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Vasopressin RNA in the neural lobe of the pituitary: Dramatic accumulation in response to salt loading

(axonal transport/pituicytes/osmoregulation)

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ABSTRACT **The peptides vasopressin (VP) and oxytocin are derived from preprohormone precursers encoded by highly homologous linked genes that are expressed in discrete groups of hypothalamic neurons. The mature hormones are released into the peripheral circulation from the neural (posterior) lobe of the pituitary and have also been implicated in the regulation of anterior lobe. We have used Northern blotting and *in situ* hybridization to RNA in tissue sections to describe the presence, anatomical localization, and regulation of VP and oxytocin RNAs in the pituitary gland itself. We were unable to detect VP transcripts in the anterior and intermediate lobes of the pituitary. Rather we found low levels of VP RNA in the neural lobe. Furthermore, the osmotic stimulation of a 2% (wt/vol) NaCl drinking diet resulted in a marked accumulation of VP RNA in the neural lobe. We suggest that VP, locally synthesized in pituicytes, may have paracrine effects on VP receptors in the neural lobe.**

The vasopressin (VP) gene (1) is expressed in two major hypothalamic neuronal groups directly concerned with pituitary function (2–7): magnocellular cells, which project to the neural lobe of the pituitary and neurosecrete VP directly into the peripheral circulation in response to osmotic or cardiovascular stimuli, and parvocellular cells, which project to the median eminence from which VP travels in the portal blood to act on corticotrophs as a corticotropin releasing factor (6, 8). There is also evidence that another peptide product of the VP gene, a 39-amino acid glycopeptide, may function as a prolactin releasing factor (7).

There have been a number of reports that VP (2, 3), VP–neurophysin (2, 4, 9), and VP RNA (2) can be detected in the anterior pituitary glands of several species and it has been suggested that locally synthesized VP could be involved in the paracrine regulation of anterior lobe function (2). We have used Northern blotting and *in situ* hybridization to analyze the anatomical distribution of VP RNA in pituitaries of water-replete and dehydrated rats and mice.

MATERIALS AND METHODS

Materials. Enzymes and hybridization membranes were purchased from Amersham. Chemicals were obtained from Sigma. Oligonucleotides used in this study were made by Ben Li (National University of Singapore).

Animals. Male Sprague–Dawley (200–250 g) rats or male CBA mice (8–10 weeks old) in a controlled environment were allowed free access to food and either tap water (control) or a 2% (wt/vol) NaCl solution. At the times indicated, animals were decapitated and the brains and pituitary glands were removed. The neurointermediate lobe of the pituitary (NIL) was carefully dissected away from the anterior lobe such that no cross contamination of cells could occur.

Total Cell RNA Preparation. Tissues were homogenized in 500 μl of a solution of 4 M guanidinium isothiocyanate, 25 mM sodium citrate (pH 7), 100 mM 2-mercaptoethanol, and 0.5% sarkosyl by suction through a 25-gauge needle and extracted by the sequential addition of 50 μl 2 M sodium acetate (pH 5.5), 500 μl of water-saturated phenol, and 100 μl of chloroform/isoamyl alcohol, 49:1 (vol/vol). After mixing, incubation on ice for 10 min, and centrifugation (Eppendorf model 5415 microcentrifuge, 5 min, 14,000 rpm), nucleic acids were precipitated from the upper aqueous phase by incubation with 1 ml of 100% ethanol at −70°C for 1 hr. The pellet was recovered by centrifugation, resuspended in 250 μl of homogenization solution, and reprecipitated with ethanol. The pellet was washed with 75% ethanol, dried, and resuspended in water.

Northern Blotting. RNA was fractionated in formaldehyde/agarose gels and transferred to Hybond N according to the instructions of the manufacturer (Amersham). Filters were air-dried and baked at 80°C for 1 hr. RNA was fixed to the filter by UV irradiation for 2 min by using a 312-nm transilluminator and then washed in 50 mM sodium phosphate (pH 6.8) containing 0.1% SDS, equilibrated for 5 min at 65°C in 0.5 M sodium phosphate (pH 6.8) containing 1 mM EDTA, 7% (wt/vol) SDS, and 15% (vol/vol) formamide and hybridized overnight at 65°C with ^{32}P-labeled probes at 10 ng/ml. Oligonucleotide probes to oxytocin (OT) and VP have been described (10) and were 5′-end-labeled using T4 polynucleotide kinase. Fragments of the cloned proopiomelanocortin (POMC) (11) and growth hormone (12) genes were labeled using random primers (13). Filters were washed for 5 min at room temperature and for 15 min at 65°C with 50 mM sodium phosphate (pH 6.8) containing 0.1% SDS and exposed to Kodak K-Omat XAR5 film. Autoradiographs were analyzed using an LKB Ultrascan XL laser densitometer.

Removal of Poly(A) Tails. Poly(A) tails were removed by hybridization of the total cell RNA to oligo(dT) followed by digestion with RNase H, as described (14).

***In Situ* Hybridization.** Oligonucleotide probes were hybridized to RNA in tissue sections as described (10, 15).

RESULTS

Localization of VP Transcripts in Control Rats and Mice. Contrary to an earlier report (2), we were unable to detect either VP or OT transcripts in RNA extracted from anterior pituitary tissue that had been carefully separated from NIL cells and assayed by Northern blotting (for rat, Fig. 1*a*, lane 2; for mouse, Fig. 1*b*, lanes 5 and 9). Rather, in both rodent species, we found VP and OT transcripts in RNA extracted

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Abbreviations: VP, vasopressin; OT, oxytocin; NIL, neurointermediate lobe of the pituitary; POMC, proopiomelanocortin.

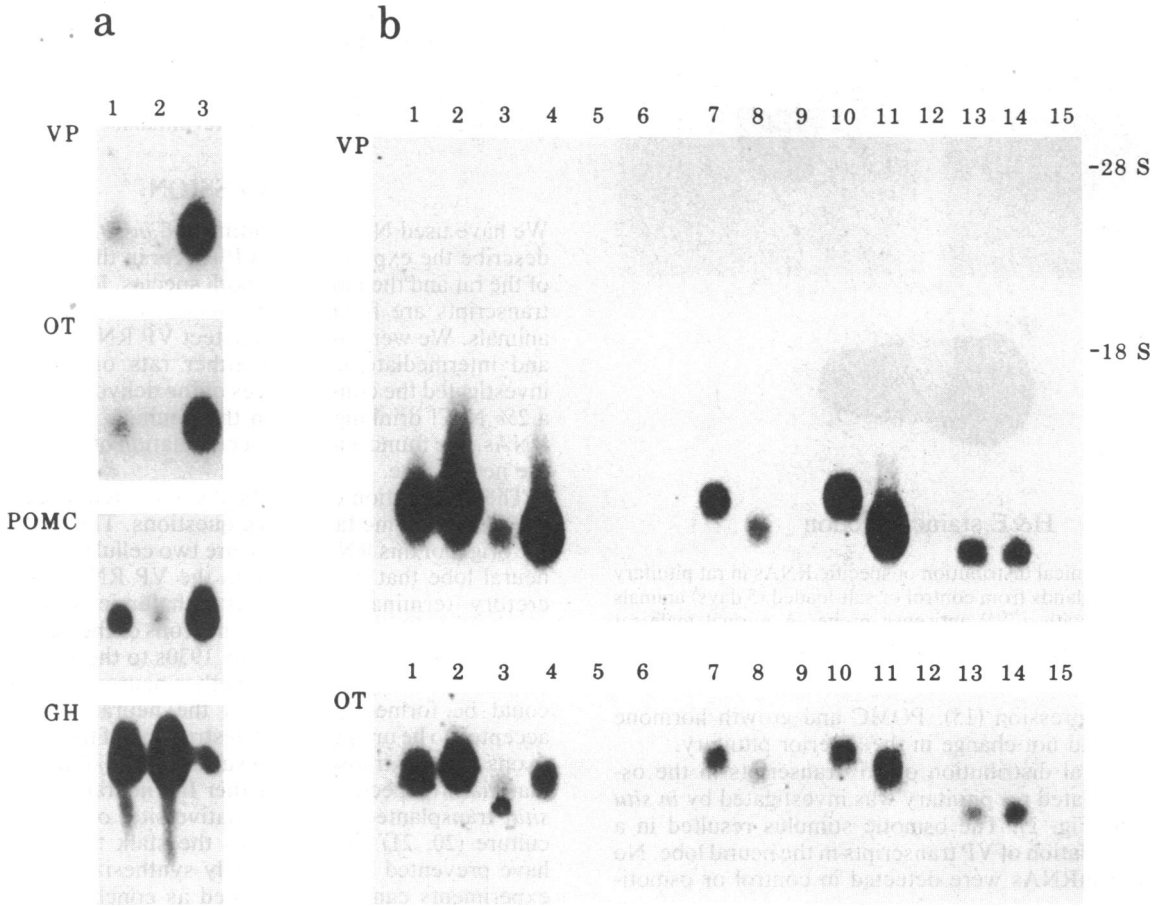

FIG. 1. Northern blot analysis of rat and mouse pituitary RNA. (*a*) Distribution of VP and OT transcripts in the rat pituitary compared to the distribution of POMC and growth hormone (GH) mRNAs. Lanes: 1, 15 μg of whole pituitary total cell RNA; 2, 15 μg of anterior lobe total cell RNA; 3, 15 μg of NIL total cell RNA. (*b*) Distribution of VP and OT transcripts in the mouse hypothalamus–pituitary system—comparison of poly(A) tail lengths and of transcript levels in control mice and in animals subjected to 5 days of salt loading. Lanes: 1 and 2, 5 μg of hypothalamic total cell RNA from control mice and salt-loaded mice, respectively; 3 and 4, 5 μg of NIL total cell RNA from control mice and from salt-loaded mice, respectively; 5 and 6, 5 μg of anterior lobe total cell RNA from control mice and from salt-loaded mice, respectively. Total cell RNAs from control animals. Lanes: 7, 2 μg of hypothalamus; 8, 5 μg of NIL; 9, 50 μg of anterior lobe. Total cell RNAs from salt-loaded animals. Lanes: 10, 2 μg of hypothalamus; 11, 5 μg of NIL; 12, 50 μg of anterior lobe. Control mouse total cell RNAs subjected to RNase H digestion in the presence of oligo(dT). Lanes: 13, 2 μg of hypothalamus; 14, 5 μg of NIL; 15, 50 μg of anterior lobe.

from the NIL (for rat, Fig. 1*a*, lane 3; for mouse, Fig. 1*b*, lanes 3 and 8). The anatomical distribution of VP transcripts in the pituitary gland was then investigated by *in situ* hybridization to RNA in tissue sections. In Fig. 2, a low level of hybridization reveals that VP transcripts are confined to the neural lobe of control male rats. A VP sense probe did not hybridize to any part of the pituitary (data not shown). An identical distribution was observed in the mouse (data not shown).

VP Transcripts in the NIL Are Shorter Than Their Hypothalamic Counterparts as a Consequence of Differential Polyadenylylation. The hypothalamic VP and OT transcripts are longer than those in the NIL in both rat and mouse; here we present our data from the mouse (Fig. 1*b*, compare lanes 7 and 8). It has been shown that the hypothalamic VP and OT transcripts possess unusually long poly(A) tails compared to the mRNAs encoded by the same genes expressed in peripheral tissues (16, 17) and to the average length of hypothalamic mRNA poly(A) tracts (14). Similarly, the difference in size of the NIL and hypothalamic VP and OT RNAs was shown to be a consequence of differential tissue-specific polyadenylylation. RNA samples were subjected to Northern blotting after digestion with RNase H in the presence of oligo(dT), a process that removes poly(A) tracts. The VP and OT RNAs from NIL and hypothalamus from rat (data not shown) and mouse (Fig. 1*b*, lanes 13 and 14) comigrated after this treatment.

Physiological Regulation of VP Transcripts in the Pituitary. Since steroid feedback (18) and osmotic stimuli (10) are known to regulate the expression of the VP gene in the parvocellular and magnocellular hypothalamus, respectively, we asked whether adrenalectomy or the osmotic stimulus of salt loading might also modify VP gene expression at the level of the pituitary gland. After adrenalectomy there was no significant change in NIL VP or OT RNA levels, as assayed by Northern blotting (data not shown). Surprisingly, however, Northern analysis revealed a dramatic increase in VP and OT transcripts in both rat and mouse NIL in response to the hyperosmotic stimulus. Indeed, in the mouse, conditions that evoked a 2-fold increase in hypothalamic VP and OT transcript abundance (Fig. 1*b*, lanes 1 and 2) resulted in an 8-fold increase in NIL VP transcript levels and a 4-fold increase in NIL OT RNA levels (Fig. 1*b*, lanes 3 and 4). A time course of the increase in VP and OT transcript levels in the rat is shown in Fig. 3. Over a 10-day period of salt loading, the NIL levels of VP RNA increased 25-fold and the OT RNA levels increased 8-fold. As a control, POMC mRNA levels were also measured and these fell by 50% in the NIL during the saline load—a change probably related to the accumulation of dopamine in the NIL during osmotic stimulation (19). Dopamine is a well-established inhibitor of intermediate lobe

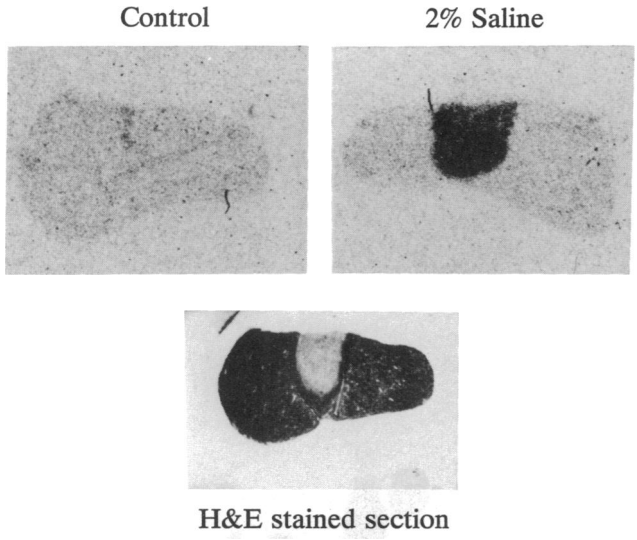

FIG. 2. Anatomical distribution of specific RNAs in rat pituitary glands. Pituitary glands from control or salt-loaded (5 days) animals were hybridized with a VP antisense probe. A normal male rat pituitary stained with hematoxylin/eosin (H&E) is shown for comparison.

POMC gene expression (15). POMC and growth hormone mRNA levels did not change in the anterior pituitary.

The anatomical distribution of VP transcripts in the osmotically stimulated rat pituitary was investigated by *in situ* hybridization (Fig. 2). The osmotic stimulus resulted in a marked accumulation of VP transcripts in the neural lobe. No prodynorphin mRNAs were detected in control or osmotically stimulated neural lobes (data not shown), although this transcript increases in abundance in parallel with OT and VP transcripts in the hypothalamus during salt loading (10), and the mature peptide actually coexists with VP in the same magnocellular cells and nerve terminals.

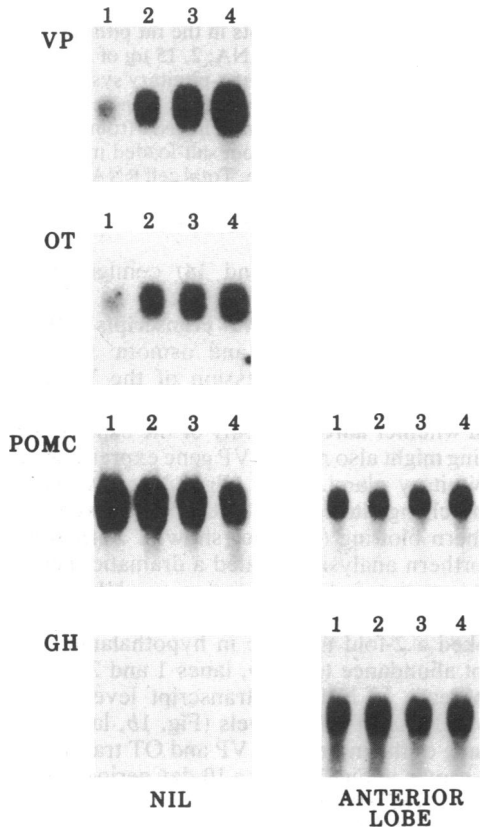

FIG. 3. Northern blot of total cell RNAs extracted from NILs (10 μg) and anterior lobes (25 μg) of male rats subjected to a 2% saline drinking diet for 2 days (lane 2), 5 days (lane 3), or 10 days (lane 4) and compared to control animals (lane 1). GH, growth hormone.

DISCUSSION

We have used Northern blotting and *in situ* hybridization to describe the expression of VP RNAs in the pituitary glands of the rat and the mouse. In both species, low abundance VP transcripts are found in the neural lobes of water-replete animals. We were unable to detect VP RNAs in the anterior and intermediate lobes of either rats or mice. We then investigated the consequences of the dehydration stimulus of a 2% NaCl drinking diet on the pituitary expression of VP RNAs. We found a marked accumulation of VP transcripts in the neural lobe.

The observation of osmotically activated induction of VP RNA raises some fascinating questions. The first deals with the origin of this RNA. There are two cellular elements in the neural lobe that could contain the VP RNA—the neurosecretory terminals of the hypothalamic supraoptic and paraventricular magnocellular neurons or the neural lobe glial cells (the pituicytes). From the 1930s to the 1970s there was considerable dispute as to whether neurosecretory material could be formed *de novo* in the neural lobe. This was accepted to be unlikely since destruction of the magnocellular axons by stalk transection resulted in loss of neurosecretory material irrespective of whether the neural lobe was left *in situ*, transplanted to an alternative site, or placed in tissue culture (20, 21). However, as the stalk transection would have prevented access of newly synthesized mRNA, these experiments cannot be regarded as conclusive. More convincing is the general observation that, although dendrites have been shown to contain mRNA and protein synthetic machinery, axons are devoid of both (22).

We favor the pituicytes as the source of the neural lobe VP transcripts and two lines of evidence support this. (*i*) The VP RNAs in the neural lobe do not have the long poly(A) tail of their hypothalamic counterparts. This suggests that different size classes are synthesized in different cell types as a result of differential polyadenylylation. (*ii*) The neural lobe axon terminals are derived from magnocellular hypothalamic perikarya that accumulate dynorphin mRNAs as well as VP and OT transcripts in response to an osmotic stimulus (10). However, no increase in prodynorphin RNAs was detected in the neural lobe.

It is of considerable interest that an osmotic stimulus activates neural lobe VP RNA expression. There is good evidence that the pituicytes themselves are osmosensitive (23, 24). During periods of low hormone demand pituicytes engulf axon terminals and interpose processes between the terminals and the basement membrane. However, when hormone demand is high (for example, during an osmotic stimulus), pituicytes retract. This morphological response to an osmotic stimulus can be reproduced *in vitro* on isolated NILs without stimulated hormone release, suggesting a direct response of the cells themselves to changes in osmolality (25). It will be of interest to determine whether VP and OT gene expression can be similarly modulated *in vitro* by an osmotic stimulus.

Specific VP receptors have been identified in the neural lobe of the pituitary (26, 27), possibly on or in VP terminals, that may be associated with feedback mechanisms regulating the release of peptides. We suggest that a source of ligand for such receptors could be the pituicytes.

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