Desensitization of tumour Leydig cells by lutropin: evidence for uncoupling of the lutropin receptor from the guanine nucleotide-binding protein

Clive J. DIX, Matthias SCHUMACHER* and Brian A. COOKE

Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, University of London,
8 Hunter Street, London WC1N 1BP, U.K.

(Received 20 October 1981/Accepted 3 December 1981)

Purified rat Leydig tumour cells were pretreated with lutropin and the effect on the subsequent response to lutropin was determined. Maximal cyclic AMP production was achieved with the same concentration of lutropin in control and lutropin-pretreated cells; however, the maximum stimulated level in pretreated cells was only 30% of controls. The sensitivity to lutropin was decreased in lutropin-pretreated cells [ED_{50} (dose that produces a response that is 50% of the maximum response) 60 ± 5.7 ng/ml and 8 ± 1.8 ng/ml (mean $\pm$ s.d., $n = 3$) for controls], as was the rate of maximal cyclic AMP production (0.58, compared with 1.89 pmol/ 10^6 cells per min for controls). However, cholera-toxin-stimulated cyclic AMP production was not decreased by lutropin pretreatment, and a potentiation was seen at all time points studied (up to 6 h). Pre-incubation with lutropin caused a decrease in specific 125 I-labelled human choriogonadotropin binding; however, this decrease was abolished if the cells were washed under acidic conditions (pH 3.0 for 2 min at 4°C), indicating that occupation but not loss of the lutropin receptors had taken place. The effect of pretreating the cells with lutropin on adenylate cyclase activity in purified plasma membranes was also investigated. In plasma membranes from control cells both guanosine 5'-[β , γ -imido]triphosphate [p(NH)ppG] plus lutropin and NaF plus lutropin caused a 50–60-fold linear increase in cyclic AMP production over 40 min compared with 15-fold with p(NH)ppG and 6-fold with lutropin alone. In plasma membranes isolated from lutropin-treated cells the NaF-plus-lutropin- and the p(NH)ppG-stimulated cyclic AMP production rates were unchanged but no effect of lutropin could be demonstrated with or without added p(NH)ppG. In contrast the plasma membranes from dibutyl cyclic AMP-treated cells had similar cyclic AMP production rates to control cells with all stimulants studied. The present evidence obtained from studies both with intact cells and with isolated plasma membranes indicates that the initial lutropin-induced desensitization of the rat Leydig tumour cell is due to a lesion in the hormone-receptor coupling to the guanine nucleotide regulatory protein. This process is apparently not mediated by cyclic AMP.

The initial action of lutropin or choriogonadotropin on the testis Leydig cell is to stimulate steroidogenesis, a response that is mediated through the production of cyclic AMP (Cooke *et al.*, 1976; Dufau & Catt, 1978). However, apart from this stimulatory action, lutropin and choriogonado-

tropin also cause a refractoriness or desensitization of that same steroidogenic response (Catt *et al.*, 1979). This hormone-induced loss of lutropin responsiveness may involve a loss of plasma-membrane receptor for lutropin (Hsueh *et al.*, 1977; Sharpe, 1977; Purvis *et al.*, 1977; Haour & Saez, 1977), an uncoupling of the lutropin receptor from the adenylate cyclase (Saez *et al.*, 1978; Jahnsen *et al.*, 1981), an increase in the metabolism of cyclic AMP due to increased phosphodiesterase activity (Purvis & Hansson, 1978) and a decrease in the

Abbreviation used: p(NH)ppG, guanosine 5'-[β , γ -imido]triphosphate.

* Present address: Abt. für Klin. u. Exp. Endokrinologie, Universitäts-Frauenklinik, Martinstr. 52, 2000 Hamburg 20, Federal Republic of Germany.

activities of some of the enzymes in the steroidogenic pathways from pregnenolone to testosterone (Tsuruhara *et al.*, 1977).

All of these studies have been carried out *in vivo*. To study the mechanisms involved we have investigated the effects of lutropin *in vitro*, using cells from a Leydig cell tumour, which have similar characteristics to normal Leydig cells (Cooke *et al.*, 1979). It was found that exposure of these cells to lutropin caused a decrease in lutropin-stimulated cyclic AMP production, which was followed by an apparent loss of lutropin receptors. These desensitizing effects of lutropin were prevented by the addition of the protein-synthesis inhibitor cycloheximide (Dix & Cooke, 1981). It was subsequently shown (S. N. Levi, C. J. Dix, M. G. Thomas & B. A. Cooke, unpublished work) that adenylate cyclase in plasma membranes prepared from the tumour Leydig cells could be stimulated by lutropin and that this stimulation was enhanced by guanine nucleotides and Mg^{2+} . However, continued exposure of the plasma membranes to GTP [but not the non-hydrolysable analogue p(NH)ppG] caused a desensitization of the adenylate cyclase, which was enhanced by lutropin (S. N. Levi, M. Schumacher, C. J. Dix, M. G. Thomas & B. A. Cooke, unpublished work).

In the present study the site and mechanism of the lesion of lutropin desensitized adenylate cyclase in intact cells has been investigated. The lutropin and cholera-toxin-induced cyclic AMP production of lutropin and dibutyryl cyclic AMP-treated cells and the adenylate cyclase activity of plasma membranes isolated from these cells incubated with a combination of lutropin, p(NH)ppG and NaF were determined. Specific ^{125}I -labelled human choriogonadotropin binding was measured in these pre-treated cells after washing under acidic conditions to remove previously bound lutropin.

Materials and methods

Sheep lutropin (NIH; S20 1.9 i.u. NIH SI/mg) and human choriogonadotropin (NIH CR115) were gifts from the Endocrinology study section, National Institutes of Health, Bethesda, MD, U.S.A. Na ^{125}I (carrier-free) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Methyl-cellulose, ATP (Sigma no. A3377), Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid], phosphocreatine, creatine kinase (132 units/mg), cholera toxin and bovine serum albumin (fraction V) were purchased from Sigma Chemical Co. p(NH)-ppG and N^6O^2' -dibutyryl cyclic AMP were purchased from Boehringer, Mannheim, Germany. Percoll was purchased from Pharmacia Fine Chemicals and 3-isobutyl-1-methylxanthine was

purchased from Aldrich Chemical Co., Milwaukee, WI, U.S.A.

Choriogonadotropin and the 2-*O*-succinyltyrosine methyl ester derivative of cyclic AMP were radioiodinated and characterized as previously described (Dix & Cooke, 1981).

Preparation of tumour Leydig cells

The Leydig tumour used for these studies has been previously characterized (Cooke *et al.*, 1979). The Leydig tumour was grown in Fischer rats and the cells isolated and purified using Percoll density gradients as previously described (Dix & Cooke, 1981) except that the collagenase dispersion step in the isolation procedure was omitted and the tumour fragments were dispersed by repeated aspiration through a 50 ml syringe as described by Schumacher *et al.* (1979) for the dispersion of mouse testis cells. Cells with a density of 1.070 g/ml were used in all studies.

Cell incubation

The purified Leydig cells were suspended in suspension media [Dulbecco's modified Eagles minimum essential medium: 0.1% (w/v) bovine serum albumin, 25 mM-Hepes and 0.1% (w/v) methyl-cellulose (pH 7.4)]. In all experiments cells were pre-incubated with either suspension medium alone or suspension medium containing lutropin in a shaking water bath at 32°C. After pre-incubation the cells were washed twice by centrifugation (200 g for 10 min) and then resuspended in fresh suspension medium and maintained in suspension throughout the course of the experiment. At various times after pre-incubation cells were removed and washed by centrifugation (200 g for 10 min) and then incubated in fresh medium containing 3-isobutyl-1-methylxanthine (0.5 mM) with or without lutropin (0.1–100 µg/ml) or a maximal concentration of cholera toxin (4 µg/ml) for 0–2 h at 32°C. At the end of the incubation period cyclic AMP was extracted from the cells by addition of $HClO_4$ (final concentration 0.5 M) and then neutralized with K_3PO_4 (final concentration 0.23 M). Cyclic AMP was then determined by radioimmunoassay by the method of Steiner *et al.* (1972) modified by the acetylation procedure described by Harper & Brooker (1975).

Where indicated, cells were taken after pre-incubation and incubated for 2 min at 0–4°C in 0.9% saline containing 50 mM-glycine, pH 3.0 or pH 7.4. After this 2 min incubation period the cells were diluted (1:25) with phosphate-buffered saline [0.01 M-phosphate buffer + 0.9% (w/v) NaCl + 0.1% (w/v) bovine serum albumin, pH 7.4], centrifuged (200 g for 10 min), resuspended in phosphate-buffered saline and ^{125}I -labelled choriogonadotropin binding determined using a single concentration

saturation assay the details of which have been described previously (Dix & Cooke, 1981).

Preparation of plasma membranes

The pre-incubated cells were sedimented, resuspended in bicarbonate buffer (1.0mM- NaHCO_3 , 1.0mM-dithiothreitol and 2mM- MgCl_2 , pH 7.0) allowed to swell for 2.5 min and homogenized with five strokes in a Dounce homogenizer with a tight-fitting pestle. The homogenate was immediately brought to iso-osmolarity by the addition of sucrose (final concentration 0.25 M). Plasma membranes were then isolated with the aid of a two-phase poly(ethylene glycol)/Dextran system, which was originally described by Brunette & Till (1971) and which was adapted for the purification of Leydig tumour cell membranes. The washed membranes were finally resuspended in 10mM-Tris/HCl buffer (pH 7.5) plus 1.0mM-dithiothreitol at a concentration corresponding to 0.5 mg of protein/ml (10^6 cells yield about 20 μg of membrane protein). The adenylate cyclase assay was performed immediately.

Adenylate cyclase assay and cyclic AMP determination

Adenylate cyclase activity was analysed by measuring the rate of cyclic AMP production. Incubations were performed at 30°C in a final volume of 50 μl for various times. The standard incubation mixture contained 40mM-Tris/HCl (pH 7.5), 5mM- MgCl_2 , 1mM-EDTA, 0.5mM-isobutylmethylxanthine, 1mM-dithiothreitol, 0.1% bovine serum albumin, 1.0mM-ATP, 10mM-phosphocreatine, creatin kinase (13.2 units/ml) and 5–10 μg of membrane protein. Where indicated the mixture contained lutropin (1 $\mu\text{g}/\text{ml}$) and/or p(NH)ppG (0.1mM) and/or NaF (10mM). The incubation was stopped by the addition of HClO_4 (final concentration 0.5 M). After precipitation of the acid by neutralization with K_3PO_4 cyclic AMP was analysed by radioimmunoassay as described above. Cyclic AMP standards were supplemented with adenylate cyclase assay mixture and treated in the same way as the samples. Cyclic AMP production was expressed in pmol/mg of membrane protein.

Protein determination

Membrane protein was determined by the method of Lowry *et al.* (1951) with the modification described by Markwell *et al.* (1978) and with bovine serum albumin as standard.

Results

Fig. 1 shows the effect of pre-incubation of purified rat Leydig tumour cells with lutropin (1.0 $\mu\text{g}/\text{ml}$) on the subsequent response to increasing concentrations of lutropin. Maximum cyclic AMP

Fig. 1. Effect of lutropin pretreatment of lutropin-induced cyclic AMP production

Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated in the absence (●) or presence (○) of lutropin (1000 ng/ml) for 1 h at 32°C, washed and incubated for a further 1 h at 32°C in the presence of different concentrations of lutropin. The cyclic AMP content was then determined as described in the Materials and methods section. Results are means $\pm$ S.E.M. (represented by the bars) for triplicate determinations.

production was achieved in control and lutropin-pretreated cells with 1 μg of lutropin; however, the maximum stimulated level of pretreated cells was only 30% of that of control cells (Fig. 1). In other experiments (results not shown) this decreased maximum varied between 22 and 40%. The sensitivity to lutropin was decreased in lutropin-pretreated cells with an ED_{50} (dose that produces a response that is 50% of the maximal response) value estimated from dose-response curves of $60.0 \pm 5.7 \text{ ng/ml}$ for pretreated cells compared with $8.4 \pm 1.8 \text{ ng/ml}$ (mean $\pm$ S.D.; $n = 3$) for control cells (Fig. 1). Kinetic studies indicate that the reduced capacity of lutropin-pretreated cells to accumulate cyclic AMP was due to a decreased rate of production (Fig. 2). Although the initial levels of cyclic AMP were higher in pretreated cells the

Fig. 2. *Effect of lutropin pretreatment on the subsequent kinetics of cyclic AMP production*

Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated in the absence (○ and ●) or presence (□ and ■) of lutropin (1000 ng/ml) for 1 h at 32°C, washed and incubated for various times in the absence (○ and □) or presence (● and ■) of lutropin (10 µg/ml) and the cyclic AMP content was determined as described in the Materials and methods section. Results obtained are means ± S.E.M. (represented by the bars) for triplicate determinations.

subsequent basal and lutropin-induced cyclic AMP production was greatly reduced compared with control cells (0.38 and 1.89 pmol/10⁶ cells per min respectively for controls; and 0.08 and 0.58 pmol/10⁶ cells per min respectively for lutropin-pretreated cells). The decreased lutropin-stimulated cyclic AMP production is still evident 6 h after the pre-incubation (Fig. 3), which is in agreement with previous observations (Dix & Cooke, 1981). However, cholera-toxin-stimulated cyclic AMP production is not decreased by lutropin pre-incubation (Fig. 3) and a significant potentiation of the cholera-toxin-stimulated cyclic AMP production can be seen at all time points studied (Fig. 3).

In further experiments, purified Leydig tumour cells were pre-incubated with lutropin or dibutyryl cyclic AMP followed by preparation of the plasma

Fig. 3. *The effect of lutropin pretreatment on lutropin- and cholera-toxin-induced cyclic AMP production*

Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated in the absence (○ and □) or presence (● and ■) of lutropin (1000 ng/ml) for 1 h at 32°C, washed and incubated at 32°C for the times indicated and the lutropin- (○ and ●; 10 µg/ml) and cholera toxin-stimulated (□ and ■; 4 µg/ml) cyclic AMP content was determined as described in the Materials and methods section. Results are means ± S.E.M. (represented by the bars) for triplicate determinations. Basal cyclic AMP content has been subtracted from all the values.

membranes. The adenylate cyclase activity of the plasma membranes in the presence of lutropin, p(NH)ppG, lutropin plus p(NH)ppG and NaF plus lutropin were determined. In addition the lutropin- and cholera-toxin-stimulated cyclic AMP production of the intact cells with and without acid washing were determined. In these experiments the cells were pre-incubated for 2 h with a higher concentration of lutropin (10 µg/ml) than in previous experiments. Under these conditions, although the mean cholera-toxin-stimulated cyclic AMP production was consistently higher in the lutropin-pretreated cells these values were not significantly different from control values (Tables 1 and 2; $P > 0.05$). In agreement with the previous experiments with lutropin pretreatment, there was no decrease in the cholera-toxin-stimulated cyclic AMP production, whereas there was a decrease in the subsequent lutropin-stimulated cyclic AMP production (Tables 1 and 2). Pre-incubation with dibutyryl cyclic AMP (2 mM) had no effect on either lutropin or cholera-toxin-stimulated cyclic AMP production (Table 2). The specific ¹²⁵I-labelled choriogonadotropin binding was decreased to approx. 5% of that of control cells

Table 1. *The effect of lutropin on the lutropin-receptor content and the cyclic AMP production of Leydig tumour cells* Leydig tumour cells prepared as described in the Materials and methods section were pre-incubated with or without lutropin (10.0 µg/ml) for 2 h at 32°C. Cells were then centrifuged (200 g for 10 min) and incubated in 0.9% NaCl/50 mM-glycine at 0–4°C for 2 min at pH 3.0 or 7.4. The cells were then washed in phosphate-buffered saline (pH 7.4) and specific ¹²⁵I-labelled human choriogonadotropin binding and basal and lutropin- (10 µg/ml) and cholera-toxin- (4 µg/ml) stimulated cyclic AMP production were determined. Results are means ± S.E.M. (n = 3).

Pretreatment	Washing conditions (pH)	Specific ¹²⁵ I-labelled human choriogonadotropin binding (fmol/10 ⁶ cells)	Basal cyclic AMP production (pmol/10 ⁶ cells per 2 h)	Lutropin-stimulated cyclic AMP production (pmol/10 ⁶ cells per 2 h)	Cholera-toxin-stimulated cyclic AMP production (pmol/10 ⁶ cells per 2 h)
NaCl	7.4	7.3 ± 0.7	4.5 ± 0.5	193.9 ± 3.0	178.4 ± 1.6
NaCl	3.0	5.9 ± 0.9	3.3 ± 0.5	190.6 ± 12.7	199.8 ± 18.9
Lutropin	7.4	0.4 ± 0.1	24.1 ± 0.7	71.1 ± 7.2	217.5 ± 6.8
Lutropin	3.0	8.4 ± 0.9	9.0 ± 0.7	70.3 ± 14.7	169.1 ± 14.2

Table 2. *The effect of lutropin and dibutyryl cyclic AMP pretreatment on cyclic AMP production* Leydig cells prepared as described in the Materials and methods section were pre-incubated with lutropin (10 µg/ml), dibutyryl cyclic AMP (2 mM) or saline for 2 h at 32°C. Cells were then washed twice in suspension medium and finally resuspended in suspension medium containing isobutylmethylxanthine (0.5 mM) and the basal and lutropin- (10 µg/ml) and cholera-toxin- (4 µg/ml) stimulated cyclic AMP production were determined. Results are means ± S.E.M. (n = 3).

Pretreatment	Cyclic AMP production (pmol/10 ⁶ cells per 2 h)		
	Basal	Lutropin treatment	Cholera toxin treatment
Control	2.1 ± 0.2	253.1 ± 34.3	260.3 ± 24.8
Dibutyryl cyclic AMP	14.5 ± 1.5	214.4 ± 8.5	248.9 ± 22.6
Lutropin	43.5 ± 4.3	65.9 ± 0.5	281.5 ± 14.0

Fig. 4. *Effect of pretreatment of Leydig tumour cells on the plasma-membrane adenylate cyclase activity* Purified rat Leydig tumour cells were purified as described in the Materials and methods section and incubated for 2 h at 32°C in the presence of (a) suspension media, (b) lutropin (10 µg/ml) and (c) dibutyryl cyclic AMP (2 mM). Cells were then washed and plasma membranes prepared and the basal (●), lutropin- (○; 1 µg/ml), p(NH)ppG- (▲; 0.1 mM), lutropin- (1 µg/ml) plus p(NH)ppG- (0.1 mM) (△) and the fluoride- (10 mM) plus lutropin- (1 µg/ml) (■) stimulated cyclic AMP production determined as described in the Materials and methods section. Results are means ± S.E.M. (represented by the bars) for triplicate determinations.

by pre-incubation with lutropin (Table 1). This decrease was abolished if the cells were washed under acidic conditions (pH 3.0 for 2 min at 0–4°C) (Table 1). Although this acid-washing procedure reversed

the lutropin-induced decrease in specific ¹²⁵I-labelled human choriogonadotropin binding it had no effect on the lutropin-induced decrease in lutropin-stimulated cyclic-AMP production of these cells (Table 1).

However, the slight potentiation in cholera-toxin-stimulated cyclic AMP production seen in lutropin-pretreated cells was reversed by this acid-washing procedure (Table 1).

The results with purified plasma membranes from lutropin and dibutyryl cyclic-AMP pretreated cells are consistent with the results from intact cells. In plasma membranes from control cells both lutropin plus p(NH)ppG and NaF plus lutropin caused a 50–60-fold linear increase in cyclic AMP production over 40 min compared with 15-fold with p(NH)ppG and 6-fold with lutropin alone (Fig. 4a). In the plasma membranes isolated from lutropin-pretreated cells the p(NH)ppG- and NaF-stimulated cyclic AMP production rates were not significantly different from those of control plasma membranes, but no effect of lutropin could be demonstrated with or without the addition of p(NH)ppG (Fig. 4b). The plasma membranes from the dibutyryl cyclic AMP-pretreated cells had similar cyclic AMP production rates to the control cells with all the stimulants studied (Fig. 4c).

Discussion

Evidence obtained previously from studies *in vivo* suggested that a lesion between the lutropin receptor and the adenylate cyclase moiety develops in the rat testis Leydig cell after administration of human choriogonadotropin (Tsuruhara *et al.*, 1977; Saez *et al.*, 1978; Jahnsen *et al.*, 1981). Similar conclusions were reached with ovarian cells (Hunzicker-Dunn & Birnbaumer, 1976; Lamprecht *et al.*, 1977). The work carried out in the present study defines more clearly the site of this lesion as being between the guanine nucleotide-binding protein and the lutropin receptor under conditions where no loss in lutropin receptor sites occurred.

In the work of Tsuruhara *et al.* (1977) it was observed that cyclic AMP responses to cholera toxin were retained in testis interstitial cells from human choriogonadotropin-treated rats. In these studies the response to cholera toxin was measured 2 days after human choriogonadotropin treatment at a time when there had been a decrease in lutropin receptor levels. However, with the lowest dose of human choriogonadotropin used (0.2 µg) that caused a loss in lutropin receptors there had been no decrease in the response to human choriogonadotropin with respect to cyclic AMP production, indicating that the hormone receptors were still able to couple to the guanine nucleotide regulatory protein. In contrast, in the present study it has been shown that, under conditions where the apparent number of hormone-binding sites is unaltered, lutropin produced a decrease in lutropin stimulation of adenylate cyclase in both intact cells and purified plasma membranes, whereas the cholera toxin response of intact cells

and the fluoride and p(NH)ppG responses of purified plasma membranes were not decreased.

Although the mechanism of receptor–cyclase coupling is not fully understood, a guanine nucleotide-dependent regulatory protein is known to be involved (Ross & Gilman, 1980). Since it has been shown that cholera toxin exerts its action through ADP ribosylation of this regulatory protein (Gill, 1977; Cassel & Pfeuffer, 1978) and p(NH)ppG activates the adenylate cyclase through this regulatory protein, the present results clearly demonstrate that the coupling between the regulatory protein and the adenylate cyclase moiety is intact in lutropin-desensitized cells. They furthermore suggest that the lesion lies at, or proximal to, the coupling of the guanine nucleotide regulatory protein to the lutropin receptor. Such a conclusion is in agreement with those of other workers investigating the isoprenaline-induced desensitization of S49 lymphoma cells (Iyengar *et al.*, 1981; Green & Clark, 1981). This lesion may be necessary before the internalization of the hormone–receptor complex can occur (Ascoli & Puett, 1977; Conn *et al.*, 1978).

Although little is known of the molecular events that cause desensitization, Hunzicker-Dunn *et al.* (1979) and Ezra & Salomon (1980) have suggested that desensitization of the ovarian adenylate cyclase system by lutropin involves a phosphorylation reaction. It is of interest to speculate that lutropin-induced desensitization of rat Leydig tumour cells involves phosphorylation either of the lutropin receptor or of the guanine nucleotide-dependent regulatory protein, and that this phosphorylation impairs the coupling between the hormone receptors and the regulatory protein. However, in the present experiments dibutyryl cyclic AMP did not cause desensitization of the Leydig cells and therefore if a phosphorylation step is involved it appears not to be cyclic AMP-mediated. Experiments carried out with isolated Leydig cell tumour plasma membranes suggest that it may be GTP-mediated (S. N. Levi, M. Schumacher, C. J. Dix, M. G. Thomas & B. A. Cooke, unpublished work). In contrast in mouse Leydig cells (Schumacher *et al.*, 1981) gonadotropin-induced desensitization of the cyclic AMP response appears to be a cyclic AMP-mediated event.

A further point of interest is that lutropin-desensitized cells show an increased cholera-toxin-stimulated cyclic AMP production (Tsuruhara *et al.*, 1977; the present work). Similar potentiation of isoprenaline-stimulated cyclic AMP production in S49 lymphoma cells by cholera toxin has been reported (Insel *et al.*, 1981). Dufau *et al.* (1980) have shown that lutropin causes an increase in the availability of the binding sites for GTP on the regulatory protein in rat testicular cell membranes. Such an increase in GTP-binding sites may explain

the potentiation of the action of cholera toxin in lutropin-desensitized Leydig tumour cells and the loss of this potentiation when the bound lutropin is removed.

In conclusion, the present study clearly indicates that the initial events of lutropin-induced desensitization involve an uncoupling of the lutropin receptor from the guanine nucleotide-dependent regulatory protein before any loss of lutropin receptors occurs. Whether these events are receptor specific or whether there is a reduced sensitivity to other ligands in the lutropin-desensitized plasma membranes has yet to be determined, along with the detailed molecular events of this process. However, the ability to prepare large numbers of pure cells and pure plasma membranes from the tumour cells used should facilitate the elucidation of these molecular events.

We are grateful to the Wellcome Trust for financial support and the National Pituitary Agency, Bethesda, MD, U.S.A., for gifts of ovine lutropin and human choriogonadotropin. M. S. was supported by a grant from the Deutsche Forschungsgemeinschaft, SFB 34.

References

- Ascoli, M. & Puett, D. (1977) *FEBS Lett.* **75**, 77–82
- Brunette, D. M. & Till, J. E. (1971) *J. Membr. Biol.* **5**, 215–224
- Cassel, D. & Pfeuffer, T. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2669–2673
- Catt, K. J., Harwood, J. P., Aguilera, G. R. & Dufau, M. L. (1979) *Nature (London)* **280**, 109–116
- Conn, P. M., Conti, M., Harwood, J. P., Dufau, M. L. & Catt, K. J. (1978) *Nature (London)* **274**, 598–600
- Cooke, B. A., Lindh, M. & Janszen, F. H. A. (1976) *Biochem. J.* **160**, 439–446
- Cooke, B. A., Janszen, F. H. A., van Driel, M. J. A. & van der Molen, H. J. (1979) *Biochim. Biophys. Acta* **583**, 320–331
- Dix, C. J. & Cooke, B. A. (1981) *Biochem. J.* **196**, 713–719
- Dufau, M. L. & Catt, K. J. (1978) *Vitam. Horm. (N.Y.)* **36**, 461–592
- Dufau, M. L., Baukal, A. S. & Catt, K. J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5837–5841
- Ezra, E. & Salomon, Y. (1980) *J. Biol. Chem.* **255**, 653–658
- Gill, D. M. (1977) *Adv. Cyclic Nucleotide Res.* **8**, 85–118
- Green, D. A. & Clark, R. B. (1981) *J. Biol. Chem.* **256**, 2105–2108
- Haour, F. & Saez, J. M. (1977) *Mol. Cell. Endocrinol.* **7**, 17–24
- Harper, J. F. & Brooker, G. (1975) *J. Cyclic Nucleotide Res.* **1**, 207–218
- Hsueh, A. J. W., Dufau, M. L. & Catt, K. J. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 592–595
- Hunzicker-Dunn, M. & Birnbaumer, L. (1976) *Endocrinology* **99**, 185–197
- Hunzicker-Dunn, M., Derda, D., Jungmann, R. A. & Birnbaumer, L. (1979) *Endocrinology* **104**, 1785–1793
- Insel, P. A., Darfler, F. J. & Kennedy, M. S. (1981) *FEBS Lett.* **128**, 59–62
- Iyengar, R., Bhat, M. K., Riser, M. E. R. & Birnbaumer, L. (1981) *J. Biol. Chem.* **256**, 4810–4815
- Jahnsen, T., Purvis, K., Torjesen, P. A. & Hansson, V. (1981) *Arch. Androl.* **6**, 155–162
- Lamprecht, S. A., Zor, U., Salomon, Y., Koch, Y., Ahren, K. & Lindner, H. R. (1977) *J. Cyclic Nucleotide Res.* **3**, 69–83
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Markwell, M. A. K., Haas, S. M., Breber, L. L. & Tolbert, N. E. (1978) *Anal. Biochem.* **87**, 206–210
- Purvis, K. & Hansson, V. (1978) *Arch. Androl.* **2**, 89–91
- Purvis, K., Torjesen, P. A., Haug, E. & Hansson, V. (1977) *Mol. Cell. Endocrinol.* **8**, 73–80
- Ross, E. M. & Gilman, A. G. (1980) *Annu. Rev. Biochem.* **49**, 533–564
- Saez, J. M., Haour, F. & Cathiard, A. M. (1978) *Biochem. Biophys. Res. Commun.* **81**, 552–558
- Schumacher, M., Schäfer, G., Lichtenberg, V. & Hülz, H. (1979) *FEBS Lett.* **107**, 398–402
- Schumacher, M., Schwarz, M., Lichtenberg, V. & Pahnke, V. (1981) *Acta Endocrinol.* **97**, Suppl. **243**, abstr. 384
- Sharpe, R. M. (1977) *Biochem. Biophys. Res. Commun.* **75**, 711–717
- Steiner, A. L., Parker, C. W. & Kipnis, D. M. (1972) *J. Biol. Chem.* **247**, 1106–1113
- Tsuruhara, T., Dufau, M. L., Cigorraga, S. & Catt, K. J. (1977) *J. Biol. Chem.* **252**, 9002–9009