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CELL SWELLING INDUCED BY THE PERMEANT MOLECULES UREA OR GLYCEROL INDUCES IMMEDIATE HIGH AMPLITUDE THYROTROPIN AND PROLACTIN SECRETION BY PERIFUSED ADENOHYPOPHYSEAL CELLS

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Summary. The permeant molecules, urea and glycerol, evoked a prompt secretory burst of TSH and PRL when added to the extracellular medium of acutely dispersed anterior pituitary cells. Secretion of both hormones was proportional to the concentration of urea or glycerol between 26 and 104 mM ($r>0.89$, $P<0.001$). Equivalent concentrations of the impermeant molecule, mannitol, did not induce secretion. The acute TSH and PRL secretory responses to TRH, hyposmolarity, and permeant molecules were qualitatively indistinguishable. These data support our hypothesis that cell swelling and resultant plasmalemma expansion is a potent inducer of hormone secretion. Since the secretory response to permeant molecules was not reduced in a Ca^{2+}-free medium containing 0.1 mM EGTA, an increase in Ca^{2+} transport across the plasmalemma to raise cytosol Ca^{2+} concentration does not appear involved.

Introduction. In perifused adenohypophyseal cells, medium hyposmolarity stimulated an immediate high-amplitude secretion of LH and all other hormones measured proportional to the degree of hyposmolarity (1). Hyperosmolarity also induced a similar proportional high amplitude secretory burst but this occurred only with the return of the medium to isosmolarity ("off" response) (2). We postulated that absolute or relative medium hyposmolarity causes cell swelling which induces transient expansion of the plasma membrane. This draws preformed secretory vesicles to the cell surface where they fuse with the plasmalemma and discharge their contents exocytotically. If this hypothesis is correct, any procedure that induces cell expansion may cause hormone secretion.

Urea and glycerol are permeant molecules which readily pass through the plasma membrane and cause cell swelling by transiently increasing intracellular osmotic pressure. Both urea (3) and medium hyposmolarity (4) produce maximal cell swelling within 2 min, with a return to the initial cell volume within 10 min in spite of continuous exposure. This time course is very similar to that of the acute burst of hormone secretion induced by either osmolar changes or by TRH (1,2,5-7). We therefore considered urea and glycerol ideal substances to further test our hypothesis (1) that cell expansion by itself is a potent inducer of hormone secretion.

Materials and Methods. Enzymatic dispersion and perifusion of rat anterior pituitary cells were performed as previously described (1) with Dulbecco's Modified Eagle's Medium (DMEM) or

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Krebs-Ringer-Bicarbonate (KRB). The flow rate was 0.5 ml/min and fractions were collected at 1-min intervals. All experiments were performed at least twice and the results of all repeated experiments were essentially identical.

In some experiments two parallel cell columns were perifused simultaneously. The sequence of various materials perifused in each of the two chambers was identical except that in one of the chambers Ca^{2+}-free KRB (Ca^{2+} not present in KRB which also contained 0.1 mM EGTA) was perifused in the first half of the experiment and normal KRB (Ca^{2+} present and no EGTA) in the second half. The perifusion sequence in the other chamber was reversed; KRB was perifused in the first half and Ca^{2+}-free KRB in the second half.

Urea and glycerol (3.25-162.5 mM) were dissolved in hypotonic solution so that the final osmolarity was the same as that of the medium. In some experiments urea and glycerol were dissolved in normal DMEM (325 mOsm) so that the final osmolarity was additive. Osmolarities of the solutions were measured with an Advanced DigiMatic Osmometer Model 3D2 with a range of 0-2000 mOsm and a sensitivity of 1 mOsm.

TSH and PRL were measured by radioimmunoassay using protocols and specific reagents supplied by the National Hormone and Pituitary Program. All samples from each experiment were measured in a single assay to avoid interassay variation. The intra-assay coefficient of variation was <10%. The lower and upper ranges of sensitivity of the assays for TSH and PRL were 0.2-14 and 1-52 ng/ml, respectively.

Results. Isosmolar urea and glycerol produced prompt high-amplitude spikes in both TSH and PRL secretion which lasted 1-2 min and returned to near baseline within 5-10 min. The minimum detectable dose-response was 6.5 mM for urea and 26 mM for glycerol. Both the amplitude of the secretory pulse and the total amount of TSH and PRL secretion were proportional to the medium concentration of urea and glycerol between 26 and 104 mM ($r>0.89$, $P<0.001$). Fig 1 shows a representative dose-response experiment. Only data from experiments using urea and in which TSH was measured are shown in these figures but the data from experiments using glycerol and/or measuring PRL were essentially identical.

Urea or glycerol were dissolved directly in DMEM so that the final osmolarity of the solution was that of DMEM plus the added solutes. No secretion was induced by such hypertonic

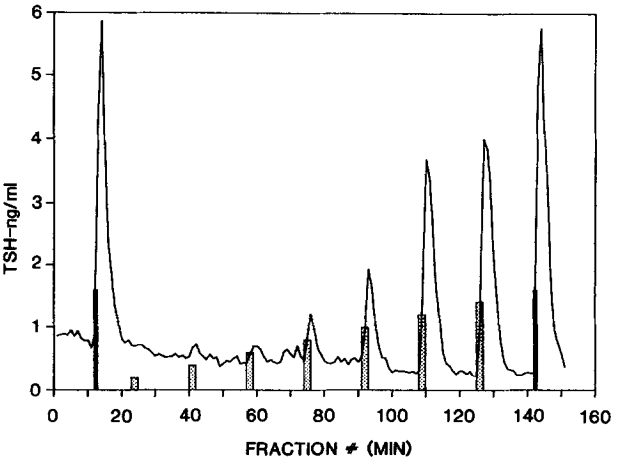

Fig 1. TSH secretion by dispersed pituitary cells. The vertical black bars at 12 and 142 min indicate 1-min perifusions of 10 nM TRH. The vertical stippled bars indicate 2-min perifusions of isosmolar concentrations of 3.25, 6.5, 13, 26, 52, 104, and 162.5 mM urea. In this and the other figures the relative solute concentration is indicated by the bar height.

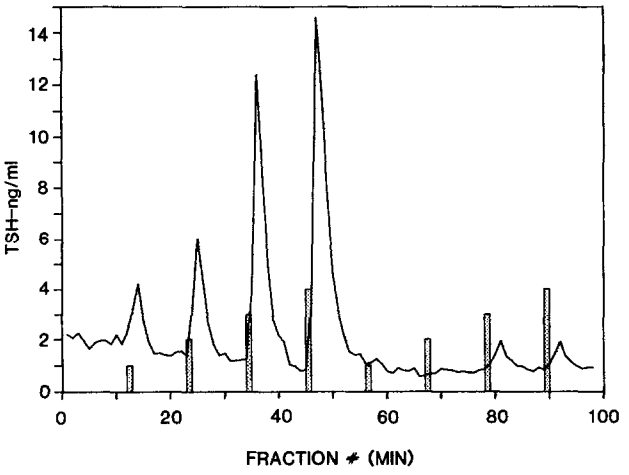

Fig 2. Effect of 1-min perifusion of 26, 52, 104, and 162.5 isosmolar urea on TSH secretion (0-50 min) and the same concentrations of urea added directly to DMEM to make hyperosmolar solutions (50-100 min). The slight secretory pulse seen after termination of perifusion of the highest hyperosmolar urea concentrations is temporally characteristic of the "off-response" seen following termination of hyperosmolar perifusions (2).

solutions even with a urea concentration of 162.5 mM (Fig 2). These data indicate that secretion is not induced by a direct action of urea or glycerol but by the cell swelling induced by the relative intracellular hyperosmolarity produced by penetrance of the molecule into the cytosol.

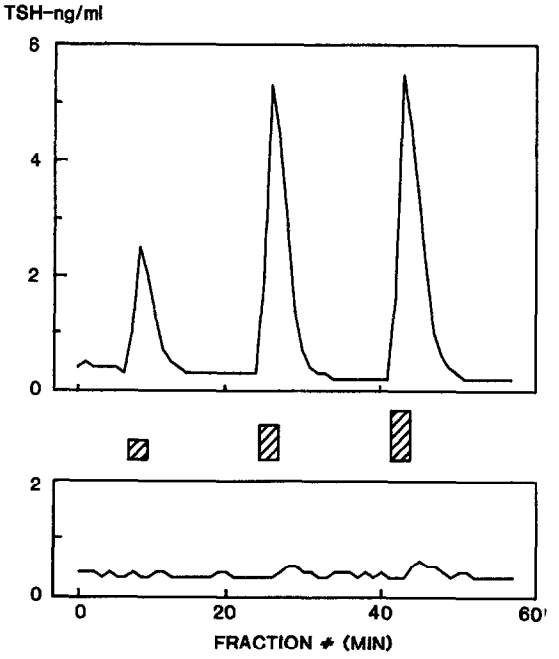

Fig 3. Comparison of the effect of isosmolar urea (upper panel) and mannitol (lower panel) on TSH secretion by dispersed pituitary cells. The hatched bars between the panels indicate simultaneous 2-min perifusions of 49, 97.5, or 162.5 mM isosmolar urea or mannitol in the two respective columns.

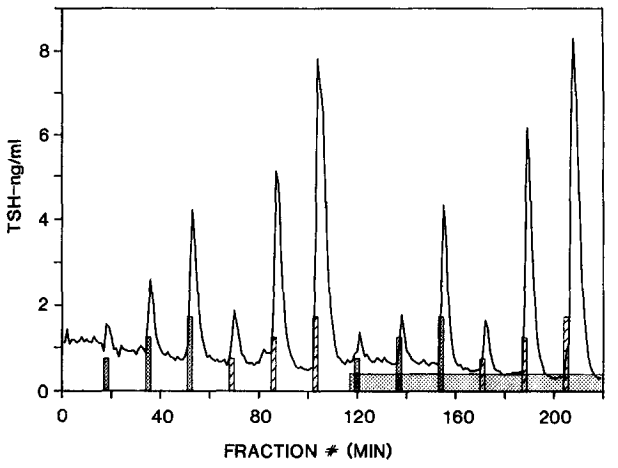

Fig 4. Independence from medium Ca^{2+} concentration of hyposmolar- or urea-induced TSH secretion by dispersed pituitary cells. Two min pulses of KRB diluted with 5, 10, or 20% water (stippled bars) or with isosmolar KRB containing 49, 97.5, or 162.5 mM urea (hatched bars) were given during perifusion of normal KRB containing Ca^{2+} or with Ca^{2+}-free KRB containing 0.1 mM EGTA, indicated by the horizontal stippled bar from 116-220 min. A parallel column run simultaneously in which the Ca^{2+}-free KRB was perifused in the first half and Ca^{2+}-containing KRB in the second half gave identical results.

Parallel pituitary columns were perifused with various concentrations of urea or mannitol in hypotonic DMEM so that the osmolarity of all solutions was maintained at 325 mOsm (Fig 3). TSH secretion was induced only by urea solutions, indicating that secretion was not induced by changes in medium osmolarity or ionic strength or by dilution of one or more essential elements of DMEM.

Parallel columns were perifused with normal or Ca^{2+}-free KRB; the two kinds of media were reversed at the midpoint of the experiment (Fig 4). Secretion induced by either hyposmolarity or urea was not depressed when the medium Ca^{2+} was profoundly reduced but TRH-induced secretion (data not shown) was nearly completely abolished.

To evaluate whether release of hormone by urea might be due to a non-specific toxic effect of the urea with release of hormone coming from dead or dying cells, we incubated acutely dispersed adenohypophyseal cells for 2 h in DMEM containing 162.5 mM urea. Cell viability was checked by the cell uptake of trypan blue. Both before and after the 2 h incubation in urea the quantity of cells/ml was the same and 95% of the cells were viable.

Discussion. This is the first demonstration of hormone secretion induced by permeant molecules. Both urea and glycerol induced an immediate high-amplitude secretion of TSH and PRL which was indistinguishable from that produced by TRH (1,2,5-7) or by hyposmolarity (1). As has previously been shown for hyposmolar stimulation (1), this release of hormone is not due to cell toxicity. Although we have not directly observed the pituitary cells to see if urea or glycerol produce acute cell swelling as reported by Wong *et al* (3), we have directly documented with cinematography that cell swelling induced by medium hyposmolarity occurs over the same 1-2 min period in which the high-amplitude secretion occurs (unpublished). It seems relatively certain that permeant molecules produce the same type of cell swelling as does medium

hyposmolarity since addition of either urea or glycerol in a hyperosmotic medium did not induce secretion, presumably because the relative osmotic pressure on both sides of the plasmalemma was maintained approximately equal or was hyperosmolar on the extracellular side.

Although the dynamics of secretion induced by permeant molecules, hyposmolarity, and TRH are quite similar (1,2,5-7), there is an obvious difference in their mechanism of action. Stimulation of TSH or PRL secretion by acutely dispersed or cultured normal pituitary cells can be induced equally well in a normal or Ca^{2+}-free medium by hyposmolarity or permeant ions whereas stimulation of secretion of these hormones by TRH is strikingly reduced in a Ca^{2+}-free medium (8,9).

In contrast, in the GH cell lines derived from rat pituitary tumors, induction of PRL secretion by TRH is much less dependent on medium Ca^{2+} than is the case in normal pituitary cells (10). Surprisingly, in GH cells secretion induced by hyposmolarity is markedly depressed in a Ca^{2+}-free medium (9). There thus appears to be a significant qualitative difference in the dependence on medium Ca^{2+} for secretion induced by cell swelling or TRH between normal and tumor-derived pituitary cells.

It has been inferred from many studies that Ca^{2+} plays a critical role in the secretory phenomenon (11-14), but it is clear that swelling of acutely dispersed or cultured normal pituitary cells induced by either medium hyposmolarity (9) or entry of permeant molecules into the cytosol does not induce secretion by transport of extracellular Ca^{2+} across the plasmalemma to increase intracellular Ca^{2+} concentration, although this is probably a component of the action of TRH in these cells (8).

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