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Comparison of Hyposmolar and Hyperosmolar Effects on *in Vitro* Luteinizing Hormone Secretion by Anterior Pituitary Cells (41979)

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Abstract. It has previously been described that perifusion of acutely dispersed adenohypophyseal cells with hypotonic medium causes an immediate high-amplitude "on" burst of luteinizing hormone (LH) secretion. In the present report the converse study with hyperosmolar solutions has been made. Perifusion with hypertonic medium depressed LH secretion; return to isotonicity caused an immediate high-amplitude "off" burst of LH secretion closely resembling that induced by hypotonic perifusion. The data give further support to the theory that exocytotic secretion may involve expansion of the outer cell membrane, thus drawing secretory granules to the cell surface where their contents are extruded. © 1985 Society for Experimental Biology and Medicine.

During our current studies of the dynamics of *in vitro* hormone secretion of adenohypophyseal cells induced by various secretagogues, we have observed that diluting the medium with as little as 4% distilled water induces a stimulation of luteinizing hormone (LH) secretion (1). Secretion is proportional to the degree of hyposmolarity and is not due to dilution of the medium ingredients since no secretion is induced if isosmolarity is maintained by dilution with 5% aqueous mannitol. The dynamics of the secretory response to hyposmolarity are very similar to those of the LH response to LHRH and of the TSH and PRL response to TRH in the same *in vitro* preparations, except that hyposmolarity simultaneously stimulates secretion of all hormones we have measured (GH, PRL, TSH, LH, ACTH) whereas the hypothalamic hormones more specifically stimulate their respective target cells (1–3).

So far as we are aware, this phenomenon has not been described previously. We had expected that any efflux of hormone induced by dilution of the medium with water would most likely be due to osmotic lysis of the cells. However, repeated hyposmolar stimulation over a 5-hr experimental period produced similar secretory responses each time and did not depress the stimulation of TSH or PRL secretion induced by the same dose of TRH given at the beginning, middle, and end of the experimental period.

The mechanism by which hyposmolarity induces secretion is unknown, but may be related to the transient swelling of cultured leukemia cells induced by continuous exposure to hyposmolarity. Such exposure induces an immediate increase in cell volume lasting 1–2 min with a return to the initial volume within 5–10 min (4). This closely resembles the temporal pattern of secretion induced by continuous exposure to a given concentration of either water or specific hypothalamic secretogogues (1–3). Our tentative hypothesis is that secretion is induced by expansion of the outer cell membrane which draws preformed secretory granules to the surface where their contents can be discharged (1). Specific secretagogues and hyposmolarity may have the same general mechanism of action except that the response induced by the former is presumably related to phenomena induced by coupling to specific receptors on the cell surface.

If this hypothesis is correct, exposure of the pituitary cells to a hypertonic solution might cause a transient decrease in cell volume associated with a decrease, or at least no stimulation, of pituitary secretion. The cellular mechanisms for homeostatic control of cell volume would be stimulated by the osmotic loss of water induced by the hypertonic external environment to pump water into the cell to restore and maintain a normal cell volume. A return of these "conditioned" cells to an isotonic medium might cause the same transient increase in cell volume and associated secretion observed when the medium is changed from isotonic to hypotonic.

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The data reported herein document that this tentative prediction can be experimentally confirmed.

Materials and Methods. Perifusion of pituitaries from 2-month-old female Simonsen Sprague–Dawley rats in unselected stages of the estrous cycle was performed as described previously (1). An initial equilibration perifusion of Dulbecco's modified Eagle's medium (DMEM) alone was performed for 45 min before the start of the experiment. The DMEM was continuously gassed with 95% O_2–5% CO_2 beginning 1 hr before the experiment. Flow rate was 0.5 ml/min. Fractions were collected at 1-min intervals. The DMEM reservoir and perifusion chamber were maintained at 37°C in a water bath. The DMEM was made hypotonic by diluting with the same glass-distilled, deionized water used to constitute the DMEM. Mannitol was dissolved in DMEM in concentrations between 0.25 and 7.5% to produce hyperosmolarity. Osmolarity of the solutions was determined by freezing-point depression with an Advanced Instruments Model 3W Wide Range Osmometer with a sensitivity and accuracy of 1 mOsm.

LH was measured with a rat radioimmunoassay kit supplied by the National Hormone and Pituitary Program, NIADDK. The stated potency of the standard supplied was utilized in the calculations. All samples from each individual experiment were analyzed in the same single-point assay to avoid interassay variance. All experiments were performed at least twice, with essentially identical results each time.

Results. As in our previous experiments (1), hyposmolarity caused an immediate stimulation of LH secretion characterized by an initial high-amplitude burst lasting 1–2 min followed by a lower level of secretion (usually about double the basal secretory rate) which was maintained as long as the hyposmolar perifusion continued. The amplitude of the secretory peak was significantly correlated ($P < 0.01$) with the degree of hyposmolarity. Return to isosmolar perifusion resulted in a prompt return to the basal secretory rate.

In contrast, perifusion of a hyperosmolar solution induced no change or a depression of secretion during the time the hypertonic medium bathed the cells. Upon return to an isotonic perifusate, a secretory burst proportional to the previous hyperosmolarity occurred. When the hypertonic solution had been perifused for only 1 min, there was a prompt return to the basal secretory rate following the initial spike (Fig. 1). However, more prolonged exposure for 5–20 min depressed secretion during hyperosmolar peri-

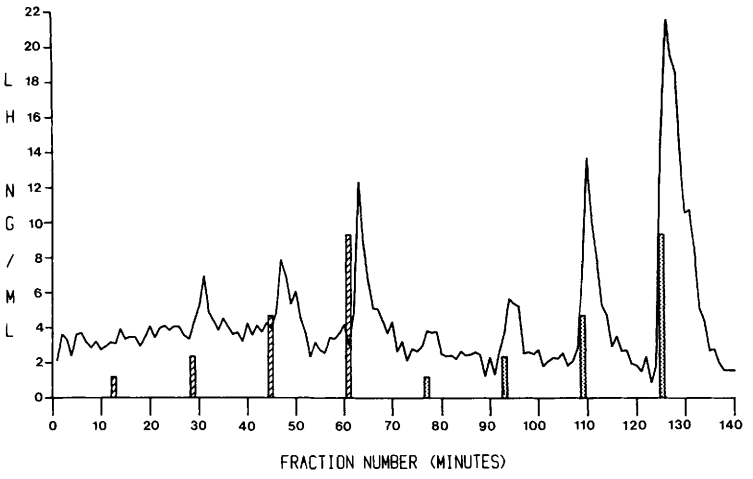

FIG. 1. Effect of 1-min graded hyperosmolar (339, 349, 379, 433 mOsm, hatched bars) and hyposmolar (312, 299, 273, 221 mOsm, stippled bars) perifusion on LH secretion by dispersed adenohypophyseal cells otherwise perifused with DMEM. The relative difference from the osmolarity of DMEM (325 mOsm) is indicated by the height of each bar.

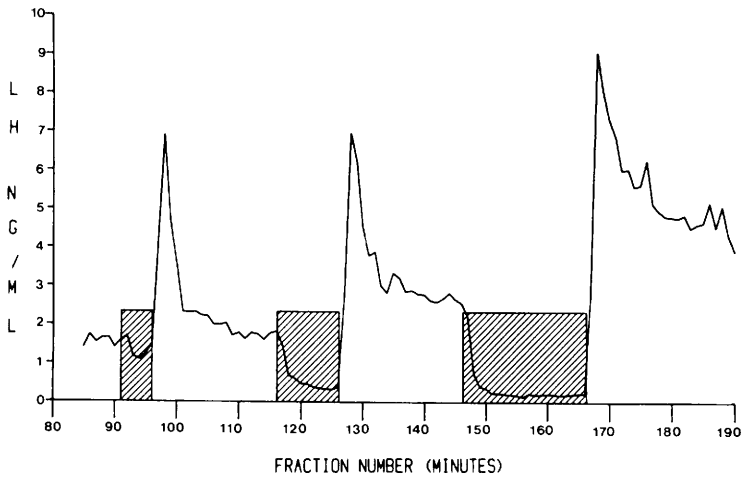

FIG. 2. Effect of perifusion for 5, 10, or 20 min of a 433 mOsm solution (hatched bars) on LH secretion.

fusion. Return to isosmolar perifusion caused the same immediate secretory "spike" seen after a 1-min hyperosmolar exposure, but there was an elevation of subsequent "basal" secretion which was proportional to the period of previous exposure to the hyperosmolar solution (Fig. 2). Basal secretion gradually drifted toward the initial level, but had not achieved this by 30 min.

Extended perifusion of a hypotonic solution conversely induced a depressed rate of basal secretion after return to isosmolar DMEM (Fig. 3). Perifusion of a hyperosmolar medium during this period of depressed basal secretion did not further depress secretion. However, following an initial secretory spike, basal secretion increased > twofold after return to isotonicity. Application of a 1-min hyposmolar stimulus at this time induced a typical secretory spike followed by a return to a lower level of basal secretion.

Sequential perifusion of a hyposmolar and hyperosmolar solution or vice versa gave a secretory response to each compatible with

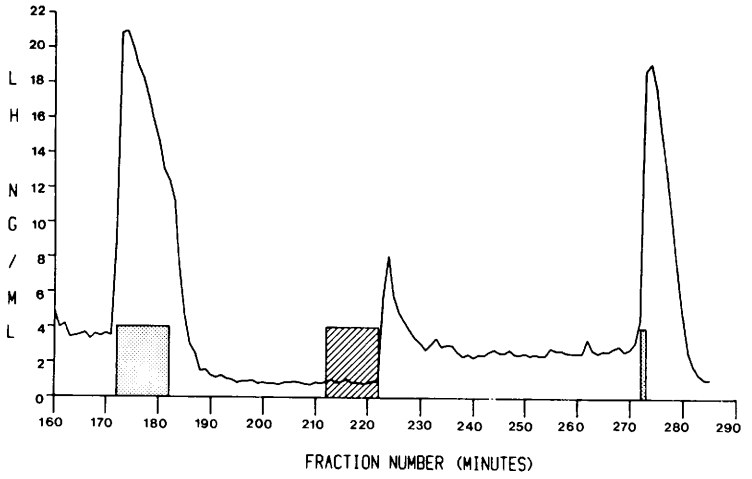

FIG. 3. Sequential perifusion of hyposmolar (214 mOsm, stippled bars) and hyperosmolar (433 mOsm, hatched bar) solutions on LH secretion. A series of hyperosmolar solutions was perifused between 90 and 140 min, accounting for the elevated basal secretion prior to the first hyposmolar perifusion illustrated.

the response to perifusion of either alone (Fig. 4).

Discussion. The present data are compatible with our theory that external hyposmolarity induces adenohypophyseal secretion by producing an increase in cell volume and an expansion of the outer cell membrane. This expansion draws secretory granules to the outer membrane where they are extruded through some undelineated exocytotic process. Contraction of cell volume induced by a hyperosmotic external environment draws the secretory granules away from the outer cell membrane and generally tends to reduce secretion. The relative rather than absolute osmolarity of the external environment appears to be the critical factor, since returning cells to a normal osmotic milieu from a hypertonic environment induces a secretory pattern very similar to that observed if the medium is changed from normal to hypotonic. Although other explanations are possible for our data, we have been unable to think of any more plausible.

The present experiments were concerned with LH secretion. However, similar phenomena have been observed when other hormones were measured (unpublished observations). *In vitro* secretion of several hormones by beef adenohypophyseal slices has been shown to be inversely correlated with medium osmolarity (5), but acute dynamic responses were not examined. We have preliminary data which suggest there may be certain subtle qualitative differences between the release of various hormones to the same change in osmotic environment, but the general pattern is the same for all hormones we have measured.

Fluctuations of plasma osmolarity of ±20%, a range which produces profound changes in pituitary hormone secretion in our *in vitro* system, are compatible with survival in mammals, including man. It is not known whether such fluctuations in plasma osmolarity induce significant changes in pituitary secretion *in vivo*. The secretory pulses would be ephemeral even with continued departure from isosmolarity if *in vivo* changes were similar to those we have observed *in vitro*.

We know of no evidence that localized osmotic changes in the extracellular fluid bathing pituitary cells *in vivo* occur or would have any direct effect on hormone secretion. However, the temporal and qualitative pattern of secretion induced by hyposmolarity and specific secretagogues such as gonadotropin-releasing hormone and thyrotropin-releasing hormone (1–3) are remarkably similar. We have therefore tentatively proposed that specific hypothalamic secretagogues may in-

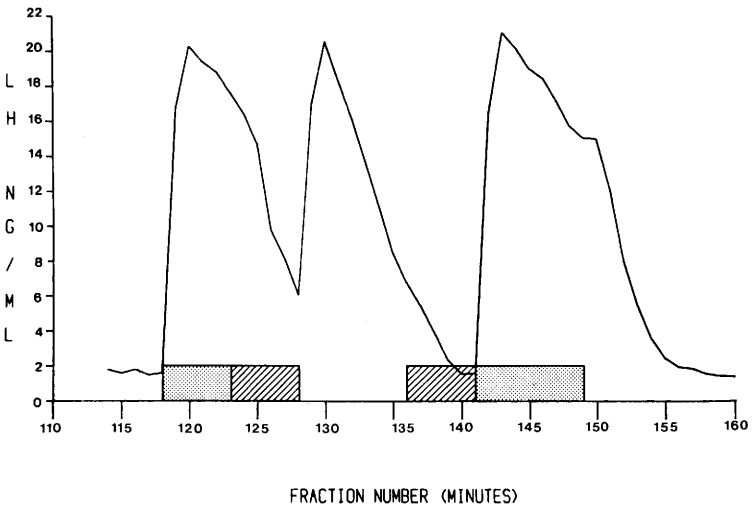

FIG. 4. Effect on LH secretion of immediate sequential perifusion of hyposmolar (220 mOsm, stippled bars) and a hyperosmolar (673 mOsm, hatched bars) solution and vice versa.

duce exocytotic secretion through a receptor-mediated localized expansion of the outer cell membrane (1).

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