Significant Qualitative Differences Exist between Thyrotropin and Prolactin Secretory Dynamics Induced by Pituitary Cell Swelling (43297)

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Abstract. **Cell swelling produced by a variety of techniques is a potent stimulus intensity-related inducer of an immediate secretory burst of thyroid-stimulating hormone (TSH) and prolaction (PRL) secretion from anterior pituitary cells. A 2-min "square wave" exposure to either hyposmolarity or isotonic urea induced stimulus intensity-correlated TSH and PRL secretory bursts peaking within 3 min, but the PRL zenith occurred 1 min later than that of TSH. With continuous exposure to these stimuli, TSH secretion rapidly decreased and remained only slightly above the unstimulated rate after 5 min. PRL secretion fell to and remained below the unstimulated level after 10 min. After stopping the stimulus, another secretory burst ("off" response) occurred with PRL, but not with TSH. A progressive "ramp" increase in stimulus intensity over 18 min induced a corresponding gradual increase in TSH secretion; there was a progressive depression, rather than increase, in PRL secretion during the stimulus ramp, with an off response secretory burst when the stimulus was discontinued. Removal of extracellular Ca^{2+} or addition of verapamil to the medium did not alter the dynamics of hyposmolarity-induced TSH secretion, but markedly altered those of PRL secretion; there was no off response PRL secretion and a hyposmolar ramp induced a corresponding gradual increase in PRL secretion, with a return to baseline after removing the stimulus. The dramatic qualitative differences in the response of the thyrotroph and lactotroph may reflect differences between the cell types in the size of secretory vesicles, membrane potential, the mechanism of exocytosis, and/or the role of Ca^{2+} influx across the plasmalemma.**
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The details of the mechanism by which exocytotic hormone secretion occurs are still cloudy. Many studies of the phenomenon have utilized clonal rat pituitary tumor-derived cells whose secretory mechanism may differ considerably from normal cells in important aspects (1). Cell swelling produced by various techniques induces hormone secretion from pituitary cells which closely resembles that induced by specific secretagogues in many respects; this phenomenon is not caused by dilution of essential ingredients in the medium or cell damage (2, 3). Since there are marked differences between normal and tumor-derived cells in the role of extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) in osmotically induced secretion (2–6), cell swelling may activate different transducing systems in tumor and in normal cells.

In evaluating the effect of osmotic changes of a magnitude which would be expected to occur *in vivo* on secretion of various hormones, we noted that there seemed to be certain striking differences in the secretory dynamics of thyroid-stimulating hormone (TSH) and prolactin (PRL), both of which share the property of being very sensitive to pharmacologic stimulation by thyrotropin-releasing hormone (TRH). In this study, we have therefore compared in detail the secretory response to cell swelling of acutely dispersed lactotrophs and thyrotrophs from normal rat pituitaries and the role of extracellular Ca^{2+}. We have also evaluated the effect of a gradual change in the osmolar stimulus

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achieved with a gradient ramp over a period of minutes rather than with an abrupt change of medium, since osmotic changes probably occur with this more leisurely transition period *in vivo*. The data indicate that there are marked differences between TSH and PRL secretory dynamics induced by cell swelling, and, in the effect of $[Ca^{2+}]_e$, even though both cell types respond similarly to TRH stimulation. Thus even in normal adenohypophyseal cells the mechanism of exocytotic secretion appears nonidentical in different cell types.

Materials and Methods

Enzymatic dispersion and perifusion of anterior pituitary cells with Dulbecco's modified Eagle's medium (DMEM) or Krebs-Ringer-bicarbonate medium were performed, as described previously (2, 7). Six to eight adult male Sprague-Dawley rats were used to provide $2–4 \times 10^6$ cells for each perifusion chamber. At 0900–1000 hr, the rats were sacrificed by decapitation immediately after removal from their cages. The skulls were opened and the anterior pituitary lobes were carefully removed and placed in Hanks' HEPES (pH 7.3) buffer. Each pituitary was finely minced into approximately 30 pieces with fine scissors and the pituitary fragments were digested for 40 min in a shaking water bath at 37°C in 10 ml of Hanks' HEPES buffer containing 40 mg of collagenase, 20 μg of DNAase in 40 mg of bovine serum albumin. After digestion, the cells were washed three times in pH 7.4 DMEM containing 10% horse serum. Evaluation with 0.4% trypan blue indicated that >95% of the cells were alive at the time they were put into the perifusion chamber. The flow rate was 0.5 ml/min and fractions were collected at 1-min intervals. An initial equilibration perifusion of DMEM alone was made 40 min before the start of the experiment. The DMEM reservoir and perifusion chamber were maintained at 37°C in a water bath. The fractions were refrigerated until the conclusion of the experimental session, then frozen (−20°C) until assayed. All experiments were performed at least twice, with essentially identical results, as indicated in the text. Verapamil, EGTA, urea, and synthetic TRH were obtained from Sigma Chemical Co.; DMEM was obtained from Gibco. The same glass-distilled, deionized water was used to reconstitute and/or dilute the DMEM to make a hypotonic solution. Urea was dissolved in hypotonic solution to produce an osmolality the same as DMEM (325 mOsm/kg). In some experiments, a gradient ramp was used to gradually increase hyposmolarity to 30% by progressively diluting the medium with water over a period of 18 min. Ca^{2+}-free medium was made by adding 3 m*M* EGTA to DMEM, or using Krebs-Ringer-bicarbonate medium containing 0.1 m*M* EGTA and in which equimolar amounts of NaCl had been substituted for $CaCl_2$. TSH and PRL were measured by radioimmunoassay using protocols and specific reagents supplied by the National Hormone and Pituitary Program, National Institutes of Diabetes, Digestive, and Kidney Diseases. The volume of effluent assayed in each fraction was 30 μl for PRL, and 200 μl for TSH and luteinizing hormone (LH). TSH and PRL, and in some experiments LH, were measured in the same samples. All samples from each experiment were measured in a single assay to avoid interassay variation. The intra-assay coefficient of variation was <12%. The upper and lower ranges of sensitivity of the assay were 1–52 ng/ml for PRL, 0.05–12.5 ng/ml for TSH, and 0.3–12 ng/ml for LH. Student's *t* test was used for statistical analysis of the data.

Results

Short-term Exposure to the Permeant Molecule Urea. A 2-min "square wave" exposure to isotonic urea induced stimulus intensity-correlated TSH and PRL secretory bursts that peaked within 3–4 min and returned to baseline within 5–10 min (Fig. 1). With the higher concentrations of urea, the zenith of PRL secretion occurred about 1 min later ($P < 0.05$, $n = 6$) and

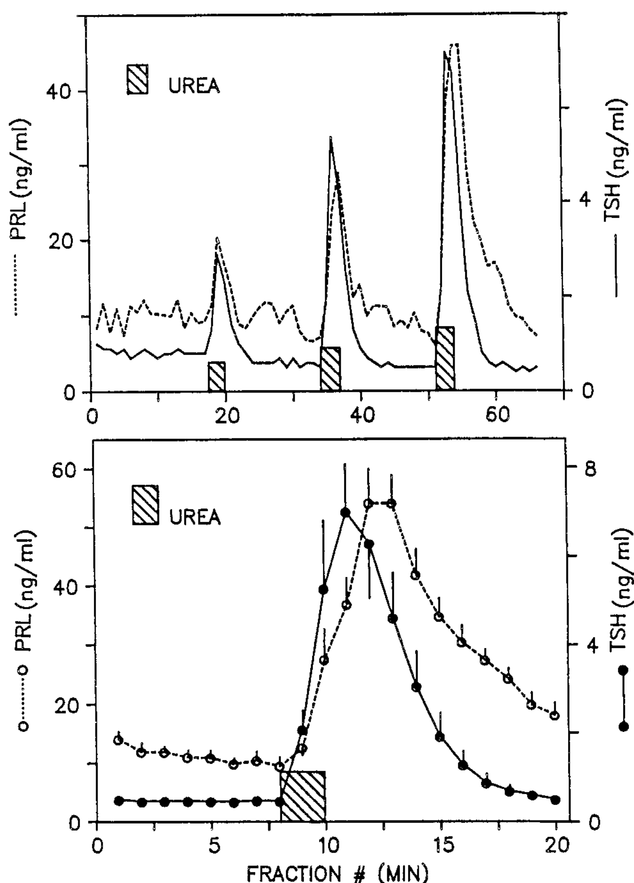

Figure 1. Effect of perifusion of 54, 104, and 162.5 m*M* isotonic urea on TSH and PRL secretion. The vertical hatched bars indicate 2-min exposure; the relative stimulus intensity is indicated by the bar height. The bottom panel shows 2-min perifusion of 162 m*M* urea. The zenith of PRL secretion occurred 1 min later than did that of TSH. The upper panel shows the mean of three experiments; the bottom panel shows mean ± SE of three experiments.

returned to baseline more slowly than that of TSH.

Prolonged Exposure to Hyposmolarity or Urea. With continuous exposure ≥10 min to 104 m*M* urea or 30% hyposmolarity, TSH secretion rapidly decreased and remained slightly above baseline after 5 min (Fig. 2). PRL secretion fell to and remained below the baseline after 10 min. After stopping the stimulus, another secretory burst ("off" response) occurred with PRL, but not TSH. When the stimulus duration was <5 min, the secretory dynamics of TSH and PRL were similar. The response to TRH remained the same at the end as at the beginning of each experiment (Fig. 2), indicating that 20-min exposure to urea or hyposmolarity did not depress cell viability or function.

Effect of a Prolonged Progressive Increase in Hyposmolarity. A gradual increase from 0 to 30% hyposmolarity over 18 min induced a corresponding gradual increase in TSH secretion, with a return to baseline after removing the stimulus (Fig. 3). In contrast, there was a progressive depression, rather than increase, in PRL secretion during the stimulus ramp with an off response secretory burst when the stimulus was discontinued.

Effect of $[Ca^{2+}]_e$. Either removal of extracellular Ca^{2+} or addition of the Ca^{2+}-channel blocker verapamil to the medium decreased basal PRL secretion, but not basal TSH secretion (Fig. 4). There was no significant effect of blocking Ca^{2+} influx on the initial burst of PRL or TSH secretion induced by hyposmolarity, but the off PRL secretory response which occurs on the return to isotonicity after perifusion >20 min with hyposmolar medium was partly or completely blocked. These data indicate that the hyposmolarity-induced "on" and "off" PRL secretory responses have different mechanisms.

Preventing Ca^{2+} influx had no significant effect on TSH secretory dynamics induced by a progressive "ramp" increase in hyposmolarity, but dramatically altered the PRL secretory dynamics so induced to make them indistinguishable from those of TSH (Fig. 5).

Comparison of 60-min Perifusion of 10 n*M* TRH and 30% Hyposmolarity. Continuous perifusion of 10 n*M* TRH for 60 min induced a similar pattern of both TSH and PRL secretion, with a rapid high amplitude pulse falling within 5–10 min to a level approximately twice the basal secretory level, where it remained until removal of stimulation (Fig. 6). Hyposmolarity only induced the first-phase high amplitude pulse of TSH and PRL secretion, which rapidly fell to (TSH) or below (PRL) baseline. An off secretory response of PRL secretion occurred, as with the earlier experiments with hyposmolar exposure ≥10 min.

Effect of $[Ca^{2+}]_e$ on Hyposmolarity-Induced LH Secretion. Secretion of LH induced by hyposmolarity was examined in a few experiments (Fig. 7). Its relation to $[Ca^{2+}]_e$ most closely resembled that of TSH rather than PRL, as might be expected from the closely related subunit structure of the glycoprotein hormones. However, a slight off response after prolonged continuous exposure to a constant stimulus was sometimes seen (Fig. 7). Removal of medium Ca^{2+} did not alter the

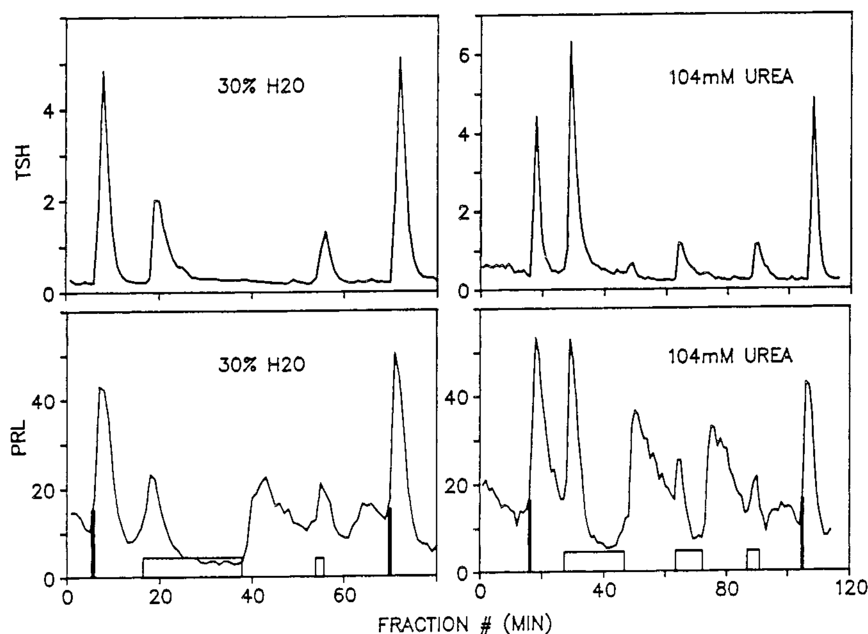

Figure 2. Effect of perifusion with a 30% hyposmolar medium for 2 and 20 min (left panel) or with 104 m*M* urea for 5, 10, or 20 min (right panel) on TSH (upper panels) or PRL (lower panels) secretion. The vertical black bars indicate 10 n*M* TRH perifusion for 1 min. The open bars indicate hyposmolar medium or urea perifusion. The length of the stimulus exposure is indicated by the bar width. The figure shows one of three experiments with similar results. The scale on the ordinate indicates effluent concentration of TSH or PRL nanograms per milliliter.

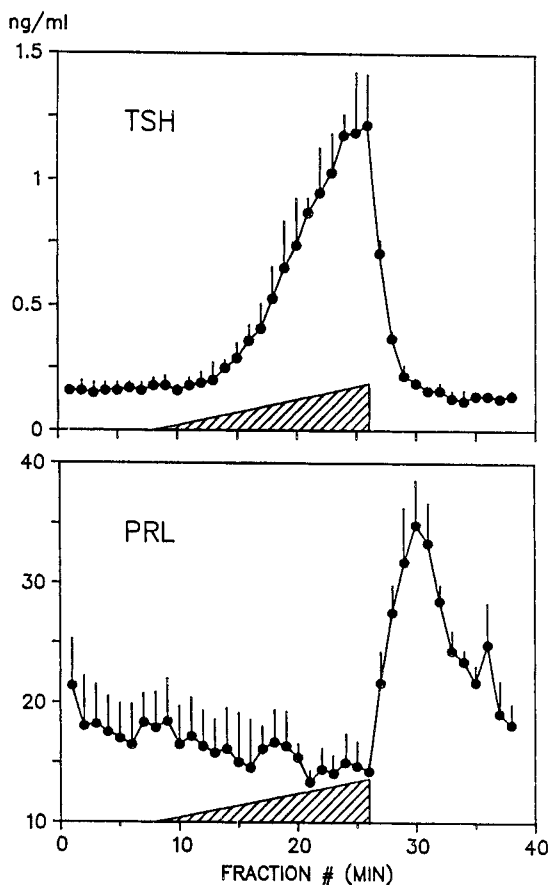

Figure 3. The triangles in the panels indicate 18-min perifusion of gradually increasing hyposmolarity from 0 to 30%. Mean ± SE of three experiments are shown.

general dynamics and did not depress the LH secretory response to hyposmolarity.

Discussion

Cell swelling, produced by relative extracellular hyposmolarity (2, 5) or isotonic permeant molecules (3, 4), is a nonspecific stimulus of adenohypophyseal hormone secretion. The transduction mechanism by which it induces secretion is unclear. It has been studied most extensively in clonal tumor-derived GH_4C_1 rat pituitary cells and in normal pituitary cells, primarily with evaluation of PRL secretion. In GH_4C_1 cells, Ca^{2+} influx through the plasmalemma seems to be the trigger that induces hormone secretion (4, 5, 8). But in normal pituitary cells Ca^{2+} influx seems to be inhibitory, since secretion of both TSH and PRL induced by cell swelling is either unaffected or significantly augmented when Ca^{2+} influx is blocked by employing a Ca^{2+}-free medium (9).

Early studies employing relatively short-term exposure to osmolar changes indicated that secretion induced by hyposmolarity was nearly identical to that induced by specific secretagogues (2, 3). This suggested that the latter might induce cell swelling as the initial step of their action, with specificity being conferred by discrete receptor-ligand interactions at the cell membrane since hyposmolarity stimulated secretion of all pituitary hormones measured (2, 10). However, although cell swelling produced by either relative extracellular hyposmolarity or the isosmolar addition of permeant molecules to the medium will induce characteristic responses in either normal or tumor-derived cells, there are significant differences in the characteristics of secretion between normal and tumor-derived cells (6), and even in secretion of different types of hormones from normal pituitary cells (11). Differences in transduction mechanisms may account for these discrepancies, but a complete explanation is not currently possible. The heterogeneity might reflect variations among cell types in membrane potential (12), the size and mobility of intracellular secretory granules (13), or the organization of the intracellular transduction chain between stimulus and secretion, especially in the role of Ca^{2+} influx (1, 6, 11).

The secretion kinetics of labeled PRL from GH cells suggest that continuous unregulated exocytosis of PRL represents more recently synthesized hormone than does the PRL secreted in response to secretagogues, indicating that there are at least two intracellular PRL pools in lactotrophs (14–16). Our data seem in accord with this hypothesis and additionally suggest that the two PRL pools may each be affected differently by cell swelling and Ca^{2+} influx.

The response to cell swelling is less complex in thyrotrophs than in lactotrophs. This may mean that there is only one intracellular TSH pool or that the transduction mechanism is less complicated in thyrotrophs.

The difference between PRL and TSH secretory dynamics that we observed may be due to differences in basal activity between the two cell types. Twenty to 30% of prolactinoma cells display spontaneous action potentials (17, 18). Spontaneous action potentials may be responsible for high basal GH or PRL secretion (18), which is decreased by Ca^{2+}-channel blockers (19). Hyposmolarity induces cell membrane hyperpolarization in clonal osteosarcoma cells (20) and may similarly induce hyperpolarization in lactotrophs, inhibiting the high rate of spontaneous action potentials and thus decreasing basal PRL secretion. In thyrotrophs, spontaneous action potentials have not been reported. Basal TSH secretion is low and not affected by Ca^{2+}-channel blockers (19). Preincubation of cells with 30% hyposmolarity for $\geq$10 min inhibited basal PRL secretion, but had no effect on basal TSH secretion. Lidocaine, which blocks membrane depolarization, inhibits basal PRL but not basal TSH secretion (7). Thus, differences in the control or spontaneous activity of the plasma membrane potential may play a role in the difference between the responses of the lactotrophs and thyrotrophs to cell swelling. The differences may also be

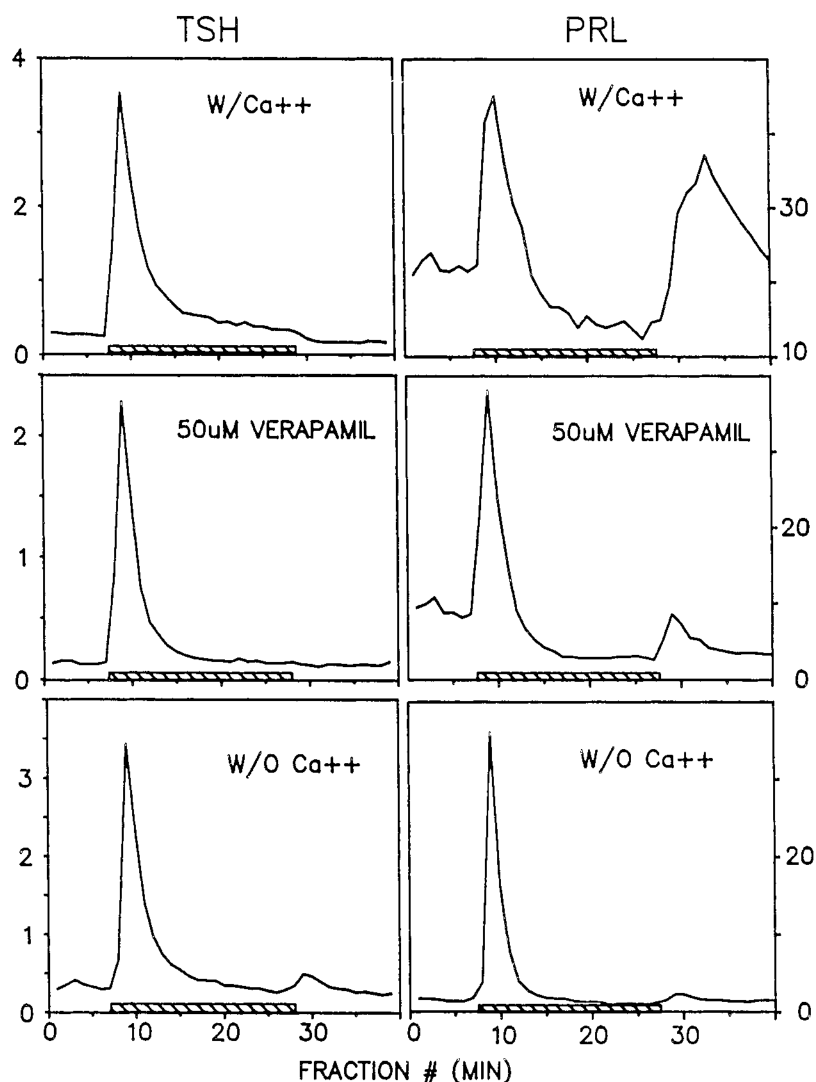

Figure 4. Effect of 50 μM verapamil or Ca^{2+}-free medium on 30% hyposmolarity-induced PRL secretion. (Upper panels) Normal medium Ca^{2+} concentration; (middle panels) 50 μM verapamil; (lower panels) medium without Ca^{2+}. In all panels, the horizontal hatched bar indicates perifusion of the medium diluted with 30% H_2O. The mean of two experiments is shown. The scale on the ordinate indicates effluent concentration of TSH or PRL in nanograms per milliliter.

related to the fact that the former are under tonic inhibition of secretion *in vivo*. This inhibition is removed when the cells are studied *in vitro*, resulting in a high level of basal secretion.

Another factor to consider is that the secretory granules in lactotrophs are five times larger than in thyrotrophs (13). Since secretory granules may need to swell before fusion can occur with the plasmalemma to produce exocytosis (21), the size of the secretory granules may account for some of the difference between the two cell types. If the ease of fusion with the plasmalemma is directly related to the absolute size of the secretory granules, one would expect that PRL secretion would be more easily induced by osmotic changes. However, our data indicate that TSH secretion occurs more rapidly than PRL secretion (Fig. 1). Since this later onset of peak PRL secretion was seen only with the higher degrees of stimulation and short exposure times, it is possible that it may be related in part to a combination of both on and off responses with PRL, whereas TSH had only an on response.

Ca^{2+} plays a critical role in secretion (22, 23). In the present studies, abolishing Ca^{2+} influx across the plasmalemma by depleting medium Ca^{2+} or adding 50 μM verapamil to the medium did not depress the initial TSH or PRL secretory burst induced by cell swelling, consistent with our earlier studies (3, 9). However, the off PRL burst upon return from a hyposmolar to an isosmolar medium was abolished by preventing Ca^{2+} influx. This is different from PRL secretion in GH_4C_1 cells, in which the on response to cell swelling is dependent on Ca^{2+} influx (4, 5, 8). Some transformation in the Ca^{2+} requirement for the acute secretory response to cell swelling has thus occurred in the tumor-derived

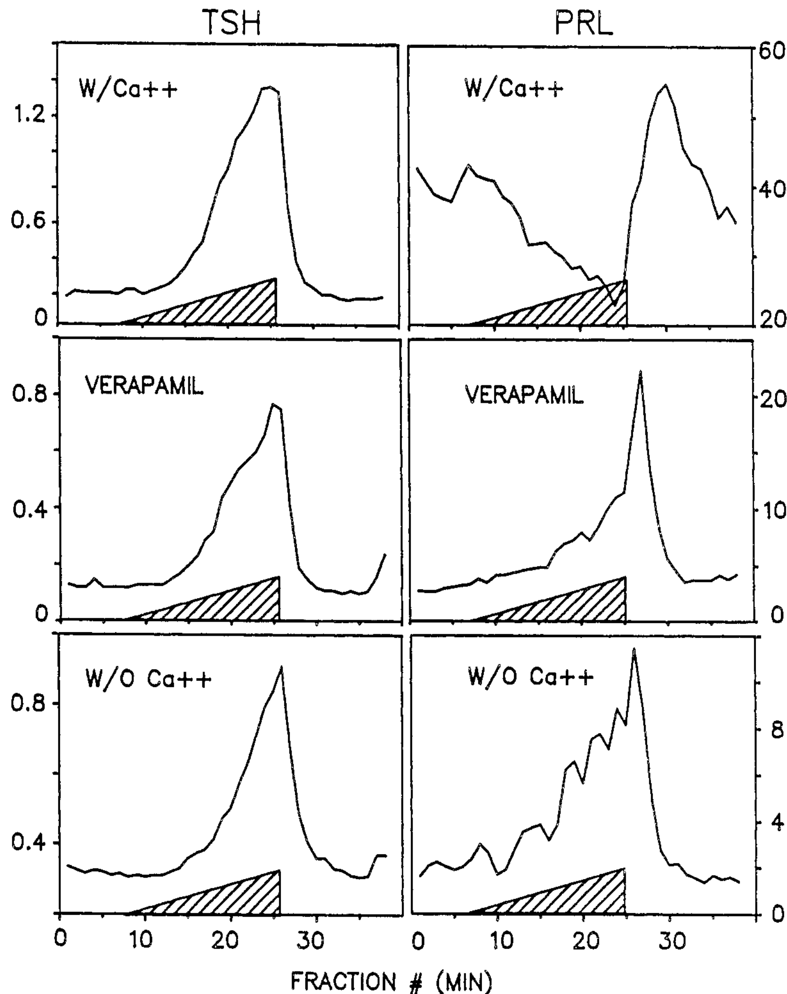

Figure 5. Effect of 50 μM verapamil or Ca^{2+}-free medium on 30% hyposmolarity-induced PRL secretion. (Upper panels) Normal medium Ca^{2+} concentration; (middle panels) medium containing 50 μM verapamil; (lower panels) medium without Ca^{2+}. In all panels, the triangles indicate perifusion of medium diluted progressively with 0–30% H_2O over 18 min. The mean of two experiments is shown. The scale on the ordinate indicates effluent concentration of TSH or PRL in nanograms per milliliter.

GH cells. This does not seem to be specific for this particular cell line, since a PRL secretory response to cell swelling also requires Ca^{2+} influx in the independently derived clonal MMQ cells (24) (unpublished data). We have not yet evaluated in sufficient detail whether the off response occurring on return to basal conditions after prolonged exposure to hyposmolarity is Ca^{2+}-dependent in growth hormone cells.

It is intriguing that preventing Ca^{2+} influx abolishes the qualitative difference between the secretory responses of the lactotrophs and thyrotrophs to cell swelling. However, some differences in the response to TRH were brought to light with this technique. Blocking Ca^{2+} influx with verapamil depressed TRH-induced TSH but not PRL secretion (11). There are also differences in Ca^{2+} requirements in forskolin-induced PRL secretion between normal and tumor-derived pituitary cells (6). The role of Ca^{2+} in exocytosis may thus be cell specific.

The dynamics of TSH and PRL secretion induced by TRH or cell swelling are different. Continuous exposure to TRH elicits a biphasic pattern of PRL secretion (25). The first phase may primarily reflect release of stored hormone, whereas the second includes the release of newly synthesized hormone (25). Hyposmolarity induced only an initial high amplitude burst of TSH and PRL secretion, which fell to or below the basal level after 10 min. Repetitive applications of the same 2-min hyposmolar stimulus at 5-min intervals gave essentially identical secretory responses over a period of several hours (9), suggesting that the failure of continuous exposure to hyposmolarity to maintain TSH and PRL secretion is not due to exhaustion of stored hormone. It is unknown whether cell swelling induces new hormone synthesis.

In the limited comparison we have made to date, the secretory dynamics of LH are very similar to those of TSH, as would be expected from their closely related structure. The dynamics of growth hormone secretion are probably correspondingly similar to those of PRL,

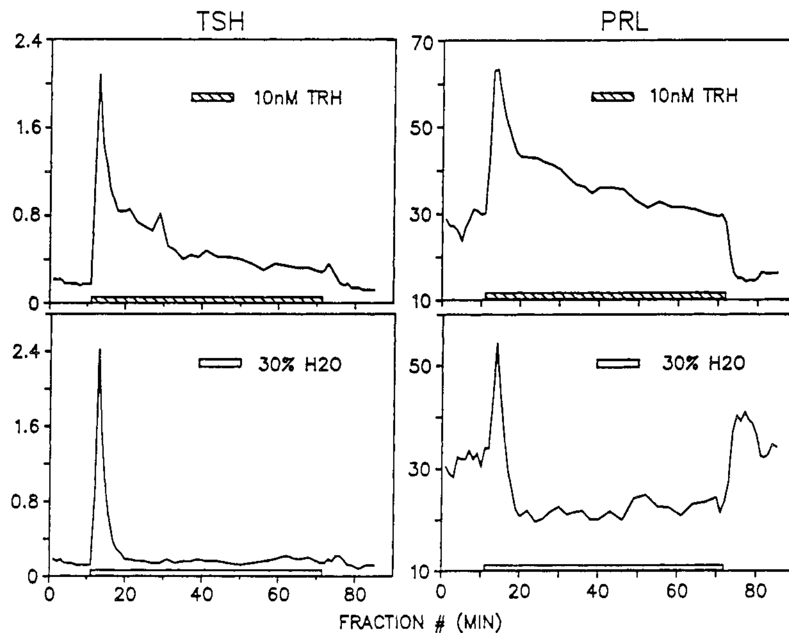

Figure 6. Comparison of prolonged TRH- and hyposmolarity-induced TSH and PRL secretion. The horizontal bars in the upper panel indicate 60-min perifusion with 10 μM TRH and the bars in the lower panel indicate 60-min perifusion with 30% hyposmolarity. The data from one of three experiments with similar results are shown. The scale on the ordinate indicates effluent concentration of TSH or PRL in nanograms per milliliter.

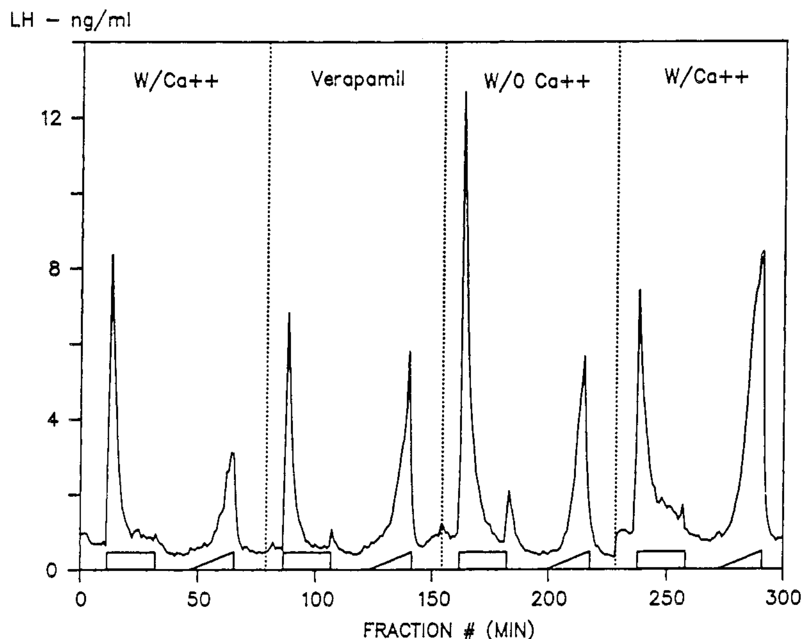

Figure 7. The dynamics of LH secretion induced by hyposmolarity in Krebs-Ringer-bicarbonate medium with a normal Ca^{2+} content (with Ca^{2+}), containing 50 μM verapamil, or Ca^{2+}-free medium (without Ca^{2+}). The horizontal rectangles indicate perifusion of 30% hyposmolar medium for 20 min. The triangles indicate gradually increasing hyposmolarity from 0 to 30% over 18 min. The vertical dotted lines indicate when the medium was changed. The data from a single experiment are shown.

since these two hormones are closely related, but we have not yet had an opportunity to examine this hypothesis.

The major conclusions to be drawn from our study are that thyrotrophs and lactotrophs (and probably other pituitary cells) have characteristic secretory mechanisms and/or transduction systems that are not the same for each cell type, even though both are stimulated by the same secretagogues (e.g., TRH, cell-swelling). Ca^{2+} plays an important role in each cell type, but its exact role is cell specific.

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