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Acute change in the cyclic AMP content of rat mammary acini *in vitro*

Influence of physiological and pharmacological agents

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1. The cyclic AMP content of acini, freshly prepared from mammary tissue of lactating rats, was measured during incubation *in vitro*. 2. Neither adrenergic agonists nor cyclic AMP phosphodiesterase inhibitors alone caused a change of more than 2-fold in the basal cyclic AMP content of acini. Together, however, these agents provoked increases of around 20-fold in acini cyclic AMP content. Forskolin caused similar effects. 3. The relative potency of adrenergic agonists in increasing cyclic AMP in acini, together with the ability of selective antagonists to oppose such rises, indicated that β_2-adrenergic receptors were involved in mediating the effects. 4. Receptor-binding experiments using [^{3}H]dihydroalprenolol and selective β-antagonists confirmed the predominant presence of β_2-adrenergic receptors on acini membranes and on membranes prepared from purified mammary secretory epithelial cells. 5. These results elucidate some previous findings [Robson, Clegg & Zammit (1984) *Biochem. J.* **217**, 743–749; Williamson, Munday, Jones, Roberts & Ramsey (1983) *Adv. Enzyme Regul.* **21**, 135–145], questioning the role of cyclic AMP in the regulation of lipogenesis in mammary acini.

Acute regulatory adjustments of the metabolic activity of isolated mammary acini have been described in response to relatively few hormones and agents. Insulin stimulates lipogenesis (Robson *et al.*, 1984; Williamson *et al.*, 1983) and under certain circumstances (Munday & Williamson, 1981; Agius *et al.*, 1980) affects the activity of pyruvate dehydrogenase (EC 1.2.4.1). Neither β-adrenergic agonists (Williamson *et al.*, 1983; N. A. Robson, R. A. Clegg & V. A. Zammit, unpublished work) nor glucagon (Robson *et al.*, 1984; Williamson *et al.*, 1983) are anti-lipogenic towards this preparation, and glucagon has been further shown to be without effect on lactose synthesis by isolated acini (Wilde & Kuhn, 1981).

The relationship between insulin's effects and the cellular cyclic AMP status is unclear. However, as has been observed in other insulin-responsive tissues (Manganiello & Vaughan, 1973; Loten *et al.*, 1978), a high-affinity cyclic AMP phosphodiesterase (EC 3.1.4.17) is stimulated by insulin in mammary acini (Aitchison *et al.*, 1984). Glucagon and β-adrenergic agonists achieve their physiological effects by a stimulatory coupling to adenylate cyclase (EC 4.6.1.1) in the surface membrane of receptor-competent cells, leading to an increase in the intracellular concentration of cyclic AMP. Such an increase can also be brought about by treatment of cells and tissues with agents which more directly stimulate adenylate cyclase or inhibit cyclic AMP phosphodiesterases. In consequence, these agents typically mimic the effects of glucagon or β-adrenergic agonists. Methylxanthines inhibit cyclic AMP phosphodiesterases, and have been shown to cause inhibition, in mammary acini, of both lactose synthesis (Loizzi, 1978; Wilde & Kuhn, 1981) and lipogenesis (Robson *et al.*, 1984). Wilde & Kuhn (1981) have argued, however, that the effect of theophylline on lactose synthesis may be a secondary consequence of the inhibition of glucose transport by this agent, and these authors emphasize that dibutyryl cyclic AMP itself has only small effects even at very large concentrations in their own experiments and those of Loizzi (1978). The same explanation for the action of theophylline in acini may apply to its inhibition of lipogenesis, observed by Robson *et al.* (1984). That this may not be so, however, is suggested by the finding that acetyl-CoA carboxylase (EC 6.4.1.2; normally the rate-determining enzyme for lipogen-

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esis) purified from mammary gland is phosphorylated and inactivated *in vitro* in the presence of ATP by the catalytic subunit of cyclic AMP-dependent protein kinase (Hardie & Guy, 1980), an enzyme which is itself abundant in mammary tissue (Munday & Hardie, 1984). Other protein kinases, not dependent on cyclic AMP, are also found in mammary tissue extracts (Munday & Hardie, 1984), and in such extracts the phosphorylation and consequent inactivation of acetyl-CoA carboxylase is apparently not catalysed by a cyclic AMP-dependent kinase (McNeillie *et al.*, 1981).

The roles of cyclic AMP, and of physiological agents which change its effective intracellular concentrations, in acute metabolic regulation of mammary tissue are therefore unresolved. The observation that rat mammary acini lack receptors for glucagon (Robson *et al.*, 1984) explains the absence of observable effects of this hormone on lipogenesis in isolated acini (Robson *et al.*, 1984; Williamson *et al.*, 1983). The purpose of the present work was to investigate whether a similar explanation would also be tenable for the insensitivity of acini to adrenergic agonists, and to examine the effects of such agents on intracellular cyclic AMP concentration in isolated acini. We also report the effects of selective adrenergic antagonists, of forskolin, a stimulatory effector of adenylate cyclase in a wide variety of cell types (Seamon & Daly, 1981), and of inhibitors of the cyclic AMP phosphodiesterases which we have characterized in rat mammary tissue (Mullaney & Clegg, 1984). A preliminary account of parts of this work has been reported (Mullaney & Clegg, 1985).

Materials and methods

Rats

Wistar rats were obtained and maintained as previously described (Mullaney & Clegg, 1984; Robson *et al.*, 1984).

Preparation and incubation of mammary acini

Acini were prepared from mammary tissue of lactating rats, 10–12 days after they had given birth to their first litters, exactly as described by Robson *et al.* (1984). For incubation *in vitro* they were dispensed into stoppered conical polycarbonate flasks containing Krebs bicarbonate-buffered saline (Krebs & Henseleit, 1932) with 5mM-glucose, 4% (w/v) fatty acid-free bovine serum albumin and 2% (w/v) Ficoll, and with other additions as indicated, under an atmosphere of O_2/CO_2 (19:1). The concentration of acini in suspension was determined as the average wet weight of the pellets obtained after centrifuging duplicate 1ml samples of the suspension in weighed micro-centrifuge tubes for 10s at 15000*g*, discarding the supernatants and blotting away residual liquid from the side walls of the tube. Incubations normally contained between 0.25 and 0.35g wet wt. of acini in a total volume of 7.5ml. Flasks were shaken (180 strokes/min) in a water bath at 37°C. At appropriate times, 1ml samples were withdrawn from incubations and transferred into tubes containing $HClO_4$ such that its final concentration was 5% (w/v). These were immediately mixed, and then frozen by immersion in liquid N_2. After such treatment, samples could be stored at −80°C for up to 4 weeks without any effect on their content of cyclic AMP.

The proportion of cyclic AMP in the incubation medium itself was checked by following the above protocol after first sedimenting acini from 1.2ml samples of incubations for 10s at 15000*g* in a micro-centrifuge and further processing 1ml samples of the resulting supernatants. Under all experimental conditions examined in the present work, extracellular cyclic AMP measured in this way accounted for less than 10% of the total cyclic AMP present.

Processing of samples for the assay of cyclic AMP

On the day of assay, samples were thawed and homogenized for 40s with a Polytron homogenizer fitted with a PTA 10S probe operating at setting no. 4. This and all further handling of samples were done at 0–4°C. The homogenized samples were then centrifuged for 6min at 15000*g* in a micro-centrifuge; supernatants were transferred to fresh tubes and precipitates were discarded. Solid $KHCO_3$ was added to the supernatants until neutrality was achieved, and they were again centrifuged for 3min as above. The resulting supernatants were then assayed for cyclic AMP content.

Determination of cyclic AMP

The concentration of cyclic AMP in samples prepared as above was determined by using the competitive radioligand-binding assay of Gilman (1970) as modified by Tovey *et al.* (1974). Each assay (final volume 0.2ml) contained 0.025μCi of cyclic [^{3}H]AMP. A preparation of cyclic AMP-dependent protein kinase was used, at a final concentration of 25μg of protein/ml, as the source of cyclic AMP-binding protein. Equilibrium binding of [^{3}H]cyclic AMP in samples containing up to 20pmol of cyclic AMP was achieved in 3h at 0°C.

[^{3}H]Dihydroalprenolol binding to membrane preparations

Membrane suspensions were prepared from acini after washing and homogenization in 0.25M-sucrose/1mM-EDTA/10mM-Tris/HCl, pH7.5, as described for adipocytes by Malbon *et al.* (1978), except that acini were homogenized with a

Polytron as above for 15 s at setting no. 4. Binding of [^{3}H]dihydroalprenolol to these membranes was determined exactly as described by those authors; radioactively labelled membranes were collected, after equilibration with [^{3}H]dihydroalprenolol for 15 min at 37°C, on 25 mm-diameter glass-fibre filters (Whatman GFC), which were then washed with ice-cold incubation buffer. For some experiments, membranes were prepared from mammary secretory epithelial cells, which had been isolated from collagenase digests of rat mammary tissues by the method of Soloff *et al.* (1980), and from adipocytes prepared as described by Robson *et al.* (1984).

Chemicals and radiochemicals

The following were purchased from Sigma Chemical Co.: (−)-isoprenaline, clonidine, DL-propranolol (all as hydrochloride salts), atenolol, 3′,5′-cyclic AMP (sodium salt), cyclic AMP-dependent protein kinase (from bovine heart), (−)-adrenaline, (−)-noradrenaline (both as bitartrate salts), and activated charcoal. Forskolin was purchased from Calbiochem–Behring Corp. and Pentex fatty acid-free bovine serum albumin from Miles Laboratories.

ICI 118,551 (hydrochloride salt) was generously given by ICI Pharmaceuticals Division, and Ro 7-2956 by F. Hoffman-La Roche and Co., Basle, Switzerland. 3′,5′-Cyclic [8-^{3}H]AMP (sp. radioactivity 28 Ci/mmol) and L-[4,6-*propyl*-^{3}H]-Dihydroalprenolol ([^{3}H]dihydroalprenolol; sp. radioactivity 77 Ci/mmol) were purchased from Amersham International.

The sources of all other chemicals were as previously reported (Mullaney & Clegg, 1984; Robson *et al.*, 1984).

Results

Cyclic AMP content of acini in vitro

When acini were dispensed into incubation medium directly after preparation, and sampled immediately for the determination of cyclic AMP, a value of $215 \pm 17(17)$ pmol/g wet wt. was obtained. This underwent no statistically significant change (Student's *t* test for paired observations) during a subsequent 45 min of incubation, although, as shown in Fig. 1, a trend towards a decrease in cyclic AMP content with increasing time of incubation was observed. Fig. 1 also shows that the presence, in the incubation medium, of forskolin (the diterpene activator of adenylate cyclase) or of a combination of isoprenaline and the cyclic AMP phosphodiesterase inhibitor Ro 7-2956 caused an enhancement of intracellular cyclic AMP in acini (examined in greater detail below), which was measureable even after exposure of acini for the shortest duration experimentally achievable with the techniques employed (several seconds), i.e. in the 'zero-time' samples. Incubation of acini in forskolin-containing medium resulted in an increase in their cyclic AMP content which was greater after 15 min than after 3 min (Fig. 1) or 5 min (results not shown). This increase persisted at well above control values for at least a further 30 min, although, as in the untreated controls, some decline in cyclic AMP content occurred during that time. The effects of forskolin and other agents were most frequently monitored, in subsequent experiments, after exposure of acini to them for 15 min at 37°C. Under these conditions, enhancement of the cyclic AMP content of acini was a function of forskolin concentration, as shown in Fig. 2.

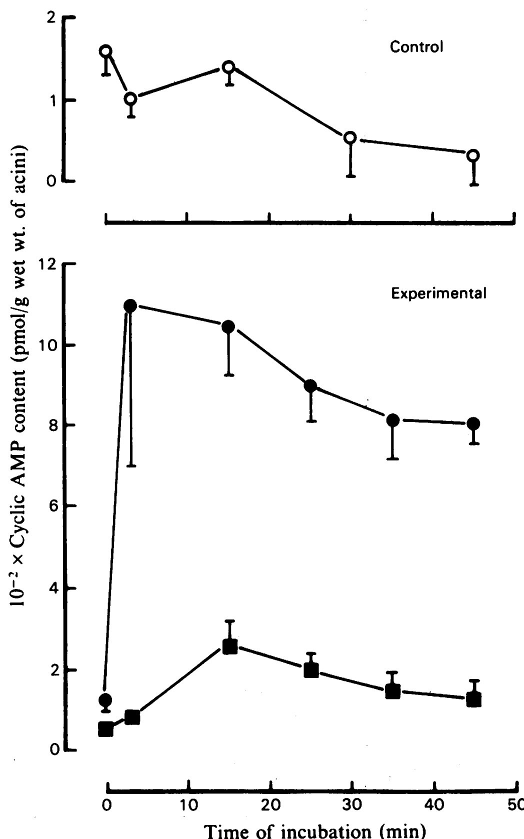

Fig. 1. *Cyclic AMP content of mammary acini incubated in vitro: response to effectors of adenylate cyclase*
Acini were incubated as described in the Materials and methods section in medium without additions (○), with 10 μM-forskolin (■) or with 1 μM-isoprenaline plus 1 mM-Ro 7-2956 (●). Results shown are mean values (error bars show S.E.M.) determined with four (control) or six (experimental) separate preparations of acini.

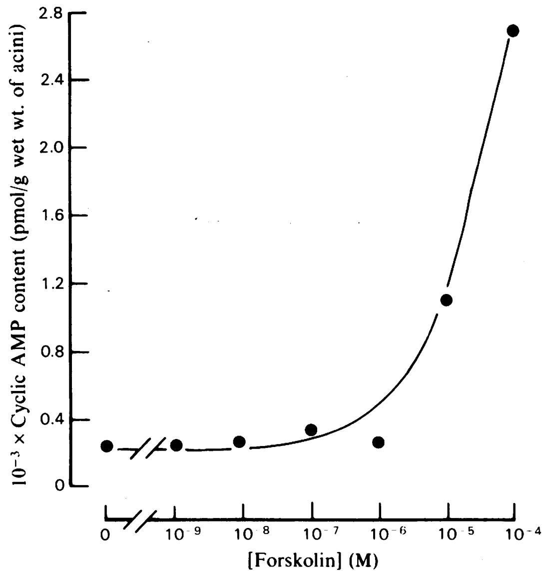

Fig. 2. *Concentration-dependence of the effect of forskolin on the content of cyclic AMP in mammary acini incubated in vitro*

Cyclic AMP was measured after incubation of acini for 15 min in the presence of various concentrations of forskolin. Ethanol (the solvent used for preparing stock solutions of forskolin) was present in all incubations at a final concentration of 0.1% (v/v). A representative experiment using a single preparation of acini is illustrated.

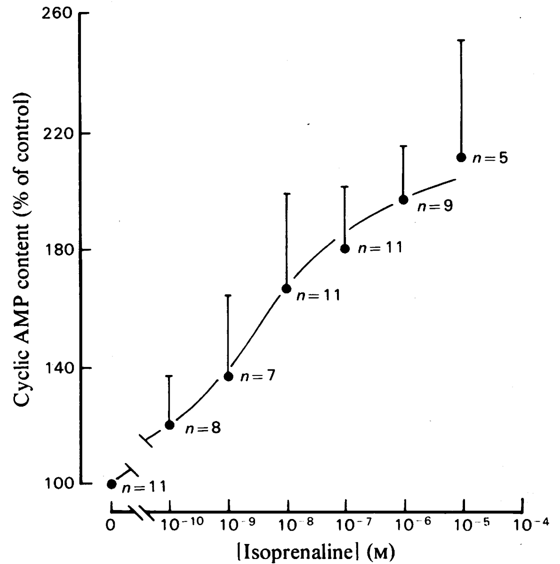

Fig. 3. *Concentration-dependence of the effect of isoprenaline on the cyclic AMP content of acini in vitro*

Conditions of incubation and sampling were as in Fig. 2, except that isoprenaline was substituted for forskolin. Results plotted are mean values (error bars show S.E.M.) for the numbers of determinations (n) indicated beside each point. The absolute value of the control group mean (±S.E.M.) was 283±33 pmol of cyclic AMP/g wet wt. of acini. Statistical significance of the difference between paired control (no isoprenaline) and experimental samples (Student's paired t test) was not attained with isoproterenol concentrations of < 10 nM. At 0.1 and 1 μM significant differences were attained ($P<0.001$), and significance persisted at 10 μM ($P<0.05$).

The inhibitor Ro 7-2956 (at a concentration of 1 mM) was without significant effect (by Student's t test for paired data) on the cyclic AMP content of acini (group mean values ± S.E.M. for 11 observations: 283 ± 33 pmol of cyclic AMP/g wet wt. of acini in the absence of inhibitor; 428 ± 95 pmol of cyclic AMP/g wet wt. in the presence of Ro 7-2956). Similarly, the methylxanthine inhibitors of cyclic AMP phosphodiesterase, isobutylmethylxanthine and theophylline (also at 1 mM) did not change the basal cyclic AMP content of acini incubated for 15 min in their presence (results not shown).

In confirmation of previous findings that acini bind little or no glucagon (Robson *et al.*, 1984), this peptide hormone in the presence of Ro 7-2956 provoked only a doubling of cyclic AMP in acini. Addition of adenosine deaminase (80 munits/ml) to incubations of acini in the presence or absence of any of the agents used in the present study had no effect on their content of cyclic AMP (results not shown).

Treatment of acini with the adrenergic agents adrenaline, noradrenaline and isoprenaline resulted, in all three cases, in a slight increase in their content of cyclic AMP. This achieved statistical significance (Student's t test for paired data) only with isoprenaline (Fig. 3). The values in Fig. 3 were measured after exposure of acini to isoprenaline for 15 min. In other experiments (results not shown) the effect of 10 μM-isoprenaline on cyclic AMP in acini after 0, 1, 2, 3, 5, 10 and 15 min in the presence of the agent was monitored. These experiments revealed no evidence for a rapid 'spike' in cyclic AMP content in response to the β-agonist, such as might be expected for a responsive but rapidly desensitized system (Terasaki *et al.*, 1978). Values in the experimental samples remained approximately double those of control (no isoprenaline) samples at all time-points tested. The effect of these adrenergic agents on the cyclic AMP content of acini was much more pronounced when Ro 7-2956 was also present to prevent the breakdown of cyclic AMP by phosphodiesterases. Fig. 4 illustrates the dose–response relationships between these three agents and cyclic AMP in Ro 7-2956-

treated mammary acini. Half-maximally effective concentrations (EC_{50}) of isoprenaline, adrenaline and noradrenaline were 0.2, 0.7 and $>1\,\mu$M respectively. The corresponding EC_{50} value for isoprenaline in the absence of Ro 7-2956 (Fig. 3) was around 10nM, although no statistically significant increase in cyclic AMP over the control emerged at this concentration of isoprenaline. This ranking of potencies suggested that a β_2-type adrenergic receptor was involved in modifying these effects (Lefkowitz *et al.*, 1983). Further information on receptor subtype of rat mammary acini was obtained by monitoring their cyclic AMP content in response to specific adrenergic agonists, and to combinations of a non-specific agonist (isoprenaline) and specific antagonists (Table 1). The α_2-agonist clonidine was without significant effect on control cyclic AMP values. Similarly, neither the α_1-antagonist prazosin, nor the α_2-antagonist yohimbine, was able to influence the degree of enhancement of acini cyclic AMP content caused either by forskolin or by the combined actions of isoprenaline and Ro 7-2956 (results not shown). The β_1-antagonist atenolol was also ineffective in opposing the elevation of acini cyclic AMP content caused by isoprenaline treatment, whereas the β_2-antagonist ICI 118,551 decreased cyclic AMP in the presence of isoprenaline to near-control values (Table 1).

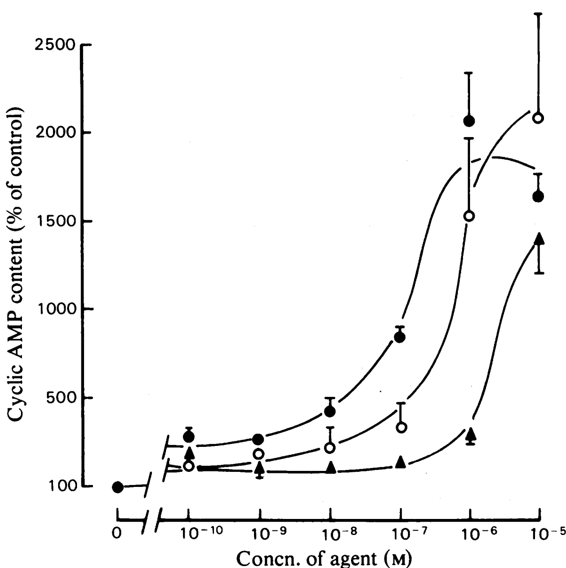

Fig. 4. *Concentration-dependence of the effects of adrenergic agents on the cyclic AMP content of acini incubated in vitro in the presence of Ro 7-2956*

Acini were incubated and sampled for cyclic AMP determination after 15min; 1mM-Ro 7-2956 was present in all incubations. Values plotted are means (error bars indicate S.E.M.) of determination made on four separate preparations for experiments with isoprenaline (●), four for adrenaline (○) and three for noradrenaline (▲). The mean value (±S.E.M.) of all 11 determinations made in the absence of adrenergic agonists was 428±95pmol of cyclic AMP/g wet wt. of acini.

These findings suggested that only β-adrenergic receptors (predominantly of the β_2 subtype) were involved in the mediation of this aspect of the biochemical response of mammary acini to adrenergic agents.

Adrenergic receptors of mammary acini: ligand-binding properties

A more direct examination of the type and number of β-adrenergic receptors on acini membranes was undertaken by measuring the binding to membranes of the radioactively labelled β-antagonist, dihydroalprenolol. [^{3}H]Dihydroalprenolol is non-selective between β_1 and β_2 receptor subtypes (Stadel & Lefkowitz, 1983). The relative abundance of these may therefore be determined by measuring the decrease in specific binding of [^{3}H]dihydroalprenolol induced by the simultaneous presence of a non-radioactive ligand capable of specific binding to only one of the receptor subtypes. An experiment of this type is illustrated in Fig. 5. Atenolol (β_1-specific) was unable to compete-out specific [^{3}H]dihydro-

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

Acini were incubated for 15min in the presence of effectors as indicated (all at 1 μM), then sampled for measurement of cyclic AMP. All incubations contained 1 mM-Ro 7-2956. Values tabulated are means (±S.E.M.) of determinations made on four separate acini preparations. Significant differences (paired *t* test) are indicated as follows: $^*P<0.01$ relative to control (no addition) samples; $^\dagger P<0.001$ relative to isoprenaline-treated samples.

Agent(s) present	Mode of action	Cyclic AMP content of acini (pmol/g wet wt.)
None		653±31
Clonidine	α_2-Agonist	805±65
Isoprenaline	Non-selective β agonist	5810±487*
Isoprenaline plus atenolol	β_1-Antagonist	6075±84*
Isoprenaline plus ICI 118,551	β_2-Antagonist	1455±214†

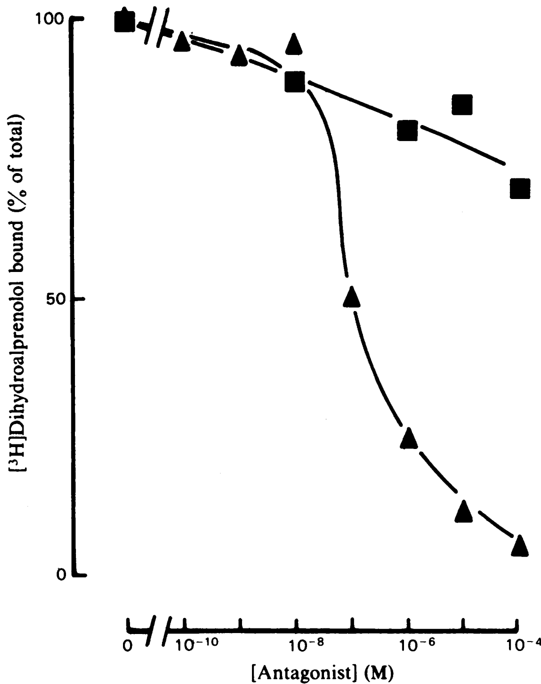

Fig. 5. *Binding of [^{3}H]dihydroalprenolol to acini membranes: displacement by β_2, but not by β_1, antagonists*
Binding of [^{3}H]dihydroalprenolol to acini membranes (0.2–0.4mg of protein/incubation) at 37°C was measured as described in the Materials and methods section. In the experiment illustrated, the concentration of [^{3}H]dihydroalprenolol during the binding incubation was 12nM and the total bound radioligand was 441fmol/mg of protein, of which 77fmol/mg of protein could not be displaced by 10μM-propranolol. Ranges of concentrations of ICI 118,551 (β_2-specific; ▲) and atenolol (β_1-specific; ■) were tested for their ability to displace the radioactively labelled ligand.

alprenolol binding, whereas the agent ICI 118,551 (β_2-specific) between 0.1 and 1μM totally displaced the specific component of the binding of 15nM-[^{3}H]dihydroalprenolol. Control experiments with rat adipocyte membranes (results not shown) yielded similar results to those of Malbon *et al.* (1978), with 361fmol of [^{3}H]dihydroalprenolol specifically bound/mg of membrane protein, when at equilibrium with 19nM-[^{3}H]dihydroalprenolol. The corresponding value for the specific binding of 12nM-[^{3}H]dihydroalprenolol to acini membranes was 397fmol/mg of protein. For both types of membrane, the non-specific binding of alprenolol (i.e. that persisting in the presence of excess non-radioactive propranolol) amounted to less than 20% of the total bound.

Discussion

No previous estimate of the cyclic AMP content of mammary acini has been published. Baldwin and co-workers have determined cyclic AMP in freeze-clamped mammary tissue of rat (Louis & Baldwin, 1975) and in tissue slices incubated *in vitro* (Plucinski & Baldwin, 1982). Their values were, respectively, 1000 and 1810pmol of cyclic AMP/g of tissue from rats at day 12 of lactation. These values, however, were not corrected for the content of milk in the preparations; since cyclic AMP is abundant in rat milk, such a correction is necessary before a valid comparison with acini can be drawn. Sapag-Hagar & Greenbaum (1974) have reported rat mammary-tissue cyclic AMP values corrected in this way. For tissue from rats in their second day of lactation, these authors found 460pmol of cyclic AMP/g of milk-free tissue, and for 19-day-lactating rats, 188pmol of cyclic AMP/g of milk-free tissue. The value reported here for acini from 10–12-day-lactating rats is consistent with the findings of Sapag-Hagar & Greenbaum (1974), suggesting that the cyclic AMP system of acini had suffered no gross disruption as a result of tissue digestion and acini isolation. Measurements of cyclic AMP in a primary culture of mammary epithelial cells from virgin mice and in a cultured human mammary carcinoma cell line have been reported (Barber *et al.*, 1984). Expression of results in that report was not in the form of absolute amounts of concentrations, so that comparison cannot be made with values reported here. However, as in the present report, Barber *et al.* (1984) noted an increase in the cyclic AMP accumulated by these cells after brief (3–5min) exposure to methylxanthines and adrenaline together, whereas neither agent alone was significantly effective.

The foregoing results leave no doubt that mammary acini possess competent adrenergic receptors which are functionally coupled to adenylate cyclase. The properties of those receptors, as characterized above, are those of the β_2-subtype. No result was obtained to suggest that any α-receptor-mediated events were involved in the observed effects of adrenergic agents on the intracellular cyclic AMP content of acini. α_1-Receptors are not thought to interact directly with adenylate cyclase, whereas α_2-receptors are functionally coupled, in an inhibitory mode, to this enzyme (Jacobs *et al.*, 1976). Since no binding studies were done with α-specific ligands, we cannot exclude the possibility that adrenergic receptors occur on the surface of mammary cells. The results reported do, however, demonstrate that, if such receptors are present, they are without influence on the contents of cyclic AMP within those cells.

The adrenergic-receptor profile of acini characterized here contrasts with that of the adipocyte, where β-mediated stimulation (Fain, 1980) is accompanied in most species, although not in the rat, by α-mediated inhibition of adenylate cyclase (Michael *et al.*, 1982). The applicability of the β_1/β_2 subdivision to adipocytes has been questioned (De Vente *et al.*, 1980; Wilson *et al.*, 1984). We have insufficient data to know whether the adrenergic receptors of mammary acini merit consideration in this debate.

The cellular composition of the mammary acini preparation is heterogeneous. Alveolar secretory epithelial cells comprise around 70% (by number) of the cells present, with minor populations of myoepithelial cells as well as fibroblasts, erythrocytes and other vascular cellular elements (Kraehenbuhl, 1977). We have considered the possibility that the receptors that we have characterized in acini membranes are not those of the secretory epithelial cells, but originate from one or more of the other cellular types present. In view of the reported preponderance of receptors on smooth-muscle cells (Lefkowitz *et al.*, 1983), we were particularly aware of the possibility that the β_2-receptors of acini might be in fact those of myoepithelial cells. This possibility was examined by further separating the cellular types in collagenase-dispersed mammary tissue and purifying the secretory epithelial cell population (Soloff *et al.*, 1980). Membranes from these epithelial cells bound [^{3}H]dihydroalprenolol: considerable variation was encountered among preparations in the extent of binding, with a mean value from five preparations of 64fmol of [^{3}H]dihydroalprenolol specifically bound/mg of membrane protein, but with the higher individual values approaching those of acini membrane preparations. The β_2-antagonist ICI 118,551 diminished the specific binding to a value of 24fmol/mg of membrane protein (mean of five preparations). In the best preparation, non-specific [^{3}H]dihydroalprenolol binding was 20% of the total bound, and ICI 118,551 decreased the specific binding to 17% of that in the absence of the antagonist. Purified secretory epithelial cells were obtained in insufficient yield from rat mammary tissue (cf. Soloff *et al.*, 1980) to allow detailed biochemical investigations of the consequences of adrenergic-receptor occupation. However, since the properties of receptors measurable on membranes from these cells resemble those, determined in this study, of acini membrane adrenergic receptors, we conclude that these properties, and the changes in mammary cellular cyclic AMP reported above, are indeed attributable, at least predominantly, to the secretory epithelial cells in the acini preparation.

Despite the presence on acini of β-adrenergic receptors in numbers comparable (per mg of membrane protein) with those found in adipocytes (Malbon *et al.*, 1978), treatment of this preparation with adrenergic agonists alone caused only very small increases in cellular cyclic AMP compared with those resulting from similar treatment of adipocytes (e.g. Butcher *et al.*, 1968). Substantial changes in the intracellular concentration of cyclic AMP after β-agonist treatment could be demonstrated only when cyclic AMP phosphodiesterase inhibitors were present. Such inhibitors alone, however, had little influence on basal cyclic AMP contents in acini (cf. similar results with adipocytes; Butcher *et al.*, 1968; Shechter, 1984), suggesting that there was little turnover of cyclic AMP in unstimulated acini. The kinetic capacity of cyclic AMP phosphodiesterases of acini (Mullaney & Clegg, 1984) to buffer the intracellular cyclic AMP against changes in concentration, which would otherwise result from adrenergic stimulation, presumably accounts for the absence of such changes, except when phosphodiesterase inhibitors were also present.

Forskolin (at $\geq 10\mu$M), in contrast with adrenergic agonists, was able to provoke a substantial rise in the cyclic AMP content of acini in the absence of phosphodiesterase inhibitors. This finding implies that, other things being equal, these concentrations of forskolin cause a greater degree of stimulation of adenylate cyclase in the mammary-epithelial-cell membrane than do saturating doses of adrenergic agonists. Measurement of adenylate cyclase activity in acini membranes incubated with and without either forskolin or isoprenaline *in vitro* in the presence of various concentrations of GTP or its non-hydrolysable analogues have confirmed this implication (Ladha *et al.*, 1985).

The present report of a small or negligible increase in the cyclic AMP content of acini caused by their treatment *in vitro* with adrenergic agents affords an explanation for the lack of effect of these agents on the rate of lipogenesis in isolated acini (Williamson *et al.*, 1983).

Acini apparently possess the necessary individual elements of a system for modifying the rate of acetyl-CoA carboxylase activity in response to changes in the intracellular concentration of cyclic AMP, and this enzyme is probably, at least under most circumstances, the rate-determining step for mammary-gland lipogenesis (Robinson *et al.*, 1978; Munday & Williamson, 1981; McNeillie & Zammit, 1982; but see also Williamson *et al.*, 1983; Bussmann *et al.*, 1984). Consequently, the inability of added cyclic AMP to cause an inhibition of lipogenesis in isolated acini (Williamson *et al.*, 1983), despite the above, remains a paradox which hampers a full understanding of acute regulation of

lipogenesis and other aspects of metabolism in mammary tissue.

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