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Prolactin Release from Rat Anterior Pituitary Cell Culture following Medium Replacement (41213)

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Abstract. The initial rapid increase in the rate of prolactin release from rat pituitary cells in monolayer cultures following medium replacement can be attributed entirely to the "mechanical" stress associated with the replacement maneuver itself.

Extended maintenance of cell cultures typically requires the periodic replacement of nutrient medium with freshly prepared medium. This is necessary in order to remove accumulated metabolites and "exhausted" buffer and to renew the supply of nutrients necessary for continued function. While monitoring prolactin production by rat anterior pituitary cells in monolayer culture, Groshong *et al.* (1) observed a dramatic surge in prolactin release within the first hour following the routine medium replacement maneuver. We have seen a similar type of effect in our culture systems and have considered several possible causes.

The surge following medium change could arise as a result of any one of several events, including (a) removal of accumulated prolactin, which suggests the operation of a negative feedback or "end-product inhibition" phenomenon, (b) removal of nonhormonal accumulated metabolites that nonspecifically suppress prolactin release, (c) introduction of a nutrient or microelement at a concentration that initially triggers release, (d) nonspecific mechanical stress, including temperature changes, associated with the medium change process. We have examined the enhanced release phenomenon and suggest that only one of these alternatives is adequate to explain the phenomenon.

Materials and Methods. *Monolayer cell culture.* Anterior pituitary glands derived from adult, male, Sprague–Dawley rats (250–300 g) were minced and dissociated with 0.47% collagenase (Type I, Worthington Biochemical Corp., Freehold, N.J.) in calcium- and magnesium-free Hanks' base (Grand Island Biological Co., Grand Island, N.Y.). The dissociated cell suspension was centrifuged and the pellet resuspended twice to remove enzymes. The cells were finally resuspended in complete medium and plated on the surface of 17.8 × 16-mm-diameter tissue culture wells (Costar, Cambridge, Mass.). The cultures, initially inoculated with $1.5-2 \times 10^4$ cells were maintained at 37 ± 0.5° and a saturated humidity in 1.2 ml of Eagle's minimum essential medium supplemented with 0.1 m*M* nonessential amino acids, 1 m*M* sodium pyruvate, 10% fetal bovine serum, and gentamicin sulfate, 40 μg/ml. pH of the medium was 7.3 ± 0.1 and maintained at that level by suitably adjusting the carbon dioxide tension in the incubator. Medium was changed every 3 days.

Assay of prolactin. Radioimmunoassays were performed using the materials and protocols supplied by the NIAMDD Rat Pituitary Hormone Distribution Program. Prolactin concentrations were interpolated from a standard curve and expressed in terms of an NIAMDD reference standard (Rat PRL-RP-1).

Experimental protocol. Well-established 9-day-old cultures were partitioned into seven groups, each represented by six culture wells. Following medium replacement, the cultures were incubated for 6 hr and 50-μl aliquots of medium were removed for radioimmunoassay during the fifth and sixth hours in order to establish pretreatment (PRE) titers of hormone and its rate of

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TABLE I. RATES OF PROLACTIN RELEASE BEFORE AND AFTER MEDIUM REPLACEMENT

Group	PRE[a]	POST[b] 0–1 hr	1–2 hr	2–3 hr	3–4 hr	4–5 hr	5–6 hr
1	124 ± 6	128 ± 10	131 ± 10	120 ± 6	118 ± 8	120 ± 11	118 ± 12
2	122 ± 7	445 ± 32*	312 ± 24*	209 ± 20*	146 ± 20	136 ± 12	132 ± 12
3	125 ± 9	430 ± 28*	287 ± 18*	156 ± 19	140 ± 13	125 ± 10	120 ± 9
4	130 ± 9	455 ± 35*	233 ± 20*	126 ± 9	133 ± 12	126 ± 10	121 ± 13
5	125 ± 10	490 ± 54*	328 ± 25*	202 ± 8*	148 ± 10	120 ± 9	117 ± 9
6	128 ± 11	505 ± 56*	350 ± 27*	248 ± 22*	147 ± 10	132 ± 10	122 ± 10
7	122 ± 7	476 ± 37*	255 ± 28*	183 ± 13*	135 ± 10	125 ± 11	126 ± 12

[a] Pretreatment release rates (ng/well/hr); mean ± SEM for six replicates.
[b] Post-treatment release rates (ng/well/hr); mean ± SEM for six replicates.
* Significantly different from PRE level ($P < 0.05$).

release. The remaining 1.1-ml quantities were removed by gentle suction from the culture wells of all but Group 1 samples which remained undisturbed. The media of Groups 2–7 were replaced with 1.1 ml of one of the following:

Group 2 Fresh medium
Group 3 Fresh medium containing 100 ng rat prolactin in 1% bovine serum albumin (BSA) buffer
Group 4 Fresh medium containing 300 ng rat prolactin in 1% BSA buffer
Group 5 "Old" medium containing 300 ng rat prolactin in 1% BSA buffer
Group 6 Fresh medium containing 1% BSA buffer only
Group 7 "Old" medium which was returned unaltered to the culture wells

Incubation was continued for 6 hr and 50-μl aliquots of medium were taken from each well at 1, 2, 3, 4, 5, and 6 hr post-treatment (POST) and subjected to prolactin radioimmunoassay.

Statistical analysis. Changes in post-treatment prolactin rates were compared with the pretreatment rate within each group using Dunnett's multiple comparison procedure (3).

Results and Discussion. Table I describes the changes in prolactin release rates following different experimental conditions. Pretreatment rates for each group were similar because of a purposefully selective process. Cultures having basal release values that clustered about a common mean were intentionally distributed in a way that insured similar basal behavior among treatment groups. Post-treatment effects, therefore, could more readily be attributed to experimental manipulations.

Group 1 cultures which underwent no medium change maneuver continued to release prolactin at approximately constant rates. Numerical differences with time among values were not significant. Group 2 cultures, which experienced a simple medium replacement, exhibited the prolactin surge similar to, but less intense than, that described by Groshong *et al.* (1). The elevation peaked during the first hour and gradually returned to the pretreatment level after 6 hr.

The incorporation of 100 and 300 ng of rat prolactin in the exchange medium (Groups 3 and 4, respectively) did not significantly alter the appearance of the early 1-hr surge nor the rates of release during the following 5-hr incubation period. Endogenous prolactin in these preparations was estimated by subtracting concentrations of administered prolactin from the total immunoassayable quantity. It was assumed that the exogenous prolactin was stable during the 6-hr treatment period.[1] Although Herbert *et al.* (2) showed that ovine prolactin could

[1] In a preliminary experiment, in which 350 ng of rat prolactin was incubated in cell-free culture medium, no significant degradation occurred during a 6-hr period. A longer period of incubation (24 hr) on the other hand reduces immunoassayable prolactin to about 60% of its initial level.

inhibit release in cultures of fetal pituitary cells, we were unable to demonstrate any such effect in our system. We conclude that a cell-level negative feedback phenomenon is not adequate to explain the surge.

The addition of 300 ng of rat prolactin to "old" medium also failed to influence the surge (Group 5), and Group 6 cultures which were exposed to the prolactin vehicle alone exhibited characteristics indistinguishable from those of Group 2. Indeed, none of the maneuvers described altered the appearance of the initial surge. The removal of "old" medium and its immediate return to the wells of Group 7 cultures clearly demonstrates that the mechanical "stress" associated with the exchange maneuver is sufficient and adequate to account for the early enhancement of prolactin release.

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