Corticotropin-releasing factor mRNA in the hypothalamus is affected differently by drinking saline and by dehydration

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Corticotropin-releasing factor (CRF) stimulates the synthesis and release of adrenocorticotropin in the anterior pituitary and may help maintain fluid and electrolyte balance. 'Salt-loaded' rats had an increase in CRF mRNA in hypothalamic magnocellular neurons of the paraventricular and supraoptic nuclei and a decrease in message in the parvocellular paraventricular neurons. After salt-loaded rats were adrenalectomized, CRF mRNA increased in the parvocellular cells. In contrast to salt loading, water deprivation lead to a decrease in CRF mRNA in magnocellular and parvocellular neurons. These results show that CRF synthesis within separate populations of hypothalamic neurons is regulated differently under various conditions.

(Paraventricular nucleus, Supraoptic nucleus) *Adrenalectomy* *Hybridization* *Pituitary*

1. INTRODUCTION

Fluid and electrolyte balance is maintained through a complex interplay of neuronal, hormonal, and osmotic signals. The adrenals are essential for this regulation [1]. Pituitary adrenocorticotropin hormone (ACTH) stimulates adrenal glucocorticoid synthesis while the amino terminus of the ACTH precursor, pro-opiomelanocortin (POMC), may stimulate mineralocorticoid production [2,3]. The synthesis of POMC in the anterior pituitary is controlled, in part, by corticotropin-releasing factor (CRF) [4].

Two major sites of CRF synthesis are the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus [5–9]. Within these nuclei, CRF is colocalized with oxytocin in about one-third of the magnocellular neurons [10,11] and with vasopressin in most of the parvocellular neurons of the PVN [12–14]. The function of the CRF synthesized by the oxytocin neurons is unknown. Presumably, the parvocellular CRF neurons, which project to the median eminence [15,16], are normally responsible for stimulating ACTH production in the anterior pituitary [4,17]. Consistent with this, CRF and CRF mRNA increase in the parvocellular neurons after adrenalectomy and hypophysectomy [7,18–20].

To gain a better understanding of the possible role of CRF in maintaining fluid and electrolyte balance, we examined the effect of giving rats saline or nothing to drink on hypothalamic CRF mRNA levels. These observations were made using in situ hybridization histochemistry with an ^{35}S-labeled synthetic oligodeoxynucleotide probe [20].

2. MATERIALS AND METHODS

Male, Sprague-Dawley rats (175–250 g, NIH) were housed with a 12 h on, 12 h off lighting schedule. Eight rats were given food and 2% NaCl ('salt-loaded') for 1 week and four rats were denied water for 3 days ('dehydrated'). We have shown previously that adrenalectomy leads to increased CRF mRNA levels in the parvocellular division of the PVN [20], so we also adrenalectomized four rats under ether anesthesia and salt-loaded them for 1 week.

The rats were killed under ether anesthesia and the brains removed, frozen on dry ice, and stored

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at −80°C. Frozen sections (12 μm) were then cut and thaw-mounted onto twice gelatin-coated slides. To avoid variation in cell distribution, only those sections containing the PVN and SON 1.8–1.9 mm caudal to the level of the bregma (see fig.3 of [7]) were used in this study.

The tissues were processed and hybridized as described [20] with minor modifications. Briefly, the sections were warmed at room temperature for 10 min and then placed in 0.25% acetic anhydride in 0.1 M triethanolamine/0.9% NaCl, pH 8, for 10 min at room temperature. They were then passed through a graded series of ethanols, chloroform, and ethanol before air-drying. Subsequently, 0.5–2.0 × 10^6 dpm of the CRF probe in 45 μl hybridization buffer (containing 0.66 M sodium and 50% formamide) were applied to each section. The hybridizations were performed for 22–24 h at room temperature or 37°C and the sections then were washed in four 15 min rinses in 2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2)/50% formamide at 40°C. After two 1 h rinses in 2 × SSC at room temperature and two rapid water dips to remove salts, the sections were dried. They were then apposed to NTB3 (1 : 1 with water, Kodak) nuclear emulsion-coated coverslips or dipped directly into the emulsion. The autoradiograms were developed after 2–6 weeks and the tissues stained with cresyl violet.

The CRF probe was complementary to the mRNA coding for amino acids 22–37 of CRF proper [21]. Details of its preparation, labeling, and specificity of hybridization have been described [20]. The specific activities of the probe used here were 3000–8000 Ci/mmol.

3. RESULTS

In agreement with our previous study [20], CRF mRNA in control animals was found in the parvocellular neurons of the PVN (fig.1A). However, when the hybridizations were performed at 37°C, labeling was also seen over magnocellular neurons in the same pattern of distribution as oxytocin neurons [22,23], surrounding the core of vasopressin neurons and in the ventral aspects of the PVN (cf. figs 1A and 2A) and in the dorsal SON (not shown). In these sections, cells containing CRF mRNA were also observed in the central nucleus of the amygdala and neocortex, in agreement with immunocytochemical studies [5,6,8,9].

After salt loading, the labeling decreased in the parvocellular portion of the PVN (figs 1B,2C) and increased in the magnocellular cells (fig.1B). The

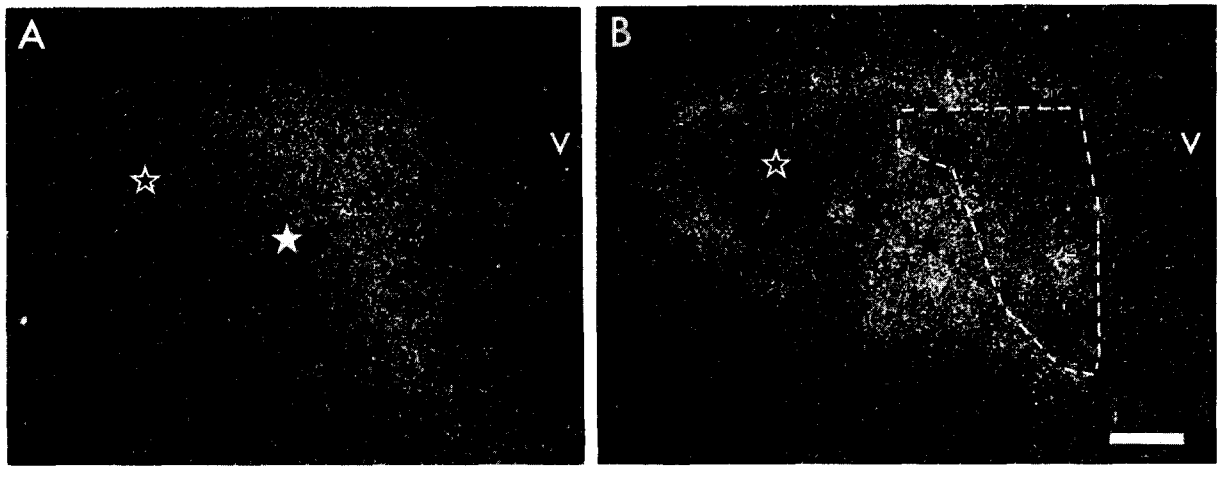

Fig.1. CRF mRNA in the PVNs from control (A) and salt-loaded (B) rats detected using an ^{35}S-labeled probe and in situ hybridization histochemistry. The hybridization was performed at room temperature. In these dark-field photomicrographs, the autoradiographic grains appear white. The parvocellular region is labeled in the control and has decreased labeling after salt loading (outlined). The hollow stars are placed over the vasopressin cores and solid stars over the regions which contain oxytocin magnocellular neurons (which also encircle the vasopressin core in B). Note the increased CRF mRNA detected in the oxytocin areas after salt loading. V is the third ventricle. Bar, 100 μm.

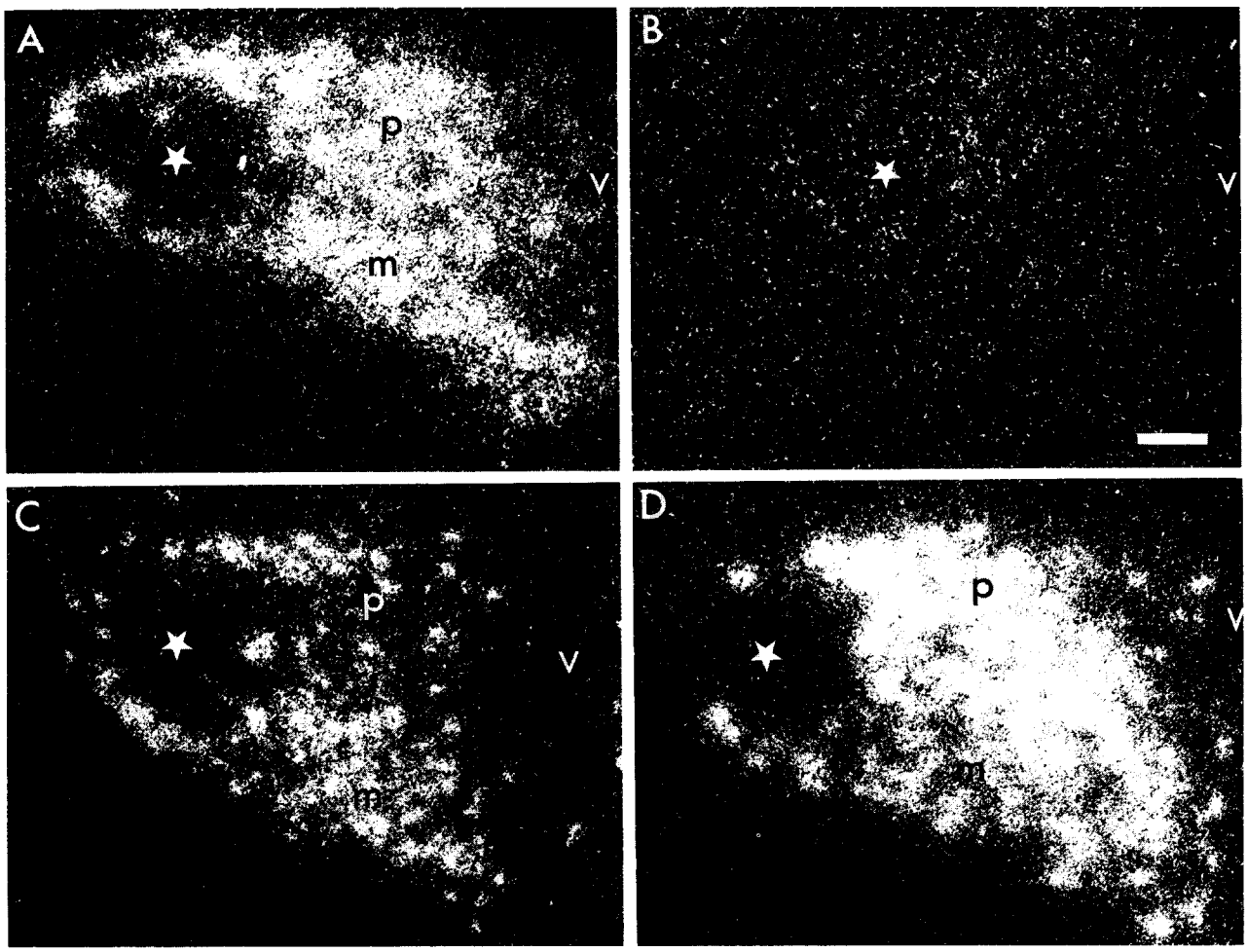

Fig.2. CRF mRNA in the PVNs from control (A), dehydrated (B), salt-loaded (C), and salt-loaded adrenalectomized (D) rats. The hybridizations were performed at 37°C. The dehydrated rat had a general decrease in CRF mRNA while the salt-loaded rat had a selective decrease in CRF mRNA in the parvocellular (p) PVN. There was also a slight increase in CRF mRNA over the magnocellular (m), oxytocin neurons which is not evident photographically (see fig.1). Adrenalectomy in the salt-loaded rat caused an increase in the parvocellular PVN to above control levels. V is the third ventricle and the star indicates the vasopressin neuron-containing core of the PVN. Bar, 100 μm.

labeling over the dorsal SON also increased after salt loading (not shown). In the salt-loaded adrenalectomized animals, the labeling over both the parvocellular and magnocellular cells was greater than controls (fig.2D). Finally, in the dehydrated rats there was a decrease in labeling over both cell types in the PVN (fig.2B) and SON.

4. DISCUSSION

At present, the factors which eventually influence CRF synthesis and the pathways through which they are effected are only partly understood [17]. Salt loading [24] and, to a greater extent, dehydration [25] lead to increases in plasma osmolality. This may be the common stimulus that leads to decreased CRF mRNA in the parvocellular PVN. It is possible that these effects are mediated through a change in adrenergic tone [26]. Apparently, this is not a powerful enough stimulus to override the effect of adrenalectomy on CRF mRNA levels there (at least when paired with salt loading).

On the other hand, the opposite effects of salt loading and dehydration on magnocellular CRF mRNA levels would suggest at least two different mechanisms are involved. Salt loading is accompanied by only a modest increase in plasma osmolality whereas dehydration produces a large increase in addition to a profound contraction of the extracellular fluid space [24,25]. These two manipulations potentially influence many components involved in maintaining fluid and electrolyte homeostasis, including peripheral and brain renin-angiotensin systems, vasopressin release, volume receptors, baroreceptors, osmoreceptors, and receptors for mineralocorticoids [27–32]. These components, in turn, relay information to the PVN through pathways originating in various brainstem nuclei (e.g. nucleus of the solitary tract, A1 cell group) and circumventricular areas (e.g. subfornical organ, anteroventral third ventricle) [29,33]. Furthermore, there also exists the possibility for interactions between peptidergic neurons within the PVN. For example, Plotsky [34] has shown that intraventricular injections of CRF reduced portal plasma levels of vasopressin and oxytocin. In view of these complexities, it is, perhaps, not surprising that salt loading and dehydration resulted in different effects.

The role of CRF in the oxytocin neurons is unknown. It may participate in local regulation within the PVN and SON as discussed above. CRF may also help regulate secretion of oxytocin and vasopressin from axon terminals in the posterior pituitary, as has been postulated for dynorphin [35]. Finally, CRF may reach the anterior pituitary via the portal circulation along with oxytocin and vasopressin [36–39], to stimulate ACTH synthesis and release. Although portal plasma levels of CRF do not increase in response to hemorrhagic stress when release from the PVN is blocked [40], the situation could well be different after salt loading. These and other questions, such as the control of CRF synthesis during pregnancy and lactation, should be amenable to study.

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