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Adenosine 3′,5′-cyclic monophosphate-mediated enhancement of calcium-evoked prolactin release from electrically permeabilised 7315c tumour cells

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1 The 7315c tumour cell was used as a model system for the investigation of adenosine 3′,5′-cyclic monophosphate (cyclic AMP)-mediated enhancement of calcium-evoked prolactin release.

2 7315c cells were permeabilised by subjecting the cells to intense electric fields. Studies investigating the penetration of the dye ethidium bromide indicated that the cells were completely permeabilised after 2 discharges of 2000 volts and that the pores remained open for at least 30 min before beginning to reseal. These permeabilisation parameters were consistent with those which gave maximal calcium-stimulated prolactin release.

3 In the absence of calcium and in the presence of EGTA (1 mM), permeabilised 7315c cells secreted prolactin at a rate of 0.23 ng min^{-1} per 10^6 cells. When EGTA was replaced by 1.5 mM calcium, permeabilised cells secreted prolactin at a rate of 2.20 ± 0.30 ng min^{-1} per 10^6 cells in the first 5 min of exposure. Maximal calcium-dependent prolactin secretion from permeabilised cells occurred at 37°C.

4 The amount of prolactin secreted, in a 5 min incubation at 37°C, from permeabilised cells depended upon the free calcium concentration in the permeabilisation medium. Calcium stimulated prolactin release from permeabilised cells in the concentration range 0.1–10 μM (half maximal = 5.8 μM). When permeabilised cells were exposed to cyclic AMP (100 μM) for 5 min prior to and during a 5 min challenge with various concentrations of calcium, the amount of prolactin secreted at each effective concentration of calcium was increased. However, cyclic AMP did not alter the potency of calcium as a stimulant of prolactin secretion.

5 The results suggest that cyclic AMP potentiates calcium-evoked secretion from 7315c cells, not by increasing the entry of calcium into the cytosol, but at a step in the secretory process, distal to calcium entry, which modulates the ability of an increase in cytosolic calcium concentration to stimulate prolactin release.

Introduction

The second messengers, calcium and adenosine 3′:5′-cyclic monophosphate (cyclic AMP), are important promoters of prolactin secretion from mammatrophs of the anterior pituitary gland (Labrie *et al.*, 1979; Albert & Tashjian, 1984). Calcium and cyclic AMP nearly always function in concert to control cellular function (Rasmussen & Barrett, 1984) and consistent with this idea are reports that cyclic AMP enhances basal and stimulated prolactin secretion (Robberecht *et al.*, 1979; Tam & Dannies, 1981; Delbeke *et al.*, 1984), both calcium-dependent phenomena. Although cyclic AMP enhances calcium-evoked secretion from a variety of tissues (Pelayo *et al.*, 1978; Collen *et al.*, 1982; Rabe *et al.*, 1982; Tsurata *et al.*, 1982; Delbeke *et al.*, 1984; Heisler, 1984; Henquin & Meissner, 1984; Malaisse *et al.*, 1984), the mechanism underlying this enhancement remains unclear. In particular, there is controversy over

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whether the cyclic AMP-mediated potentiation of hormone secretion is due to an increased calcium entry from either extra- or intracellular sources to result in an increased cytosolic calcium concentration or whether cyclic AMP acts to potentiate the effect of calcium upon the secretory apparatus.

In an earlier study from this laboratory (Frey *et al.*, 1986), it was demonstrated that, in the prolactin-secreting 7315c tumour cell, cyclic AMP potentiates calcium-evoked prolactin release without any corresponding increase in the amount of calcium entering the cytosol. Therefore, it was postulated that cyclic AMP must be acting distal to the entry of calcium into the cytosol. In the present study, the electrical permeabilisation technique (Knight & Baker, 1982) was used to test this hypothesis further. This technique permits the formation of pores in the cell membrane thus eliminating the regulation of calcium entry at this level. In addition, the intracellular milieu, in particular the secretory apparatus, is exposed to manipulation from the outside. Secretion can then be regulated by controlling the calcium concentration in the external medium with calcium-EGTA (ethyleneglycol-*bis*(β-amino- ethylether)N,N'-tetraacetic acid) buffers (Knight & Baker, 1982; Dunn & Holz, 1983; Wilson & Kirshner, 1983; Ronning & Martin, 1986). A previous report from this laboratory used this technique to investigate calcium-evoked secretion of α-MSH from dispersed cells of the intermediate lobe of the rat pituitary (Yamamoto *et al.*, 1987). The effect of cyclic AMP upon calcium-evoked prolactin release was investigated in electrically permeabilised 7315c cells. The results of the present study support the hypothesis that, in 7315c, cyclic AMP enhances calcium-dependent prolactin secretion without increasing the calcium concentration in a compartment critical for hormone secretion.

Methods

Preparation of tumour cells for experiments

Rat 7315c tumour cells were grown *in vivo* and dispersed for use in experiments as previously described (Frey & Kebabian, 1984). In preparation for each experiment, dispersed cells were incubated in a volume of 50 ml, at a density of 10^6 cells ml^{-1}, of Eagles minimum essential medium (EMEM) supplemented with 0.25% (w/v) of bovine serum albumin (EMEM/BSA), for 2 h at 37°C under a humidified atmosphere of 95% air and 5% CO_2.

Permeabilisation of 7315c tumour cells and measurement of prolacton secretion

After the 2 h preparatory incubation, the cell suspension was centrifuged (5 min, 200 *g*) and the cell pellet resuspended in 10 ml of permeabilisation buffer (Na acetate 146 mM, KCl 5 mM, Na_2HPO_4 1 mM, Mg SO_4 1 mM, Mg ATP 1 mM, HEPES 5 mM, glucose 7.8 mM, EGTA 1 mM pH 7.4; 37°C) as previously described for intermediate lobe pituitary cells (Yamamoto *et al.*, 1987). The cell suspension was centrifuged (5 min, 200 *g*) and the cell pellet resuspended in permeabilisation buffer at a cell density of 10^7 cells ml^{-1}. At this point, cells were either left intact or permeabilised as follows.

The cells were permeabilised by subjecting 1 ml of the cell suspension to intense electric fields of brief duration (Knight & Baker, 1982). The nuclear stain, ethidium bromide, is normally impermeant and fluorescent only when in contact with nucleic acid. It can, therefore, be used as an indicator of cell permeabilisation. To determine the parameters that lead to 100% peremeabilisation, the cells were subjected to varying numbers of discharges of varying voltages and subsequently stained with ethidium bromide (50 μM). The cells were washed once after staining and observed under phase contrast and fluorescence to obtain counts of total and stained cells, respectively. Under these conditions non-permeabilised cells did not take up ethidium bromide. Cells which were permeabilised and did stain with ethidium bromide still excluded the dye trypan blue and no measurable leakage of the cytosolic protein lactate dehydrogenase was detected from permeabilised cells (data not shown).

Optimum permeabilisation parameters were also determined for the ability of calcium, added to the external medium, to stimulate prolactin release from permeabilised cells. The standard protocol for the determination of prolactin secretion from permeabilised cells was as follows: 100 μl of the permeabilised cells (10^7 cells ml^{-1}) was added to 900 μl of either standard permeabilisation buffer (37°C) described above or permeabilisation buffer in which the 1 mM EGTA had been replaced by either 1.5 mM $CaCl_2$ or calcium-EGTA buffers. The incubation tubes were vortexed and placed in a 37°C shaking water bath. At this point, the zero time samples were centrifuged (10 s, 10,000 *g*) and an aliquot of the supernatant was stored upon ice until the radioimmunoassay for prolactin content was begun. The remaining tubes were incubated for 5 min at 37°C before terminating the incubation by centrifugation (10 s, 10,000 *g*) and sampling the supernatant for the determination of prolactin content by radioimmunoassay. For each condition tested, triplicate samples were run in each experiment.

Table 1 The effect of increasing the number of discharges upon the staining of 7315c cells by ethidium bromide

Number of discharges	*% of cells stained*
1	74 ± 7
2	96 ± 2
3	94 ± 4
4	96 ± 3
5	98 ± 2
10	97 ± 3

7315c cells were subjected to the indicated number of discharges at 2000 V cm^{-1} and stained with ethidium bromide as described in the methods. The results are expressed as the mean ± s.e.mean of 6 determinations. The cells were completely permeabilised after 2 discharges of 2000 V cm^{-1}.

Modifications of the standard protocol are elaborated upon in the legends to the figures and tables.

Preparation of calcium-EGTA buffers

In experiments involving the exposure of permeabilised cells to a range of free calcium concentrations in the external medium, Ca-EGTA buffers were employed to obtain the desired free calcium concentration (Portzehl *et al.*, 1964). Various quantities of volumetric 1 M $CaCl_2$ were added to the standard permeabilisation buffer to give the required free calcium concentration when in equilibrium with 1 mM EGTA and 1 mM Mg ATP (present in the standard buffer) at pH 7.4. The exact quantity of $CaCl_2$ to be added was calculated by use of a computer programme written on the basis of previously published programmes for hand-held calculators (Perrin & Sayce, 1967; Fabiato & Fabiato, 1979). The free calcium concentration of these Ca-EGTA buffers was checked by a calcium-sensitive electrode (Affolter & Siegel, 1979) and calibrated using calcium standards (Tsien & Rink, 1980). Cyclic AMP (100 μM) did not alter the free calcium concentration in the permeabilisation buffer. In addition, calcium-EGTA buffers were constructed using an EGTA concentration of 5 mM instead of 1 mM and at a pH of 6.6 instead of 7.4. These variations were found to have no effect upon the results obtained for the calcium activation curve.

Table 2 The effect of increasing the voltage upon the staining of 7315c cells by ethidium bromide

Voltage	*% of cells stained*
500	5 ± 5
1000	19 ± 1
1500	96 ± 4
2000	98 ± 1
2500	99 ± 1

7315c cells were subjected to 5 discharges at the indicated voltages and stained with ethidium bromide as described in the methods. The results are expressed as the mean ± s.e.mean of 6 determinations. The cells were completely permeabilised after 5 discharges of 1500 V cm^{-1}.

Radioimmunoassays

The radioimmunoassay for prolactin was performed as previously described (Frey & Kebabian, 1984). The amount of prolactin released by 10^6 cells was expressed as the amount present at the end of the specified incubation period less the amount present at zero time.

Materials

Permeabilisation buffer was prepared with chemicals from the J.T. Baker Co (Phillipsburg, N.J.). The following substances (with their sources) were used: cyclic AMP and MgATP (Sigma Chemical Co., St Louis, MO); bovine serum albumin (BSA fraction V), (Miles Laboratories Inc., Elkhart, IN); Eagles modified essential medium (EMEM) (Grand Island Biological Co., Grand Island, NY); rat prolactin antiserum (anti-rPRL-S-8) and rat prolactin for standards and iodonation, (the National Hormone and Pituitary Program, Baltimore, MD). Rat prolactin was iodonated by Meloy Laboratories, Inc. (Springfield, VA). Chemicals associated with the preparation of the calcium-sensitive electrodes were obtained from Fluka Chemical Co. (Hauppauge, NY). All other chemicals were readily commercially available.

Statistics

Each experiment was repeated three times, on different days. A two-sided, 0.05 level, paired *t* test was used to determine if the effect of a treatment was significant. Rates of secretion were estimated by linear regression analysis. Correlation coefficients were 94% or better.

Results

Permeabilisation estimated by ethidium bromide studies

Permeabilisation of 7315c cells resulted in staining of the cells by the fluorescent dye, ethidium bromide.

Table 3 The ability of ethidium bromide to stain permeabilised 7315c cells at various times after permeabilisation

Time after permeabilisation	*% of cells stained*
0 min	98 ± 1
30 min	98 ± 1
60 min	81 ± 7
90 min	71 ± 7

7315c cells were permeabilised by 2 discharges of 2000 V cm^{-1} and, at the indicated times after permeabilisation, stained with ethidium bromide as described in the methods. The results are expressed as the mean ± s.e.mean of 6 determinations. The cells remained fully permeabilised for 30 min.

The degree of staining, and by implication the degree of permeabilisation of the cells, was dependent upon both the number of discharges (Table 1) and the voltage applied across the cells (Table 2). The cells were completely stained after 2 discharges of 2000 V cm^{-1}. Furthermore, addition of ethidium bromide at various time intervals after permeabilisation of the cells (2 discharges at 2 kV cm^{-1}), revealed that they remained capable of staining, and so remained completely permeabilised, for at least 30 min after permeabilisation (Table 3). These results were not affected by the presence of calcium or cyclic AMP in the permeabilisation medium.

Permeabilisation estimated by calcium-evoked prolactin secretion

Electrically permeabilised 7315c tumour cells exhibited calcium-dependent prolactin secretion. The magnitude of the calcium-evoked prolactin release was, as in the case of staining of the cells by ethidium bromide, dependent upon both the voltage applied across the cells (Figure 1) and the number of discharges (Figure 2). Maximal calcium-dependent secretion was attained at 2 discharges of 2 kV cm^{-1} which, since this corresponds with those parameters that yield maximal staining of the cells by ethidium bromide, would seem to reflect 100% permeabilisation of the cells under these conditions. These optimum premeabilisation parameters were used in all subsequent experiments. Additional evidence that the cells remained permeabilised for at least 30 min after permeabilisation is provided by the observation that a challenge with 1.5 mM calcium is still able to evoke prolactin release 30 min post permeabilisation (Table 4). Calcium-dependent prolactin release declines with calcium challenge at longer times after permeabilisation.

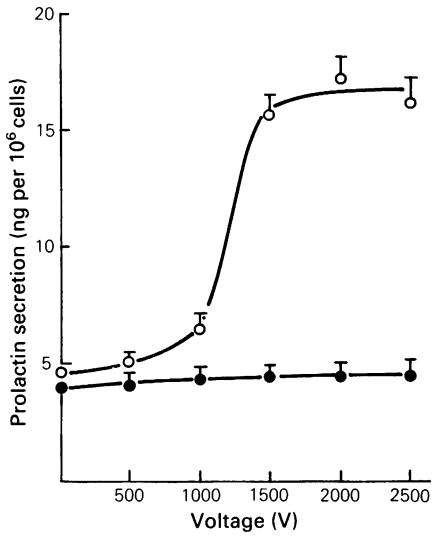

Figure 1 The effect of increasing the voltage of each discharge upon the ability of extracellular calcium to stimulate prolactin release from 7315c cells. 7315c cells were subjected to 5 discharges, at the indicated voltage, as described in the methods and incubated for 5 min in permeabilisation medium containing either 1 mM EGTA (●) or 1.5 mM Ca^{2+} (○). The amount of prolactin released into the medium, during a 5 min incubation, was determined by radioimmunoassay as described in the methods. The results are expressed as the mean from 3 separate experiments; s.e.mean shown by vertical bars. Significant calcium-dependent prolactin secretion was evident at voltages of 1000 V and above.

Table 4 Calcium-dependent prolactin release from permeabilised 7315c cells at various times after permeabilisation

Time after permeabilisation	*Prolactin secretion* (ng per 10^6 cells 5 min^{-1}) *EGTA* (1 mM)	*Ca^{2+}* (1.5 mM)
0 min	2.8 ± 0.4	13.2 ± 0.8
30 min	2.2 ± 0.3	12.9 ± 0.8
60 min	2.3 ± 0.4	11.1 ± 0.4
90 min	2.6 ± 0.6	8.4 ± 0.4

7315c cells, permeabilised (2 discharges of 2 kV cm^{-1}) as described in the methods, were incubated for 5 min, at the indicated times after permeabilisation, in either standard permeabilisation buffer containing 1 mM EGTA or buffer in which the EGTA was replaced by 1.5 mM Ca^{2+}. The results are expressed as the mean ± s.e.mean from 3 separate experiments.

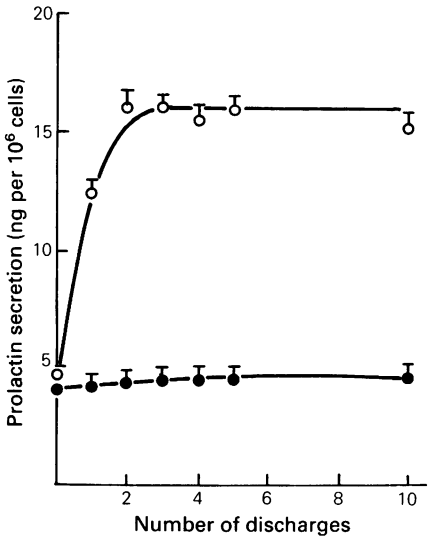

Figure 2 The effect of increasing the number of discharges upon the ability of external calcium to stimulate prolactin release from 7315c cells. 7315c cells were subjected to the indicated number of discharges of $2\,kV\,cm^{-1}$ in the cell permeabiliser as described in the methods and incubated for 5 min in permeabilisation medium containing either 1 mM EGTA (●) or 1.5 mM Ca^{2+} (○). The amount of prolactin secreted into the medium, during a 5 min incubation, was determined by radioimmunoassay as described in the methods. The results are expressed as the mean from 3 separate experiments; s.e.mean shown by vertical bars. Significant calcium-dependent prolactin release was evident after 1 discharge.

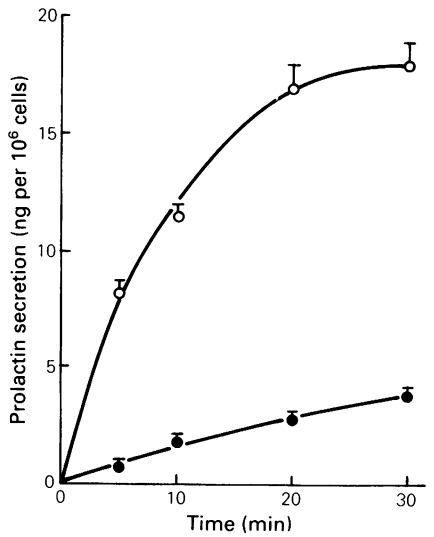

Figure 3 Time course of prolactin secretion from permeabilised 7315c cells. 7315c cells, permeabilised (2 discharges of $2\,kV\,cm^{-1}$) as described in the methods, were incubated, for the indicated time periods in permeabilisation medium containing either 1 mM EGTA (●) or 1.5 mM $CaCl_2$ (○). The amount of prolactin secreted into the medium during each time period was determined by radioimmunoassay, as described in the methods. The results are expressed as the mean from 3 separate experiments; s.e.mean shown by vertical bars. Significant stimulation of prolactin secretion by calcium was evident from 5 min.

Characterization of calcium-stimulated prolactin secretion

Permeabilised tumour cells, exposed to 1 mM EGTA, secreted prolactin at a rate of $0.23\,ng\,min^{-1}$ per 10^6 cells for up to 60 min (Figure 3). Permeabilised cells, exposed to 1.5 mM calcium, secreted hormone at an increased rate of $2.20\,ng\,min^{-1}$ per 10^6 cells during the first 5 min of exposure. Between 5 and 20 min of exposure to calcium the rate of secretion was $1.16\,ng\,min^{-1}$ per 10^6 cells. After 20 min of exposure to calcium the rate of secretion was the same as the basal rate. Intact cells secreted prolactin at rates of 0.21 and $0.26\,ng\,min^{-1}$ per 10^6 cells in the presence of 1 mM EGTA and 1.5 mM calcium, respectively (data not shown).

Calcium-evoked secretion from permeabilised 7315c was temperature-dependent (Table 5). At 4° and 20°C, calcium evoked little prolactin secretion from permeabilised cells. The maximum stimulant effects of calcium on hormone secretion were observed at 37°C.

Calcium-evoked prolactin release from permeabilised 7315c cells was dependent upon the concentration of free calcium in the permeabilisation medium (Figure 4). Calcium increased prolactin

Table 5 Temperature-dependency of calcium-stimulated prolactin secretion from permeabilised 7315c cells

Temperature (°C)	*Prolactin secretion* (ng per 10^6 cells $5\,min^{-1}$) *EGTA* (1 mM)	*Ca^{2+}* (1.5 mM)
4	0.8 ± 0.1	1.4 ± 0.1
20	2.3 ± 0.2	5.4 ± 0.3
37	4.0 ± 0.2	14.1 ± 0.7

7315c cells, permeabilised (2 discharges of $2\,kV\,cm^{-1}$) as described in the methods, were incubated for 5 min at the indicated temperature in either the standard permeabilisation buffer containing 1 mM EGTA or buffer in which the EGTA was replaced by 1.5 mM Ca^{2+}. The results are expressed as the mean ± s.e.mean from 3 separate experiments.

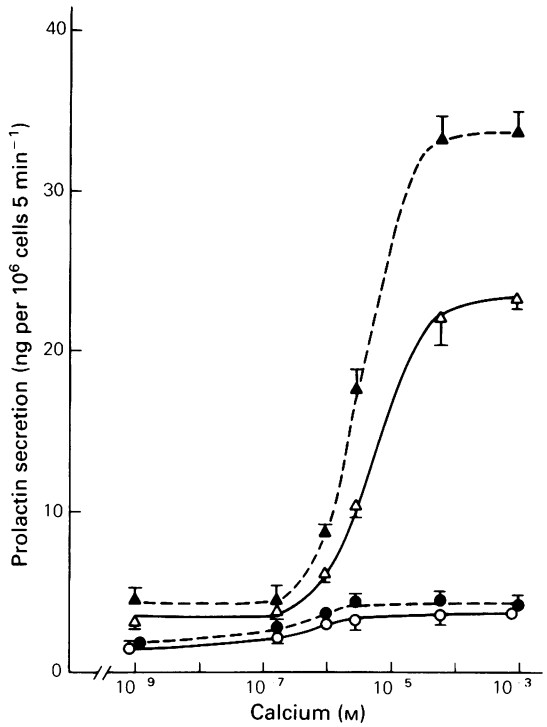

Figure 4 Cyclic AMP enhances calcium-dependent prolactin secretion from permeabilised 7315c cells. 7315c cells were left intact (circles) or were permeabilised (triangles) as described in the methods. Intact or permeabilised cells (10^7 cells ml^{-1}) were incubated in the standard permeabilisation buffer for 5 min at 37°C in the absence (open symbols) or presence (closed symbols) of 100 μM cyclic AMP. After 5 min an aliquot of 10^6 cells was added to 900 μl of permeabilisation buffer containing various proportions of calcium and EGTA, such that the indicated concentrations of free calcium resulted, and cyclic AMP, such that a final concentration of 100 μM was maintained. For both intact and permeabilised cells, the amount of prolactin secreted during the second half of the 10 min incubation was determined. The results are expressed as the mean from 3 experiments; s.e.mean shown by vertical bars. Calcium (0.9 μM) significantly increased prolactin secretion from permeabilised, but not intact cells. Cyclic AMP significantly enhanced the amount of prolactin secreted from permeabilised cells but did not significantly affect prolactin release from intact cells at any concentration of calcium tested.

secretion in a concentration-dependent manner between 0.1 and 10 μM (EC_{50} = 5.8 ± 0.6 μM).

The effect of cyclic AMP upon calcium-evoked prolactin release

Cyclic AMP enhanced calcium-dependent prolactin secretion from permeabilised 7315c cells (Figure 4). The protocol used to obtain the data shown in Figure 4 mimicked, as closely as possible, that used in a previous report from this laboratory (Frey *et al.*, 1986) in which intact cells were preincubated, with forskolin, for 5 min before a 5 min incubation with the calcium ionophore, ionomycin. When permeabilised cells were exposed to cyclic AMP (100 μM) for 5 min prior to and during a 5 min challenge with calcium, the amount of prolactin secreted, at each effective concentration of calcium, was increased (the maximum effect was a 2 fold increase). Cyclic AMP did not change the EC_{50} of the calcium concentration-effect curve (5.8 ± 0.6 μM and 3.9 ± 0.9 μM in the absence and presence of cyclic AMP, respectively) nor the concentration of calcium which caused a maximal effect. Concentrations of calcium which maximally stimulated secretion from permeabilised cells had only a small effect on secretion from intact cells (Figure 4). Cyclic AMP had no effect on prolactin secretion from intact cells at any concentration of calcium tested. The results presented in Figure 4 were similar to those obtained when the concentration of EGTA, used in the EGTA buffers, was 5 mM and when the calcium-EGTA buffers were constructed at a pH of 6.6 (data not shown).

Discussion

Conditions were obtained under which it was possible to permeabilise a population of cells which would remain fully permeabilised for at least 30 min before starting to reseal. The size of the pores created in the cell membrane is difficult to estimate from these data but were sufficiently large to admit ethidium bromide but small enough to exclude trypan blue and prevent escape of lactate dehydrogenase. This suggests that the pores formed in the cell membrane could permit free exchange of substances with molecular weights below 1000. These data permit reasonable confidence that the cells were fully permeabilised throughout the experiments detailed here and that the substances added to the external medium (i.e. cyclic AMP and EGTA/Ca^{2+}) could freely equilibrate throughout the experimental system both extra- and intracellularly.

Permeabilising 7315c cells by use of the high voltage technique does not impair their ability to undergo exocytosis. Permeabilised tumour cells maintain a calcium-dependent secretory response which, in common with intact cells, is dependent upon temperature and cytosolic free calcium concentration. Tumour cells permeabilised in the absence of calcium did not release prolactin in an amount significantly above the basal secretion of intact cells. This suggests that passive release, due to cellular

damage, was not significant. Permeabilised 7315c cells exhibited increased prolactin secretion when the intracellular calcium concentration was increased between 0.1 to 10 μM with an EC_{50} of 5.8 μM. These data are entirely consistent with those obtained from several cell types using various permeabilisation techniques (Pace *et al.*, 1980; Knight & Baker, 1982; Dunn & Holz, 1983; Wilson & Kirshner, 1983; Knight & Scrutton, 1980; 1986). In addition, the calcium concentration range of 0.1–10 μM is similar to that recently found in electrically permeabilised GH_3 cells (Ronning & Martin, 1986) and our own results in dispersed intermediate lobe pituitary cells (Yamamoto *et al.*, 1987). The results obtained with permeabilised 7315c cells were consistent with the results obtained from intact 7315c cells. In intact tumour cells, increased prolactin secretion was associated with cytosolic calcium concentrations above 0.1 μM (Frey *et al.*, 1986). The magnitude of calcium-dependent secretion from permeabilised cells was similar to that elicited by thyrotropin-releasing hormone (Aub *et al.*, 1986), ionomycin, or high extracellular potassium concentration (Frey *et al.*, 1986) from intact 7315c cells. These comparisons indicate that secretory responses from electrically permeabilised 7315c cells do not significantly differ from those obtained from intact 7315c cells and support the contention that electrically permeabilised cells are a useful system for the further investigation of the effects of cyclic AMP upon calcium-evoked prolactin release.

Cyclic AMP enhanced calcium-dependent secretion from permeabilised 7315c cells. This implies that, in permeabilised 7315c cells, cyclic AMP does not enhance hormone secretion by increasing the cytosolic calcium concentration, since such increases would be buffered by the calcium-EGTA buffers (which are designed to maintain fixed free calcium concentration). Thus, in the intact tumour cell, increased calcium entry into the cytosol from either outside the cell or from intracellular organelles may not be the principal mechanism by which cyclic AMP enhances secretion. This is a conclusion which is consistent with our previous work with the intact 7315c cell (Frey *et al.*, 1986). Also consistent with our previous work, was the observation that cyclic AMP approximately doubled, at maximum, calcium-dependent prolactin secretion from permeabilised 7315c cells. If cyclic AMP were to increase the cytosolic calcium concentration in a compartment critical for secretion, then calcium would be more potent in stimulating secretion in its presence. Thus, since this was not observed, the principle site of action of cyclic AMP in enhancing secretion from the 7315c tumour cell must be distal to the entry of calcium into the cytosol.

The precise mechanism by which cyclic AMP could produce this effect remains unknown. The fact that cyclic AMP increases the capacity of the pituitary tumour cell to release prolactin in response to a particular increment in cytosolic calcium leads to speculation that a preincubation with cyclic AMP alters a rate-limiting step in the calcium-dependent release pathway or alters the fusion of secretory vessicles with the cell membrane. It has been reported that vasoactive intestinal peptide (VIP), an agent which stimulates cyclic AMP production, and thyrotropin-releasing hormone (TRH), an agent which increases cytosolic calcium concentration and protein kinase activity, promote the phosphorylation of proteins, some of which are common to both agents, in GH_3 cells (Drust *et al.*, 1982). This suggests that there are certain proteins in the secretory pathways used by agents which use the cyclic AMP and calcium second messenger pathways in prolactin-secreting pituitary tumour cells. One of these proteins may be the site at which cyclic AMP acts to modulate the calcium-activated pathway for prolactin secretion. An alternative explanation would be that cyclic AMP may alter prolactin storage patterns making more prolactin available for release by an increase in cytosolic calcium (Dannies, 1982).

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