SHORT TERM KINETICS OF LUTEINIZING HORMONE SECRETION STUDIED IN DISSOCIATED PITUITARY CELLS ATTACHED TO MANIPULABLE SUBSTRATES

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ABSTRACT

With the use of poly-L-lysine, a method has been developed which induces acutely dissociated rat anterior pituitary cells to attach to glass and polyacrylamide surfaces. In these attached cells the recovery of the secretory response, which is impaired in acutely dissociated cells, has been followed, and it has been established that, in terms of their ability to secrete luteinizing hormone (LH) in response to the specific secretogogue luteinizing-hormone-releasing hormone (LHRH), the cells become maximally responsive after 48 h. The attached cells also allow the short-term kinetics of LH secretion to be followed with great facility; and, when cells allowed to recover for 48 h are used, it is shown that in response to LHRH the pattern of LH release is biphasic.

Dissociated cell preparations of the rat anterior pituitary have proved very useful for the study of stimulation secretion coupling because they provide a stable in vitro preparation comprised of multiple, functionally representative samples (11, 21, 24). Experience has shown, however, that the usefulness of these preparations would be further improved if, while they recover from the injurious effects of the dissociation procedure (6, 11), the dissociated cells could be (a) discouraged from reaggregating and (b) induced to attach to a manipulable substrate. These two modifications would maintain the advantage of direct access to the cell surface which is otherwise only available in the acute suspension, and they would also allow rapid and frequent changes in the environment, a prerequisite for studies on the short-term kinetics of secretory product release.

In work reported here I have found that the polycation poly-L-lysine (PL) can be used to induce acutely dissociated cells to attach and establish long-term contact with glass and certain other surfaces without significant impairment of their secretory function. I have used this device to follow the recovery of the dissociated cells in terms of their sensitivity to luteinizing-hormone-releasing hormone (LHRH) and to demonstrate, for the first time, that the pattern of luteinizing hormone (LH) release in response to LHRH stimulation is biphasic.

MATERIALS AND METHODS

Chemicals

The chemicals used were normally of analytical grade and obtained from either Sigma (London) Chemical Co., London, U.K., or British Drug Houses, Ltd., Poole, Dorset, U.K. The ionophore A23187 was generously supplied by Lilly Research Laboratories, Windlesham, Surrey, U.K.

Animals

Either male or female Sprague Dawley rats, 200 g in weight, were used. No attempt was made to record or synchronize the estrus cycle in the females.

Methods

CELL DISSOCIATION AND INCUBATION: Pituitaries were removed, chopped into small blocks, and dissociated either as described previously (11) or as described by Hymer et al. (13). The cell suspensions which were usually free of cell aggregates were freed from debris by centrifugation at 400 g through 4% bovine serum albumin. They were then counted with a hemocytometer and assessed for viability with trypan blue. Routinely, 1.75 × 10⁶ and 2.5 × 10⁶ cells/pituitary were obtained from males and females, respectively, and the viability was normally better than 95%. For postdissociation incubation of 1 h or longer, the cells were plated out in 5-cm diameter petri dishes (Sterilin Ltd., Richmond, Surrey, U.K.) in Dulbecco's medium essential medium (DMEM) (Gibco Bio-Cult Ltd., Paisley, U.K.) supplemented with 12.5% fetal calf serum, and incubated at 37°C in 5% CO_2/95% O_2.

For stimulation with LHRH (synthesized according to the sequence published by Matsuo et al. (18) by Dr. H. Gregory, I.C.I. Pharmaceuticals, Macclesfield, Cheshire, U.K.), the cells were washed with three rinses of DMEM minus serum plus 0.1% gelatin (DMEM/gel) before being incubated at 37°C in well-gassed DMEM/gel containing the peptide but lacking serum.

57 mM potassium medium was prepared by modifying the balanced salt component of Krebs-Ringer bicarbonate (23).

COLLECTION OF MEDIA AND ASSAY OF LH: Media collected from petri dishes or columns were centrifuged at 400 g for 15 min, decanted, and stored at −20°C until assayed. For assay of the hormone content of cells attached to dishes, 1 ml of 1 mM EDTA/0.25% ovalbumin was added to the drained dishes, and the dishes were frozen to −20°C. The dishes were then thawed, the cells were scraped from the surface, and the broken cell suspension was centrifuged at 400 g for 5 min. The supernate was collected and stored for assay. Cells attached to beads were treated similarly, being removed from the beads after freezing by vigorous pipetting.

LH in media and cell extracts was measured by the NIAMDD homologous double antibody radioimmunoassay (20). NIAMDD rat LH RP-1 was used as standard and Goat-anti-rabbit FAB as precipitating antibody. (The Fab fragment of anti-rabbit γ-globulin used as antigen was kindly provided by Dr. J. P. Kraehenbuhl, Swiss National Cancer Institute, Lausanne, Switzerland). The assay has a sensitivity of 2.65 ± 0.34 (SD) ng of NIAMDD rat LHRP-1 and an interassay variation of less than 10% (n = 50).

ATTACHING CELLS TO PL-COATED DISHES: 5 cm plastic petri dishes were coated with PL by rinsing them sequentially with 1 mg/ml aqueous PL and water and then allowing them to dry. Cells added in suspension in DMEM rapidly settled out and attached to the dish surface. To encourage cell attachment to the PL, suspensions were washed free of carrier protein before addition

to the dish, and serum was usually added only after the cells had attached.

PREPARATION OF POLY-L-LYSINE-COATED BEADS: Poly-L-lysine (PL) (Type 1-B, mol wt 70,000+, Sigma [London]) was covalently attached to Biogel P6 beads (minus 400 mesh) (Bio-Rad Laboratories, St. Albans, Herts, England) by way of an acyl azide Biogel intermediate according to the methods of Inman and Dintzis (14). 35 ml of preswollen gel were incubated with 15 ml of hydrazine hydrate at 55°C for 6 h and then washed overnight with 0.1 M NaCl. At 5°C the acid azide groups were generated with 1.0 M sodium nitrite in 0.6 N HCl until spot testing with sodium trinitrobenzene sulfonate for acyl azide groups indicated that activation was complete. 10 g (wet weight) of derivatized gel was then briefly washed with ice-cold 0.3 N HCl before being resuspended in 0.1 M sodium tetraborate containing 50 mg of PL and brought to pH 9.0 with 0.1 N NaOH. Incubation by inversion in a capped polycarbonate tube followed for 48 h at 5°C before the gel was washed free of unbound PL by repeated centrifugation with alternate washes of 0.15 M NaCl and distilled water. The efficiency of the attachment of PL and of the washing procedure was monitored with PL iodinated by the active ester method of Bolton and Hunter (2). 10,000 dpm of [I¹²⁵]PL was added to the polycarbonate tube at the beginning of the incubation and indicated that routinely 4 mg of PL (~80%) was bound per gram wet weight of beads.[1]

Sepharose 4B (Pharmacia G.B., Ltd., London, U.K.) was activated with cyanogen bromide and reacted with PL in 0.25 M NaHCO₃ as described by Porath et al. (22).

Before use, the derivatized gels were washed exhaustively with DMEM.

Attaching Cells to Derivatized Beads

Cells and beads can be brought into contact and will attach if they are mixed either in a petri dish agitated on a rotary shaker or in a capped polycarbonate tube subjected to slow inversion (2 rpm). However, the most effective way of attaching the cells is to allow them to percolate through a column of derivatized beads. Routinely, a 2-ml packed bed of gel supported in an inverted 2-ml syringe barrel by two discs of N10 nylon gauze was used. The column bed is equilibrated with DMEM minus serum, and the cell suspension (20 × 10⁶ cells/ml) is slowly added above the gel bed. The rate of flow is controlled by a Portex tap (700/321/010 Portex Ltd., Hythe, Kent, U.K.) fitted to the syringe barrel, and cell loss from the column is followed by collecting the eluent in centrifuge tubes, centrifuging for 15 min at 400 g and examining the pellet. Up to 40 × 10⁶ cells can be loaded on a 2-ml column of derivatized beads and washed with

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[1] When beads are derivatized with PL at concentrations below 0.5 mg of PL/g wet weight, cell loading is low and unsatisfactory.

30 ml of DMEM without significant cell loss. Sampling of the gel after loading always shows some unattached cells and a significant number of beads free of cells. By collecting the beads from the column, allowing them to settle out in excess medium and counting the free cells in the supernate, it is estimated that optimally 60% of the loaded cells can be attached to the beads.

For overnight incubation the column is equilibrated with DMEM + serum, and the beads bearing cells are transferred to petri dishes. Alternatively, the preparation can be maintained overnight in the column, although under static conditions the environment within the gel bed deteriorates and care must be taken to use fully gassed medium. Precise estimates of the viability of attached cells are not easily made, but, while the number of attached cells on derivatized polyacrylamide beads that take up trypan blue seems only marginally increased after 1- and 2-day incubations, the majority of those cells on derivatized Sepharose 4B are dead within 24 h.

ELECTRON MICROSCOPY: Cells were prepared for electron microscopy by being fixed *in situ* with Karnovsky's formaldehyde-glutaraldehyde (16). Where they were adherent, the cells were gently scraped off with a polythene policeman or detached by vigorous pipetting. All subsequent treatment thereafter was carried out as described by Hopkins and Farquhar (11).

IODINATION OF LHRH: The peptide was iodinated by the lactoperoxidase oxidation method described by Marshall and Odell (17). The ^{125}I-LHRH was then separated from free I^{125} and unlabeled peptide by car-

boxymethyl cellulose chromatography (17). The biological activity of the labeled peptide was not evaluated.

RESULTS

Efficiency of Attachment, Viability and the Secretory Response to LHRH in Cells Attached to PL-Coated Petri Dishes

Cells in suspension was aliquoted into uncoated and PL-coated dishes and incubated for 18 h with DMEM + serum (Fig. 1). After incubation the medium plus any floating cells was collected and centrifuged to separate floating cells from the medium. Viability (trypan blue) and hormone content (LH by radio-immunoassay) were estimated in (a) attached cells and (b) floating cells while the medium (supernate) was assayed for hormone only. Table I shows the results obtained. The viability of attached cells in both PL-coated and uncoated dishes was about the same, and thus the hormone content was used as an estimate of cell number. On this basis, it was evident that, in comparison with uncoated dishes, at least twice as many cells were attached to the PL-coated surface. It should also be noted that most of the unattached cells in the PL-coated dishes were dead
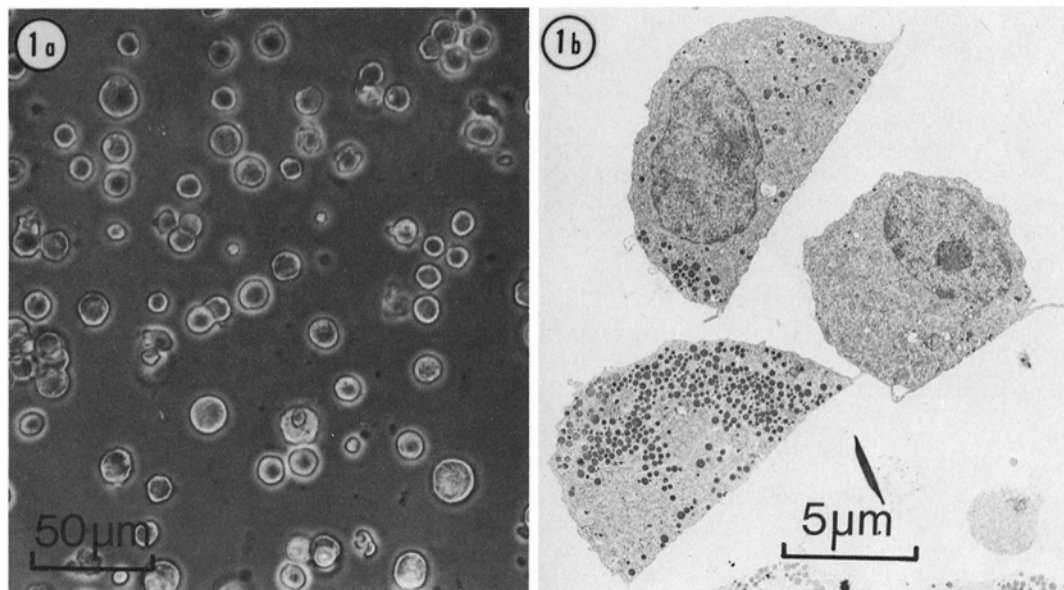

FIGURE 1 (a) Light micrograph of isolated cells attached to PL-coated surface and incubated for 24 h postdissociation. Phase-contrast optics. × 320. (b) Electron micrograph of section showing cells fixed *in situ* on PL-coated surface having been incubated for 24 h postdissociation. Note the flattened surfaces indicating areas of attachment. × 3,500.

TABLE I

Distribution of Luteinizing Hormone in Attached and Floating Cells and in the Medium of Preparations Derived from Male Donor Rats and Incubated for 18 h on Uncoated and PL-Coated Dishes

	Uncoated dishes (ng dish)	PL-coated dishes (ng dish)
Attached cells	15,200 ± 1,300	28,400 ± 1,900
Floating cells/debris	12,200 ± 500	8,700 ± 1,000
Medium (ng/ml)	12,150 ± 1,200	12,600 ± 1,250

Cells plated out at 4 × 10⁶/dish. Medium content ± SEM.

whereas those in the uncoated dishes showed good viability. On PL-coated dishes the attached cells contained more than 50% of the total LH.

Adherent cells can be displaced from PL-coated dishes within 2 h of attachment by 100 μg/ml polyglutamic acid, indicating that their initial attachment to PL relies primarily upon electrostatic interactions between the polycation and the negatively charged cell surface components. After 18-h incubation, attempts to remove adherent cells from the uncoated dishes by gentle pipetting reduced their viability to about 20% while attempts to remove the cells from PL-coated dishes after a similar interval by incubating them with polyglutamic acid induced only a few, already dead, cells to detach. Detachment of cells from PL-coated dishes by vigorous pipetting markedly reduced their viability (to about 10%) although trypsin treatment (100 μg/ml) succeeded in providing a free suspension of rounded cells with better than 90% viability.

Log dose responses to LHRH were obtained with cells plated either onto uncoated dishes or onto PL-coated dishes and incubated for 4 days. In both preparations, there was less than a 10% variation between replicate dishes, and in both preparations a half-maximal response and a maximal response were observed with doses of 2 × 10^{-9} and 10^{-7} M LHRH, respectively.

Response to LHRH with Time after Dissociation

The early attachment of dissociated cells induced by PL allows their responsiveness to LHRH to be measured within hours of dissociation, and thus the recovery of their secretory response can be readily monitored.

Cells were aliquoted from suspension at 4 × 10⁶/ dish onto PL-coated dishes and incubated for 12-98 h in DMEM + serum. A 24-h postdissociation incubation period is required before the variation

in response between replicates is below 10% and a sigmoidal log-dose response curve can be obtained (Fig. 2). After a 24-h interval, the dose response curve shows a half maximal dose at 1.5 × 10^{-8} M LHRH and a maximal level at 10^{-6} M. At 48 h and 96 h, the shape and slope of the curve remain unchanged, but the half maximal dose decreases to become optimum at 48 h (8 × 10^{-10} M LHRH) before marginally increasing to 2 × 10^{-9} M at 96 h. The response is maximal at 10^{-7} M LHRH at 48 h and remains unchanged at 96 h and up to 192 h (data not shown). After 96 h, however, the maximal amount of LH released from the preparation (between 16- and 18.5-fold over the period 24-96 h) is reduced within any single experiment by between 8- and 12-fold. In a representative experiment, the amount of LH maximally released per dish (4 × 10⁶ cells plated) at 48 h by 10^{-7} M LHRH during the 4-h period was 8 μg of a total of 80-96 μg.

Response with Time of Cells on PL-Coated Dishes to 57 mM Potassium

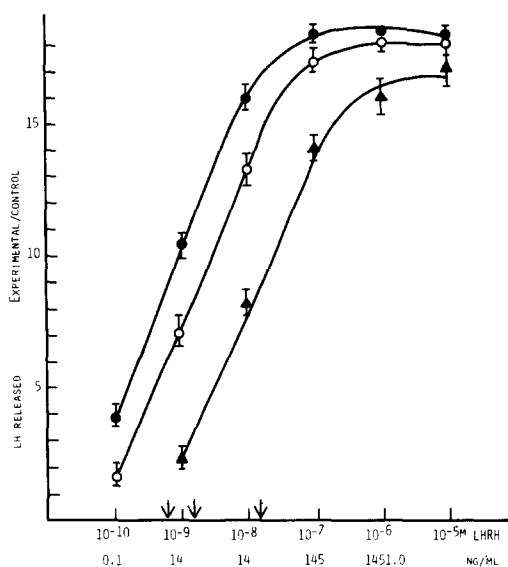Since the results with LHRH indicated that a period of about 48 h is required for the prepara-

FIGURE 2 Dose response curve for cells on PL-coated dishes incubated for varying periods of time postdissociation. At 24 h (▲—▲), 48 h (●—●) and 96 h (○—○), the preparations were washed free of DMEM + serum and incubated for 4 h with DMEM/gel containing LHRH. Results are expressed as ±SEM. Glands were obtained from male donors.

tion to maximally recover from the effects of the dissociation procedure, it was of interest to examine the ability of the preparation to release LH in response to a secretogogue that can influence LH release without requiring the direct participation of LHRH (23). The results obtained with 57 mM K^+ are given in Table II. They indicate that while LH release in response to this secretogogue is also impaired in acutely dissociated cells, the capacity to release LH is maximally recovered within 24 h.

Short-Term Kinetics of LH Release in Response to LHRH in Cells Attached to Polyacrylamide Beads

The facility with which the flow of medium through a column of beads bearing cells can be monitored should prove useful both for studies concerned with the kinetics of secretory product release and for quantitating interactions between secretogogues and the cell surface. To date, I have examined the release of LH with time in response to stimulation with LHRH. Figs. 3 and 4 show the form and arrangement of the attached cells.

To follow the short-term kinetics of LH release in response to LHRH, beads bearing approx. 20–30 × 10^6 cells were packed at 48-h postdissociation into a short column as described in Materials and Methods.

Stimulation was initiated by introducing 10^{-8} LHRH into the eluent of DMEM/gel and at the same time introducing a 100-μl pulse sample of I^{125} LHRH. The profile of radioactivity served to indicate the beginning of stimulation and, as seen in Fig. 5, an increase in the release of radio-immunoassayable LH was observed within 1 min of the I^{125} LHRH pulse becoming half maximal. This initial increase in LH release became maximal at 2 min and was followed by a sharp decline almost to prestimulation levels at 6 min. By 10 min, however, the rate of LH release increased again, albeit more slowly, until it became maximal at 90 min. This pattern of biphasic release was

consistently observed for LH release, and similar kinetics for the release of follicle-stimulating hormone have also been observed (my unpublished observations). An indication of a biphasic pattern of release was also evident when 48-h cells attached to petri dishes were used. However, so far it has not been possible to obtain a similar response with cells incubated for 96 h postdissociation. At 96 h, only a single phase which temporally corresponds with the second phase obtained with 48-h cells was observed.

LH Release in Response to LHRH over a 5-h Period

Figure 6 shows the pattern of LH release over a 5-h period with media collected at 30-min intervals from cells incubated for 48 h in PL-coated petri dishes. Only the second phase of release was observed with this protocol, but it nevertheless served to illustrate, under these conditions of continuous stimulation, that once LH release reaches a maximum in this phase it again declines to prestimulation levels. After 4-h stimulation with 10^{-7} M LHRH, no further increase in LH release above unstimulated levels can be obtained even though assay of LH content of the cells indicates that less than 10% of the total LH has been released (Table III). Also shown in Table III are the results obtained when cells that have been stimulated with LHRH for 4 h are stimulated with other potent secretagogues of LH release. Since all these agents induced release, I conclude that the preparation becomes refractory to LHRH stimulation at an early step in the sequence of stimulation-secretion coupling, probably at the level at which the peptide interacts with its receptor.

DISCUSSION

Tryptic digestion of the cell surface during dissociation clearly removes a significant proportion of plasma membrane glycopeptides and may also inflict intracellular damage on the isolated cells (10).

TABLE II

LH released in Nanogram/Milliliter with Time after Dissociation in Response to 10^{-7} M LHRH and 57 mM Potassium

	Acute	24 h	48 h
DMEM	1,870 ± 1,000	1,270 ± 400	1,240 ± 150
10^{-7} M LHRH	1,500 ± 85	8,500 ± 1,750	15,400 ± 1,200
57 mM Potassium	1,180 ± 150	5,000 ± 600	4,450 ± 660

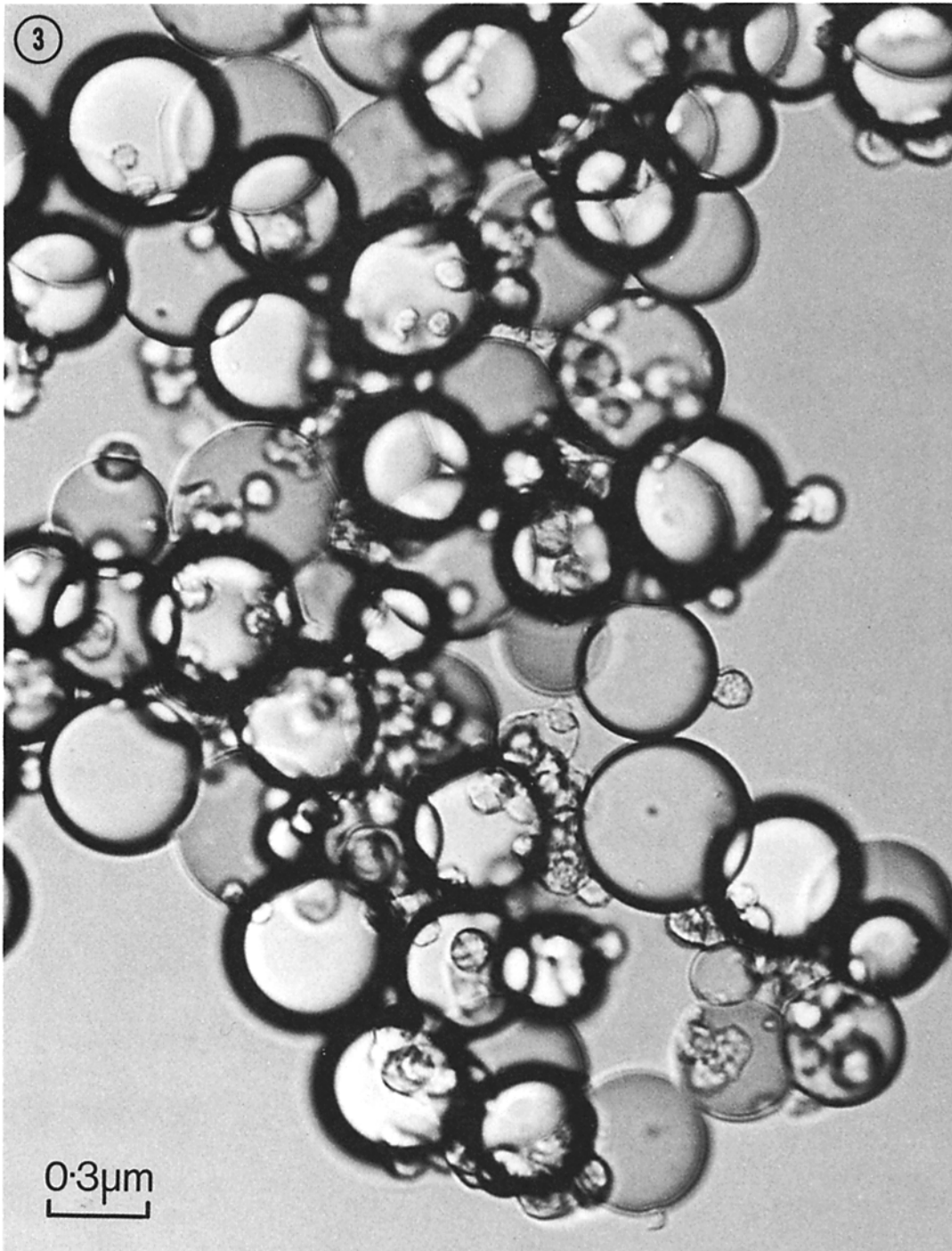Cells plated out at 8 × 10^6/dish. Medium content ± SEM.

FIGURE 3 Light micrograph showing derivatized beads bearing cells. The acutely dissociated cells were loaded onto a column of PL-derivatized Biogel beads, removed and incubated overnight in a petri dish with DMEM + serum. × 50.
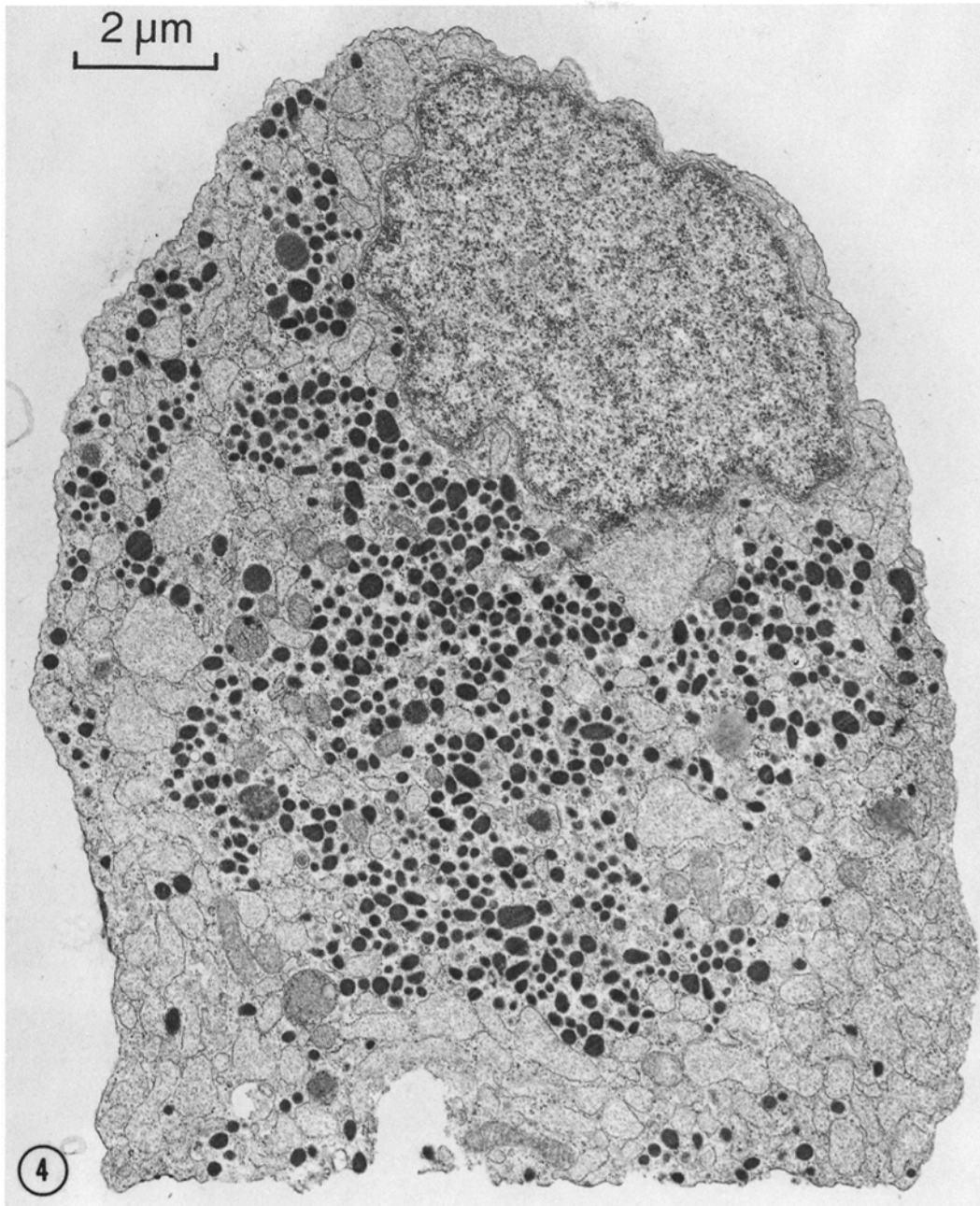
FIGURE 4   Electron micrograph of a gonadotrophin cell from a preparation in which the cells were fixed *in situ* on the surface of derivatized beads. The flattened base of the cell shows the area of attachment. Rupture of the membrane in the attachment area probably occurred when the fixed cell was dislodged from the bead surface. Otherwise, the fine structural organization is typical of this cell type. × 10,000.

However, the good viability and stability of pituitary cells in culture shows that, with time, cells are able to recover from these injurious effects, and, for optimum sensitivity in terms of their ability to respond to LHRH stimulation, my results indicate that a recovery period of at least 48 h is required.
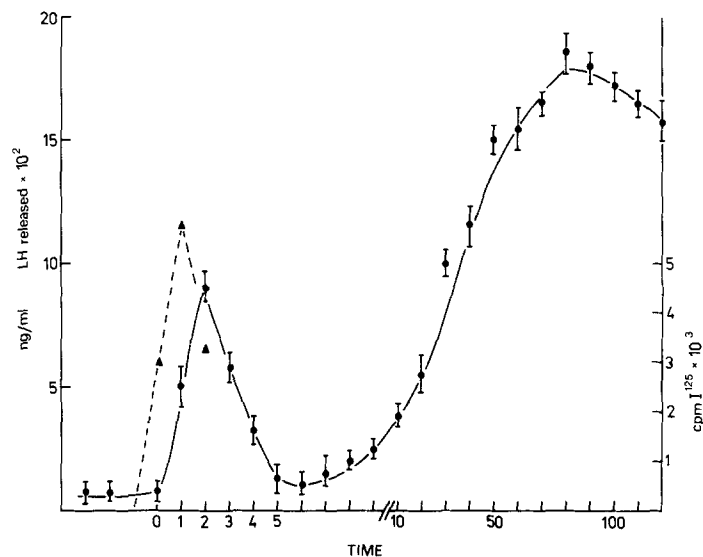
FIGURE 5 Kinetics of LH release from cells attached to PL-derivatized beads in response to 10^{-8} M LHRH. Beads bearing approx. 30×10^6 cells were loaded to form a 0.5×2.0-cm column bed, and after washing for 30 min (when the LH content was reduced to less than 25 ng/ml), 10^{-8} M LHRH + 100 μl ^{125}I LHRH was included in the perfusate. The radioactivity of the iodinated peptide (▲—▲) serves to indicate the time at which stimulation is initiated; there is a 1-min lag between the time ^{125}I LHRH and the time the LH released in response to LHRH become half maximal. The pattern of LH release is biphasic with peaks at 2 and 90 min. Flow rate is 1 ml/min. Glands were obtained from male donors.
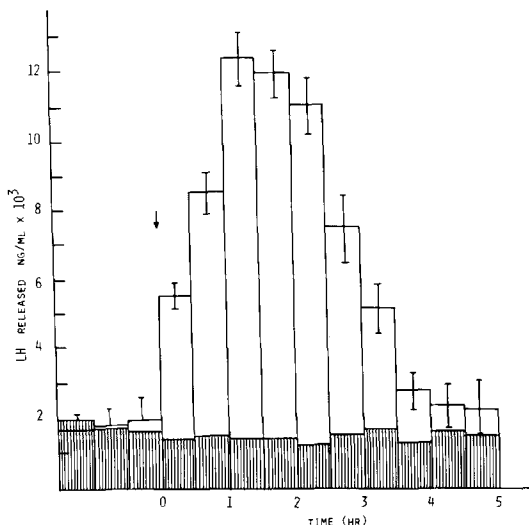

FIGURE 6 Kinetics of LH release from 48-h cells incubated for 5 h with 10^{-7} M LHRH. Media were collected at 30-min intervals. With this protocol, only a single phase of LH release is apparent which is maximal at 60–90 min. At 4 h, LH release in response to LHRH is not significantly different from that of the unstimulated control. Results expressed as ±SEM. Glands were obtained from female donors.

Recently, using a dissociation procedure that employs primarily collagenase, Nakano et al. (21) have also shown that the responsiveness of acutely dissociated pituitary cells to LHRH significantly improves during a postdissociation incubation for 22–24 h, although the authors did not report on longer intervals. They suggest that the recovery that they have obtained is better than might be expected with methods employing trypsin, and indeed after 24 h the sensitivity of their preparation is marginally better with a half maximal dose of about 5×10^{-9} M LHRH compared to the 1.5×10^{-8} M obtained in the present study. At the same time, however, it should be noted that the magnitude of the response obtained in terms of the experimental over the control with the collagenase preparation is rather less than 50% of that obtained with trypsin-dissociated cells.

My results indicate that the early attachment of cells significantly lowers the mortality that occurs in the first 24 h postdissociation. Nakano et al. (21) estimate the cell loss in unattached cells to be between 35% and 45%. With my preparation, I have not assessed cell loss in percentage terms, but the data that I have obtained by comparing cells on PL-coated dishes with those on uncoated dishes

TABLE III
LH Secretion in Response to LHRH, 57 mM K⁺, and A23187

	0-4 h		4-8 h	Cell content per dish
	ng/ml LH released		*ng/ml LH released*	*ng/LH*
Saline	240 ± 470	Saline	100 ± 4	71,750 ± 7,600
10^{-7} M LHRH	6,750 ± 470	Saline	2,500 ± 270	65,500 ± 7,600
Saline	260 ± 20	10^{-7} M LHRH	6,600 ± 540	
10^{-7} M LHRH	6,500 ± 570	10^{-7} M LHRH	2,300 ± 180	
Saline	200 ± 35	57 mM K⁺	2,500 ± 210	
57 mM K⁺	4,800 ± 400	Saline	380 ± 40	
57 mM K⁺	4,500 ± 400	57 mM K⁺	2,000 ± 210	
10^{-7} M LHRH	6,500 ± 751	57 mM K⁺	4,900 ± 475	
57 mM K⁺	4,400 ± 500	10^{-7} M LHRH	5,800 ± 550	
10^{-6} M A23187	3,000 ± 275	Saline	1,500 ± 200	
10^{-7} M LHRH	6,400 ± 620	10^{-6} M A23187	4,500 ± 410	

4×10^6 cells preincubated on PL-coated petri dishes for 48 h, and rinsed three times with DMEM/gel before being stimulated as indicated. Dishes used in triplicate; LH released expressed as ±SEM. Microscope examination and staining with trypan blue at the end of the experiment indicated that no obvious cell loss or reduction in viability had occurred during the 8-h incubation.

indicate that with attached cells the viability can be improved up to twofold. The limited period during which cells can be displaced from PL-coated surfaces with polyglutamic acid indicates that probably the secondary attachment that the cell establishes with the substratum is the important one for improved viability. The failure of cells to establish this kind of secondary attachment on PL-coated Sepharose may well be the primary reason why such a low level of survival is obtained with this substrate.

In addition to its effect in improving the viability of the preparation, PL has also, of course, the special advantage of anchoring the cells to a readily manipulable substrate. Since the functional tests that I have used indicate that PL does not adversely affect the secretory performance of the attached cells, it should now be possible to take full advantage of the free surface exposed in the dissociated preparation. Using this approach, we have recently succeeded in topographically locating the binding sites for LHRH on the dissociated cell surface (12), but from the data presented in this report it is also clear that the preparation may be used to advantage for studies in which a rapid exchange between the cells and their environment needs to be closely monitored. For the study of

gonadotrophin secretion, this is likely to be a useful advantage since it is known that the effects of LHRH are related to the frequency and magnitude of the dose (15) and that in vivo the response in terms of the release of LH is rapid and may well be pulsatile (8, 19). In these circumstances, it is to be expected that the triggering and second messenger systems relating to the initial stimulation of LH secretion by LHRH will also need to be studied over short time intervals.

The results obtained in response to a subthreshold stimulation with LHRH show a significant peak in LH release at 2 min, followed by a more gradual and sustained phase of release over the next 90 min. The early response has not been identified hitherto in vitro although it correlates well with a reported peak in the level of LH at 6 min found in the serum of rats given a single venous injection (15). The time scale of the more gradual second phase of LH release conforms to that observed in previous studies with a longer "pulse" stimulus of LHRH and in which either in vitro perfused tissue fragments were used (5) or in which in vivo serum LH levels were monitored at brief (10 min) intervals (1). In the wider context of stimulation-secretion coupling in general, it is of interest that the biphasic response observed in the

present study is closely similar to that shown to occur during glucose-stimulation insulin release from isolated rat islet preparations (4).

The hormone released in the initial release phase accounts for less than 0.1% of the total LH content of the cells, although with prolonged stimulation a maximum, which rises to a peak at 120 min, of about 10% of the total LH content is reached. It is not clear at present why only a small fraction of the total LH content is releasable. Since further release can be stimulated with other secretogogues, it is clear that the cells are still able to release hormone. Our observations (12) which show that the majority of gonadotrophin cells do bear receptors that can bind LHRH indicate that LHRH receptors are not restricted to only 10% of the cell population. Nor do we know why the cells become refractory to LHRH after 4 h of stimulation. However, since the ability of secretogogues such as 57 mM potassium and the calcium ionophore A23187 (which presumably invoke release without involving the LHRH receptor) to stimulate LH release is unimpaired after a 4-h continuous stimulation with the peptide, it is possible that at this time the limiting step may reside at the level of the LHRH receptor. This aspect may be related to the physiological regulation of receptor sites described in other systems (3, 7, 9), and we are therefore currently examining in detail the redistribution of LHRH receptors before and after stimulation.

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