present in all tight epithelia where water reabsorption is driven by large transepithelial osmotic gradients, but clearly the subject warrants further study (*27*).

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18. As used here P_{osm} refers to the product of the unit water permeability and total surface area for the individual membranes. The CCT epithelium is composed of principal and intercalated cells present in a ratio of approximately 3:1. Our P_{osm} measurements suggested strongly, but did not prove, that the intercalated cell was also responsive to ADH and that its ratio of basolateral to apical P_{osm} was at least 5:1 in the presence of the hormone. In the absence of ADH, the ratio of basolateral to apical P_{osm} in principal cells and intercalated cells is at least 27:1 and 17:1, respectively.
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20. As described previously (*2*), the optical sectioning technique in CCT does not allow us to detect volume changes of less than 6 to 7%. The mean coefficient of variation for tracing CCT optical sections was 5.7 ± 0.8% (n = 7). Volume changes of 10% or greater, however, can and have been easily detected in previous studies (*2*).
21. The elastic modulus for the basal lamina of rabbit CCT is 0.7×10^8 dyne/cm^2 (*22*). This value can be compared to the elastic modulus of cell membranes, which ranges between 10^{10} and 10^{11} dyne/cm^2 [E. A. Evans and R. M. Hochmuth, *Curr. Top. Membr. Transp.* **10**, 1 (1978)].
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24. The actual amount of cytoplasmic dilution observed in rabbit CCT in situ is probably even less than the 6 to 8% predicted by our P_{osm} measurements for ADH-stimulated tubules perfused with a 130-mos*M* solution. Under normal physiological conditions ADH is probably not released in chronic supramaximal doses as used in in vitro experiments. Furthermore, it seems unlikely that rabbit CCT are ever exposed to osmotic gradients as large as 160 mos*M* during antidiuresis. [See, for example, H. Wirz, *Helv. Physiol. Pharmacol. Acta* **14**, 353 (1956); C. W. Gottschalk and M. Mylle, *Am. J. Physiol.* **196**, 927 (1959)].
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27. Note that water reabsorption in the rat initial collecting duct and connecting segment is driven by extremely large transepithelial osmotic gradients (*24*) and that the ratio of basolateral to apical surface areas in principal and connecting cells of these segments ranges between 10 and 16:1 [B. A. Stanton *et al.*, *Kidney Int.* **19**, 36 (1981)].
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Arginine Vasopressin as a Thyrotropin-Releasing Hormone

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Although hypothyroidism (with concomitant increased levels of thyroid-stimulating hormone) has been associated with elevated plasma vasopressin, the role that vasopressin plays in controlling thyroid-stimulating hormone secretion from the adenohypophysis is not understood. In two in vitro pituitary cell systems, vasopressin caused a specific and dose-related release of thyroid-stimulating hormone from cells that was equal in potency to that elicited by thyrotropin-releasing hormone, the primary acknowledged regulator of thyroid-stimulating hormone release. When injected into the hypothalamus, however, vasopressin specifically inhibited the release of thyroid-stimulating hormone. Thus, vasopressin may exert differential regulatory effects on thyroid-stimulating hormone secretion in the hypothalamus and pituitary gland.

MORE THAN 20 YEARS AGO, VASOPRESSIN had been characterized as a thyrotropin-releasing hormone (TRH) by LaBella (*1*); however, this idea was soon disputed by Guillemin (*2*) and was explored essentially no further after elucidation of the structure of and synthesis of the thyroid-stimulating hormone (TSH)–releasing factor (TRH) (*3–6*). However, the conclusion was based on studies performed with lysine vasopressin that had been extracted from porcine posterior pituitary glands and by the indirect measurement of TSH by bioassay. Since the analog of vasopressin in most mammalian species, including the human and the rat, is the arginine version of the vasopressin nonapeptide (*7*), and, because TSH levels may now be measured directly by radioimmunoassay (RIA), we thus directly investigated the ability of synthetic arginine vasopressin (AVP) to release TSH from cells of the anterior pituitary with static incubation and perifusion of dispersed anterior pituitary cells and RIA for measurement of TSH; these tools were not available when this issue was considered earlier (*1, 2*). TSH functions in general metabolism and calorigenesis through stimulation of the thyroid gland (*8*). AVP is critical in fluid homeostasis, electrolyte balance (*9*), some behaviors (*10*), and the stress responses (*11*). Because AVP is involved in all of these actions and because AVP levels are elevated in conditions of experimental (*12*) and human (*13*) hypothyroidism when TSH levels are also high, we investigated whether AVP

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could function as a significant TSH-releasing hormone.

To determine the direct effect of AVP on TSH release, the anterior pituitary glands of male rats (350 to 400 g) of the Sprague-Dawley strain (Holtzman) were collected upon decapitation, minced into small fragments, and dispersed in the presence of trypsin (Difco) (*14*). Cells were harvested from the trypsinized medium and resuspended in fresh culture medium 199 (Grand Island Biological) with 20 m*M* Hepes buffer, *p*H = 7.4. Cells were aliquoted (4.0×10^5 cells per tube) into polystyrene test tubes in 1 ml of medium for culture at 37°C for 24 hours in room atmosphere. After this time, cells were subjected to low-speed centrifugation, the medium was withdrawn and discarded, and cells were resuspended in either 1 ml of Hepes-buffered culture medium 199 alone or 1 ml of buffered medium 199 plus synthetic AVP or control peptides (Peninsula) at doses ranging from 10^{-9} to $10^{-5}M$. This procedure was repeated in three separate trials with three different vials of synthetic AVP. In addition, in trial 1, synthetic oxytocin (Oxy) at $10^{-6}M$ was included to test for analog specificity; in trial 2, synthetic TRH ($10^{-6}M$) was used to examine cell responsiveness and as a comparison with AVP potency; and in trial 3, synthetic somatostatin (SRIF) ($10^{-6}M$) was used as a contrast to the actions of AVP and TRH. Peptide solutions were incubated with cells in 12- by 75-mm polystyrene tubes for 2 hours under air.

Results from static incubation of cells with AVP (Fig. 1) showed that doses as low as 10^{-9} or $10^{-8}M$ significantly stimulated TSH release in three trials by approximately 25%. In two of three incubations, 10^{-7} and $10^{-6}M$ significantly stimulated a greater TSH release (approximately 40%). In trials 1 and 3, $10^{-5}M$ AVP produced the same degree of TSH release as $10^{-6}M$ AVP. In trial 1, $10^{-6}M$ Oxy did not alter TSH release, whereas $10^{-6}M$ AVP significantly increased TSH secretion to levels produced by $10^{-6}M$ TRH in trial 2 (Fig. 1). SRIF at $10^{-6}M$ significantly decreased the release of TSH in trial 3. Release of prolactin (Prl), growth hormone (GH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were unaffected by any dose of AVP.

Because the accumulation of secreted products in a static incubation system may influence the response of pituitary cells to administered secretagogues, we also examined the effect of AVP on TSH secretion by another method. Cells from anterior pituitary glands of male rats were again dispersed and incubated for 24 hours as described above. Then a dynamic perifusion system was set up in which 8×10^6 dispersed cells were loaded onto each of three Bio-Gel P-2 columns (0.4 by 1.5 cm; Bio-

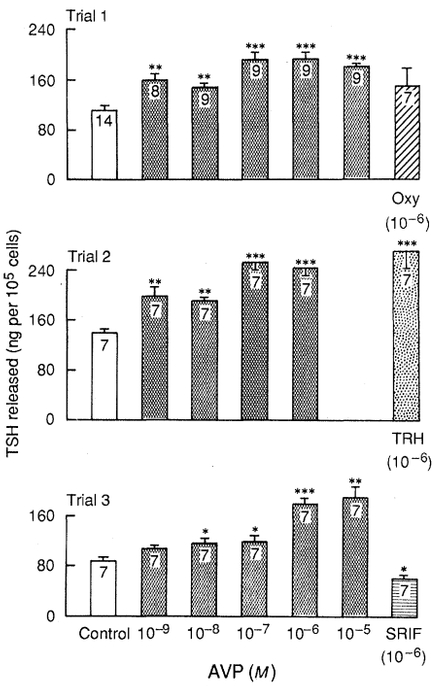

Fig. 1. Effect of increasing concentrations of synthetic AVP (hatched bars) in three separate trials on the release of TSH from trypsin-dispersed cells from the anterior pituitary glands of adult male rats (mean ± SEM). The value of *n* is at the top of each bar. Synthetic Oxy (diagonally striped bar), TRH (stippled bar), and SRIF (horizontally striped bar) were incubated with the same cell preparations at a dose of $10^{-6}M$ for comparison of maximum effects. Open bar indicates the control condition. All peptide solutions were incubated with cells in 12- by 75-mm polystyrene tubes for 2 hours in room atmosphere. Levels of significance shown are *, $P < 0.05$; **, $P < 0.025$; ***, $P < 0.001$ versus control, as determined by analysis of variance and the Student-Newman-Keuls test.

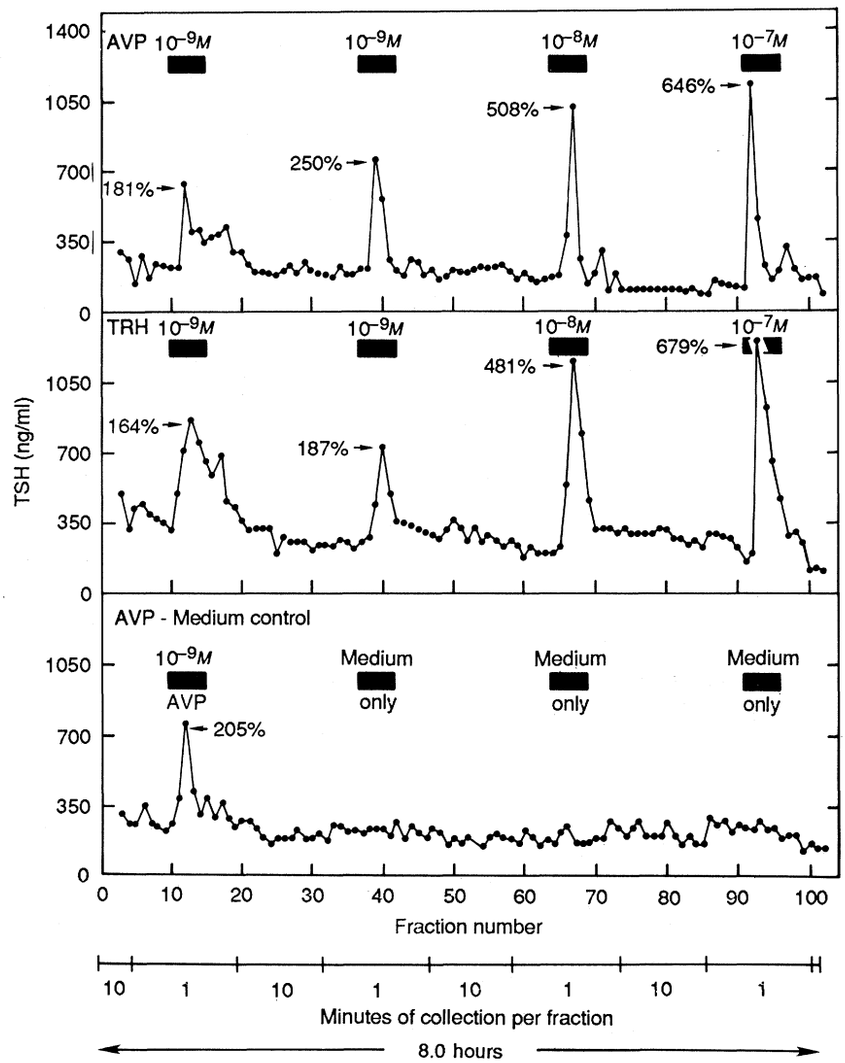

Fig. 2. Effect of perifusion of trypsin-dispersed anterior pituitary cells from male rats in the presence of synthetic AVP, TRH, or control medium alone on the release of TSH. Dispersed pituitary cells (8×10^6) were loaded onto each of three Bio-Gel P-2 columns and perifused with buffered medium 199 as described by Gillies and Lowry (*16*).

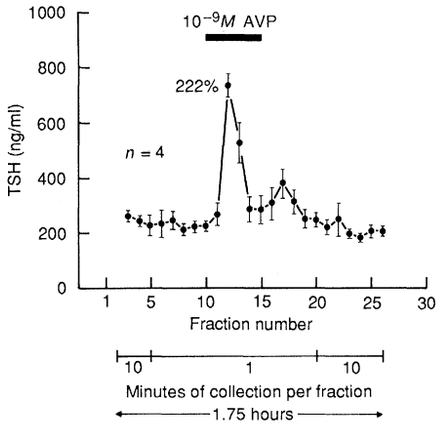

Fig. 3. Effect of perifusion of trypsin-dispersed pituitary cells with synthetic AVP at the concentration ($10^{-9}M$) found in hypophyseal portal blood on the mean levels (± SEM) of TSH secretion. The peak of release (222 ± 15.1%) was statistically significant ($P < 0.001$).

Rad) (*15*). These columns were perifused with medium 199 containing 20 m*M* Hepes buffer according to the methods of Gillies and Lowry (*16*). A period of 3 hours was allowed for perifused cells to achieve a stable baseline of hormone release. The cells were perifused at a flow rate of 0.5 ml per minute, and 1-minute fractions were collected for 5 minutes before the cells were exposed to peptide-containing medium, for 5 minutes during exposure, and for 5 minutes after exposure.

During the intervals between periods of 1-minute collections, cells were perifused with medium without peptide for 120 minutes (recovery periods); fractions were collected every 10 minutes. Perifused cells were exposed to either (i) AVP or TRH at initial doses of $10^{-9}M$ and then to successive doses of 10^{-9}, 10^{-8}, and $10^{-7}M$, or (ii) AVP at an initial dose of $10^{-9}M$ followed by control medium.

Results from the perifusions were expressed as nanograms of hormone released per milliliter of medium per fraction collected. The percentages of stimulation of TSH release were determined by comparing the mean baseline levels of the five fractions obtained before peptide introduction with the highest peak of release during perifusion with the peptide (Figs. 2 and 3).

Perifusion of pituitary cells with increasing logarithmic doses of AVP and TRH (10^{-9} to $10^{-7}M$) produced comparable dose-related peak stimulations of TSH release (Fig. 2), whereas medium alone did not affect TSH secretion (Fig. 2). Two additional perifusion studies with AVP (10^{-8} to $10^{-6}M$) produced similar dose-related stimulations of TSH from cells. The percentage of peak TSH release in Fig. 2 (up to 646% or 679% with $10^{-7}M$ AVP or $10^{-7}M$ TRH, respectively) far exceeded the approximate 40% change in TSH release elicited by 10^{-7} to $10^{-5}M$ AVP or $10^{-6}M$ TRH in static incubations. In the same perifusates, we detected no effect of AVP on the secretion of Prl. Perifusion of cells with Oxy (10^{-9} to $10^{-7}M$) did not influence TSH release.

To establish the reproducibility of the effect of the lowest stimulatory dose of AVP on thyrotrophs, four Bio-Gel P-2 columns, each containing 8×10^6 dispersed pituitary cells, were perifused simultaneously with $10^{-9}M$ AVP in buffered medium as described above. The lowest dose of AVP ($10^{-9}M$) (Fig. 3) produced a peak stimulation of TSH release (222 ± 15.1%) that was statistically significant and corroborative of the results from individual perifusions with $10^{-9}M$ AVP (Fig. 2).

To compare the hypothalamic control of AVP with its direct pituitary cell effect on TSH secretion, we microinjected synthetic AVP into the third ventricle of the hypothalamus of the conscious male rat. Silastic cannulas implanted into the right external jugular vein facilitated sequential blood sampling from undisturbed animals for hormone measurements. Small doses of AVP (5.0 and 0.5 ng), which are below threshold for motor activation (*17*), were injected intraventricularly in 2 μl of sterile 0.9% NaCl vehicle; control animals received vehicle alone. Concentrations of TSH, Prl, LH, FSH, and GH in plasma or medium were determined by RIA (*18*). Statistical significance was determined by analysis of variance and the Student-Newman-Keuls test (*19*).

Intraventricular administration of AVP significantly suppressed basal TSH secretion beginning 5 minutes after infusion. This effect lasted for 60 minutes (Fig. 4). The inhibition produced by both doses was significantly different from values before injection (0 minutes) and from those of saline-treated controls. Other plasma hormone concentrations were unchanged.

AVP releases adrenocorticotropic hormone (ACTH) from the adenohypophysis (*20*). Little has been done since the structure of TRH was elucidated (*3–6*), however, to investigate whether AVP can act as a TSH-releasing factor. One exception was the examination by Fujimoto and Hedge (*21*) of TSH release in the homozygous Brattleboro rat, which has a genetic deficiency of AVP with consequent diabetes insipidus (DI); plasma TSH was elevated in such animals. At first this seemed to contradict our finding that AVP acts directly on normal dispersed pituitary cells to release TSH in vitro. However, Fujimoto and Hedge (*21*) concluded that increased TSH levels in these abnormal animals resulted from reduced sensitivity of

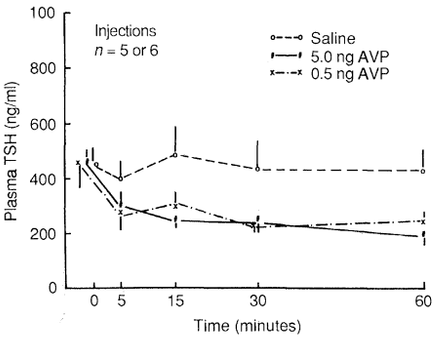

Fig. 4. Effect of injection into the third ventricle of 0.5 or 5.0 ng of synthetic AVP in 2 μl of sterile 0.9% NaCl vehicle or 2 μl of the saline vehicle alone (controls) on plasma levels of TSH (mean ± SEM) in conscious male rats. At 5 minutes, $P < 0.05$ versus 0 minutes (before injection) for both doses. At 30 and 60 minutes, $P < 0.025$ versus 0 minutes and versus saline-treated controls for both doses.

the thyroid gland to TSH, which led to decreased thyroid hormone secretion that, in turn, enhanced pituitary release of TSH. They surmised that elevated TSH was not directly related to the absence of vasopressin. Therefore, the DI Brattleboro rat, with its numerous endocrinopathies (*22*), may not be an appropriate model with which to examine the normal relation between AVP and TSH.

AVP-induced release of TSH from pituitary cells was related to dosage and specific for TSH because LH, FSH, Prl, and GH release were unaltered in static incubation and Prl secretion was unchanged during AVP perifusion. Also, the structural homolog Oxy failed to modify basal TSH release. Stimulation of TSH release by AVP was modest in static incubation when compared with the peaks of release elicited by AVP perifusion. It is possible that accumulation of TSH in static incubation media may exert a local feedback on TSH secretion at the pituitary level (*23*). The successive removal of released TSH during perifusion circumvents this problem and could account for the higher levels of secretion.

AVP is a pressor agent that, if tested by systemic injection in the whole animal, can alter cardiovascular characteristics that perturb the endocrine response of the animal. Consequently, we selected in vitro methods to examine the direct actions of AVP on pituitary TSH secretion. The release of TSH by AVP provides an explanation for the observation that TRH can stimulate AVP release in vivo in humans (*24*) and rabbits (*25*) and in vitro from hypothalamic tissue (*26*) by suggesting that endogenous TRH not only produces TSH release by direct pituitary action, but also by stimulation of AVP secretion to achieve TSH release. In addition, AVP is normally present in hy-

pophyseal portal blood (*27*) at the same concentrations (10^{-9} to $10^{-8}M$) that we find release TSH; AVP is located in the external zone of the median eminence in high concentrations (*28*), as are other releasing factors; AVP receptors are present in the anterior pituitary (*29*); and experimental (*12*) and human (*13*) hypothyroidism results in elevated basal plasma AVP. These observations provide additional support for the hypothesis that AVP is an important, but overlooked, TSH-releasing factor.

Finally, the fact that centrally administered AVP lowers plasma TSH (Fig. 4) but not other hormones is consistent with our observations that hypothalamic-releasing (*14*) or -inhibiting (*30*) factors, at the appropriate ventricular dose, produce effects that oppose their direct actions on their respective target cells of the adenohypophysis. Others (*31*) have supported this concept of negative ultrashort-loop feedback for hypothalamic releasing and inhibiting factors in vitro.

Thus, AVP at physiological concentrations acts specifically on anterior pituitary cells to enhance the release of TSH. The finding that AVP is equipotent with TRH in stimulating TSH release strongly suggests that AVP may indeed be a physiological regulator of TSH secretion. In the hypothalamus, however, AVP may function as a negative autofeedback agent to regulate signals for TSH release.

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Technical Comments

Trans-activator Gene of HTLV-II: Interpretation

We wish to inform the readership of *Science* of a problem in interpretation of our studies of the *trans*-activator gene (*tat*-II) of human T-lymphotropic virus type II (HTLV-II) described in (*1*). In recent studies of cell surface antigen expression, DNA polymorphisms of the DR-β class II major histocompatibility gene complex, and rearrangements of the T-cell receptor β-chain gene locus, we have determined that the sense *tat*-II gene was not introduced into Jurkat T cells, but rather into another T-cell line, probably HUT 78. We cannot detect either interleukin-2 (IL-2) receptor or IL-2 gene expression in the uninfected HUT 78 T-cell line carried in the Dana-Farber laboratory. Thus, the original conclusion that the *tat*-II gene induced the expression of these cellular genes may be correct. However, we have also demonstrated that this HUT 78 T-cell line, while monoclonal at the DR-β gene locus, is a polyclonal population of cells as judged by the presence of multiple T-cell receptor β-chain gene rearrangements. Therefore, it is possible that the expression of the IL-2 receptor and IL-2 genes observed in the four *tat*-II clones studied could reflect the outgrowth of undetectable subpopulations of cells that express these cellular gene products independent of the presence of the *tat*-II gene. However, in support of a specific effect of the *tat*-II protein, the National Cancer Institute and Dana-Farber laboratories have observed that this gene product induces the IL-2 receptor promoter and partially activates the IL-2 promoter in transient cotransfection assays in Jurkat T cells. In addition, using Jurkat on HSB-2 T cells, Inoue and colleagues have described activation of both the IL-2 receptor and IL-2 genes by the *tat*-I gene isolated from HTLV-I (*2*), which shares similar structural and functional properties with the *tat*-II gene. Each of our three laboratories has also confirmed activation of the IL-2 receptor promoter by the *tat*-I gene product. The reintroduction of the *tat*-II gene into Jurkat T cells is currently being attempted.

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