonist</td>
<td>4410 ± 909*</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>β-agonist</td>
<td>5810 ± 487*</td>
</tr>
<tr>
<td>Isoproterenol + atenolol</td>
<td>βi-antagonist</td>
<td>6075 ± 84*</td>
</tr>
<tr>
<td>Isoproterenol + ICI 118551</td>
<td>β-antagonist</td>
<td>1455 ± 214†</td>
</tr>
</tbody>
</table>

Vol. 13
This implies that the kinetic properties of the phospho-
diesterases of mammary secretory cells (previously
characterized by Mullaney & Clegg, 1984) ensure very
efficient 'buffering' of the intracellular cyclic AMP, thereby
quenching the transmission, to cyclic AMP-dependent
protein kinase, of the intracellular signal normally evoked by
β-adrenergic agents.

Ro 7-2956 and KCI 118551 were generously given by Roche
Products Limited and KCI Pharmaceuticals Division respectively.

173, 306 - 311
140, 325 - 333
Biochem. J. 226, 139 - 145

Presence of lipoprotein lipase in neonatal rat liver

MANUEL REINA, SENÉN VILARÓ and
MIQUEL LLOBERA
Cátedra de Fisiología General, Facultad de Biología,
Universitat de Barcelona, Barcelona, Spain

We previously reported the presence of triacylglycerol
hydrolyase activity in the neonatal rat liver (neonatal liver
lipase) (Llobera et al., 1979). Like lipoprotein lipase (LPL)
but unlike hepatic triacylglycerol lipase (HTGL), neonatal
liver lipase is inhibited by 1 M-NaCl and 3 mg of protamine
sulphate/ml in the medium (Ramirez et al., 1983).

We speculated that this activity can convert liver during
the perinatal phase into a triacylglycerol utilizer instead of
a producer. In fact, liver triacylglycerol content increases
rapidly after birth, but in postmaturity, both the appear-
ance of neonatal liver lipase and triacylglycerol accumu-
lation in the liver of the foetus disappear completely
(Ramirez et al., 1983). Furthermore, this activity increases
with postnatal starvation, perhaps a compensatory reaction
to the decrease in circulating triacylglycerol in this situ-
ation (Grinberg et al., 1985).

In the present study we attempted to obtain information
which would allow us to conclude whether this lipase
activity may be considered to be lipoprotein lipase. So we
studied the conditions for neonatal liver lipase tissue
extraction and for semi-purification by heparin-Sepharose
chromatography (Boberg & Caron, 1979), and we compared its
elution characteristics with those of adipose tissue LPL,
liver HTGL, and post-heparin plasma LPL and HTGL from
adult Wistar rats.

Neonatal rat liver was obtained from rats starved for
24 h beginning at the fifth hour after birth (Grinberg et al.,
1985). The tissues were obtained by decapitating the
animals and rapidly dissecting and freezing the livers in
liquid N₂. The tissue extracts were treated with acetone/
ethanol (Llobera et al., 1979), and re-dissolved in Veronal
buffer (0.5 M-NaCl). The supernatant of the ultracentri-
fugation (30,000 g, 30 min at 4°C) was applied to the
chromatographic column. The post-heparin plasma was
obtained by intravenously injecting 33 i. u. of heparin/100 g
body weight. To obtain chromatographic affinity we used a
0.6 cm × 5 cm column of heparin-Sepharose CL6B
(Pharmacia), eluted with Veronal buffer (5 mM, pH 7.2)
with different concentrations of NaCl (0.5, 0.7, 0.9 or
1.5 M). A solution of bovine serum albumin free of fatty
acids (Sigma) was used as an enzyme stabilizer. LPL
(Nilsson-Ehle & Schutz, 1976) and HTGL activities

Abbreviations used: LPL, lipoprotein lipase (EC 3.1.1.34);
HTGL, hepatic triacylglycerol lipase (EC 3.1.1.3).

Fig. 1. Heparin-Sepharose chromatography of different
tissue extracts

All fractions (1.5 ml) were assayed for hepatic triacyl-
glycerol lipase (——-) and lipoprotein lipase (———)
activities and protein concentration (— — —).