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Long-Term Salt Loading Impairs Pituitary Responsiveness to ACTH Secretagogues and Stress in Rats

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DOHANICS, J , K J KOVACS, G FOLLY AND G B MAKARA *Long-term salt loading impairs pituitary responsiveness to ACTH secretagogues and stress in rats* PEPTIDES **11**(1) 59–63, 1990 —Male Wistar rats were allowed to drink tap water ad lib (W), 2% saline (S) or 2% saline containing dexamethasone (S+D, 1 mg/l) for 7 days On the 8th day rats were subjected to a 3-min ether stress. Plasma ACTH, corticosterone and prolactin concentrations were determined before and after ether exposure Prestress concentrations of plasma ACTH were low and did not vary among the three groups In response to ether stress W rats exhibited twice as high plasma ACTH concentrations as did S rats. Rats of the S+D group exhibited a small but statistically significant ACTH response Plasma corticosterone concentration in S rats was increased while in S+D rats was significantly decreased under resting conditions compared to that in W rats Ether stress caused large increases in plasma corticosterone concentrations in W and S rats while a small but statistically significant increase was observed in S+D rats. Prolactin responses to ether were smaller in groups S and S+D than in group W To test whether the decreased ACTH response to ether exposure was a result of a decreased sensitivity of corticotrope cells to corticotropin releasing factor (CRF)-41 or arginine vasopressin (AVP), adenohypophysial fragments from W, S and S+D rats were incubated in the presence of different doses of CRF-41 or AVP Pituitary fragments obtained from W rats secreted larger amounts of ACTH than did pituitaries from S rats in response to either CRF-41 or AVP CRF-41 caused only a slight increase and AVP caused no significant increase in ACTH release from pituitary fragments obtained from S+D rats These results indicate that prolonged osmotic stimulation impairs ACTH and prolactin but not corticosterone responses to ether stress We suggest that the decreased sensitivity of corticotropes to CRF-41 and AVP is a possible mechanism that could account for the deficient ACTH response to ether stress in S rats.

Osmotic stimulation Ether stress ACTH Corticosterone Prolactin Dexamethasone CRF-41 Arginine vasopressin

REGULATION of adenohypophysial ACTH secretion is primarily under the control of parvocellular neurones located in the hypothalamic paraventricular nucleus (PVN) [for review see (2)]. These neurones synthesize and release corticotropin releasing factor (CRF)-41 (1,3) and arginine vasopressin (AVP) (19, 34, 37), peptides with most potent ACTH-releasing ability. While CRF-41 and AVP supplied by parvocellular neurones of the PVN are believed to regulate ACTH secretion under most conditions (4,25), other hypothalamic neurones synthesizing these peptides or other substances with ACTH-releasing ability are thought to influence ACTH secretion as well. Studies employing neurolobectomized rats showed that such animals had a decreased response to certain types of stress (7,11), suggesting a role for magnocellular hypothalamic neurones in the regulation of ACTH secretion.

Magnocellular neurones are known to respond to osmotic stimulation with an increased secretion of AVP and oxytocin (OXY) (6,33). Recent studies have shown that osmotic stimulation also increases the synthesis rate of CRF-41 in magnocellular neurones while decreasing it in parvocellular neurones (8, 21, 40), and increases neurohypophysial CRF-41 content (9,16). Despite these profound changes in parvocellular and magnocellular neuronal function, no changes were observed in basal plasma ACTH concentration in long-term osmotically stimulated rats (9). In this study we assessed the ACTH, corticosterone and prolactin responses of long-term osmotically stimulated rats to ether stress Here we report that long-term osmotic stimulation results in decreased ACTH and prolactin responses to stress and the decreased ACTH response may be due to a decreased sensitivity of corticotropes to CRF-41 and AVP

METHOD

Male Wistar rats weighing 250–300 g were kept 5 per cage

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under controlled conditions of temperature and humidity (24+1°C, 55–75% humidity). Lights were on from 600 to 1900. Rat food was available ad lib Tap water, 2% saline or 2% saline containing 1 mg/l dexamethasone (DEX) (Oradexon, Organon, Oss, Netherlands) were available ad lib for the groups labeled W, S or S+D, respectively. Seven days after rats began to drink one of these fluids, they were exposed to a single 3-min ether stress Experiments took place between 9·00 and 11·00 a.m. Blood samples for determination of basal levels of ACTH, corticosterone and prolactin were taken by decapitation from rats that had not been exposed to ether. Three min after the beginning of ether exposure blood samples for ACTH and prolactin determinations were taken by jugular puncture. Blood samples obtained by decapitation 30 min after the beginning of ether exposure were saved for corticosterone determination.

Rats drinking one of the fluids specified above for 7 days were decapitated in order to obtain anterior pituitaries Anterior pituitaries were cut quickly into 8 approximately equal pieces and put into plastic beakers containing 200 µl of Krebs-Ringer solution (100 ml of Krebs-Ringer solution consisted of. 0 9% w/v NaCl 76.9 ml; 1.15% w/v KCl 3.0 ml, 1 22% w/v $CaCl_2$ 2 3 ml, 2 11% w/v KH_2PO_4 1 ml, 3 82% w/v $MgSO_4 \times 7H_2O$ 1 ml; 1.3% w/v $NaHCO_3$ 16.1 ml, containing 200 mg glucose, 20 µmol ascorbic acid and 100 mg bovine serum albumin). The solution was saturated by a mixture of O_2 (95%) and CO_2 (5%) Beakers were rinsed during preincubation and incubation and temperature was set to 37°C. The Krebs-Ringer medium was three times renewed during the 4 hours of preincubation period. After preincubation, medium was removed and replaced by Krebs-Ringer solutions containing various concentrations of CRF-41 (rCRF-41, Bachem Feinbiochemikalien A. G., Bubendorf, Switzerland) or AVP (Peninsula Laboratories, Belmont, CA) One hour later the incubation medium was removed, centrifuged at 4°C and the supernatant saved for ACTH determination.

Adrenocorticotropin concentrations in plasma and incubation medium samples were determined by the method of Nicholson *et al.* (31) Plasma corticosterone concentrations were determined as described elsewhere (28). Plasma prolactin concentrations were assayed using reagents provided by the NIADDKD, National Hormone and Pituitary Program. The assay sensitivity was 0.1 ng using 10 µl plasma Intraassay variation was 6%; all samples were assayed in a single run.

Statistical analysis was performed on the logarithmic transforms of data using analysis of variance followed by Dunn's test for multiple comparisons (10) Mean ± SE's are presented.

RESULTS

Basal plasma ACTH concentrations averaged less than 8 pmol/l in all groups. Following ether stress, group W rats displayed plasma ACTH concentrations more than 2 times higher than did rats of group S. Rats of group S+D exhibited a small increase in plasma ACTH in response to ether, $F(5,80) = 50\ 12387$, $p<0\ 01$ (Fig. 1). Under resting conditions, mean plasma corticosterone concentration in S rats was increased, $F(5,81) = 41.38926$, $p<0.05$, while it was decreased in S+D rats compared to that in W rats, $F(5,81) = 41.38926$, $p<0.01$. Ether stress caused much larger increases in plasma corticosterone in W and S rats than it did in S+D rats [$F(5,81) = 41.38926$, $p<0.01$ for both comparisons] (Fig. 2). Plasma prolactin also increased in all groups in response to ether exposure, $F(5,80) = 23.14111$, $p<0.01$ Rats drinking water showed a larger increase in plasma prolactin than did either S or S+D rats, $F(5,80) = 23.14111$, $p<0.05$. No difference was observed in plasma prolactin responses to stress between groups S and S+D (Fig 3)

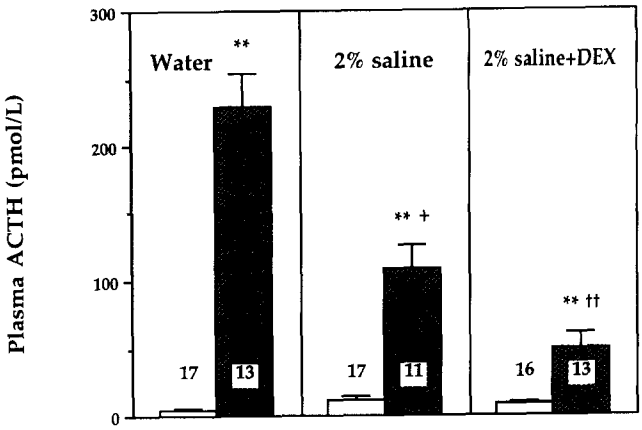

FIG 1 Plasma ACTH concentrations under resting conditions and after a 3-min ether stress in rats allowed to drink tap water ad lib, 2% saline or 2% saline containing 1 mg/l dexamethasone for 7 days Numbers in or above bars show numbers of rats $F(5,80) = 50\ 12387$, $^{**}p<0\ 01$ compared with resting controls, $+p<0\ 05$ interaction between the effects of 2% saline drinking and ether stress, $\dagger\dagger p<0\ 01$ compared with 2% saline-drinking stressed rats by analysis of variance Open bars resting, striped bars stress

Adenohypophysial fragments from rats drinking 2% saline secreted less ACTH than did those from water-drinking rats in response to CRF-41 stimulation An attenuated response in ACTH secretion by pituitary fragments from S+D rats was observed in response to CRF-41 stimulation (Fig. 4). Pituitary fragments from S rats released less ACTH than did those from W rats in response to AVP stimulation. No significant increase in ACTH secretion was produced by pituitary fragments from S+D rats when exposed to AVP stimulation (Fig. 5)

DISCUSSION

Results of this study indicate that long-term osmotic stimulation by 2% saline drinking increases basal plasma level of

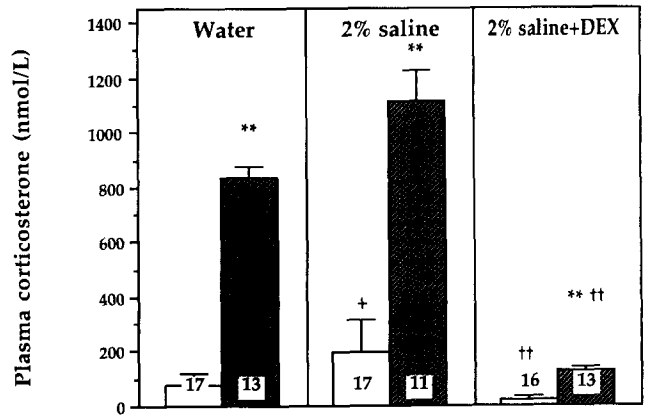

FIG 2 Plasma corticosterone concentrations under resting conditions and after a 3-min ether stress in rats allowed to drink tap water ad lib, 2% saline or 2% saline containing 1 mg/l dexamethasone for 7 days Numbers in or above bars show numbers of rats $F(5,81) = 41\ 38926$, $+p<0\ 05$ compared with water-drinking rats, $^{**}p<0\ 01$ compared with resting controls, $\dagger\dagger p<0\ 01$ compared with water-drinking rats by analysis of variance Open bars resting, striped bars stress

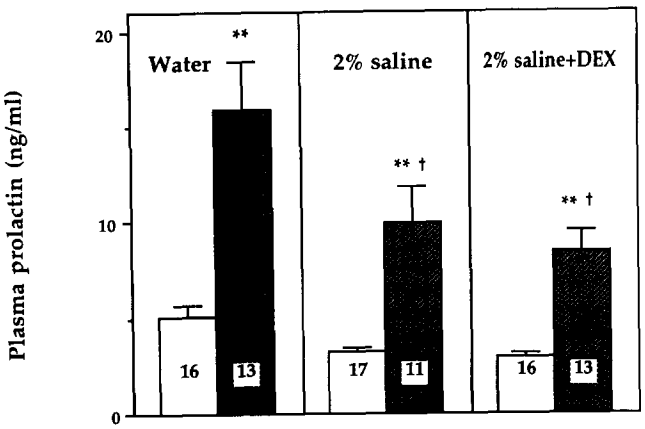

FIG 3. Plasma prolactin concentrations under resting conditions and after a 3-min ether stress in rats allowed to drink tap water ad lib, 2% saline or 2% saline containing 1 mg/l dexamethasone for 7 days Numbers in bars show numbers of rats $F(5,80) = 23\ 14111$, $^{**}p<0\ 01$ compared with resting controls, $\dagger p<0\ 05$ compared with ether-stressed, water-drinking rats by analysis of variance Open bars resting, striped bars stress

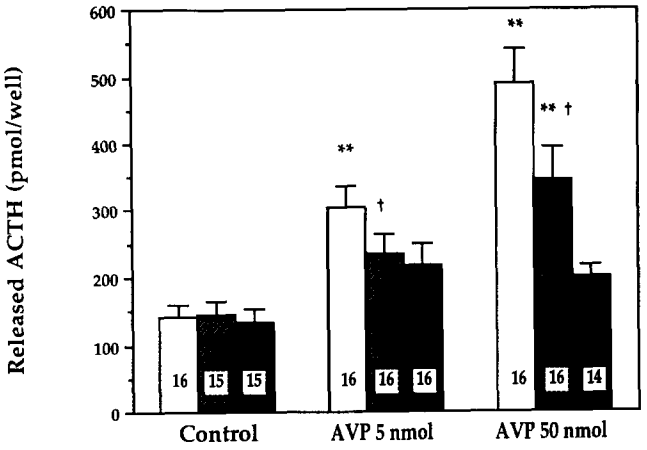

FIG. 5 AVP-stimulated ACTH release by pituitary fragments obtained from rats allowed to drink tap water ad lib, 2% saline or 2% saline containing 1 mg/l dexamethasone for 7 days. Numbers in bars show numbers of pituitary fragments $F(8,131) = 13\ 67538$, $^{**}p<0\ 01$ compared to nonstimulated controls, $\dagger p<0\ 05$ integrated response by pituitaries from W rats vs S rats by analysis of variance Open bars water, striped bars 2% saline, solid bars 2% saline + DEX

corticosterone, but not that of ACTH It also decreases plasma ACTH and prolactin but not corticosterone responses to ether stress. In addition, a decreased sensitivity of corticotropes to both CRF-41 and AVP was also observed.

ACTH secretion is regulated by a complex hypothalamic mechanism and by adrenal glucocorticoids (2). Parvocellular neurons in the PVN are a key part of this mechanism (4,25) by secreting CRF-41 and AVP into the portal circulation. Osmotic stimulation was shown to decrease CRF-41 staining of parvocellular neurones in colchicine-treated rats (8), suggesting a decreased synthesis rate (40). If, indeed, a decrease in the synthesis rate of CRF-41 occurred, it is possible that release of this peptide from parvocellular axon terminals in the external zone of the median eminence was also decreased. Such a mechanism could

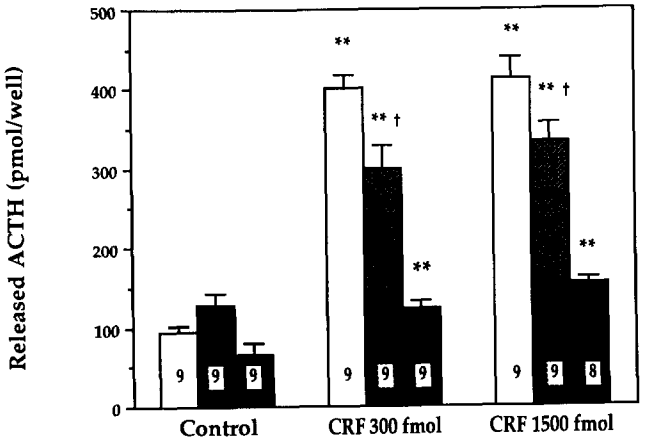

FIG 4 CRF-41-stimulated ACTH release by pituitary fragments obtained from rats allowed to drink tap water ad lib, 2% saline or 2% saline containing 1 mg/l dexamethasone for 7 days Numbers in bars show numbers of pituitary fragments $F(8,71) = 61\ 06$, $^{**}p<0\ 01$ compared to nonstimulated controls; $\dagger p<0\ 05$ integrated response by pituitaries from W rats vs S rats by analysis of variance. Open bars water, striped bars 2% saline, solid bars 2% saline + DEX

account for the deficient ACTH response to stress in osmotically stimulated rats; however, no results of portal plasma measurements of CRF-41 are available to support this hypothesis.

In addition to parvocellular neurones, magnocellular neurones have also been suggested to contribute to the regulation of ACTH secretion since neurolobectomy resulted in an impaired ACTH response to certain stressors (7,11) and electrical stimulation of the neurohypophysis caused an increase in plasma corticosterone in PVN-lesioned rats (24). Substances produced by magnocellular neurons can reach the anterior pituitary through the short portal vessels (30) and the neurohemal interface present in the internal zone of the median eminence (32) which contains magnocellular axons supplying the neurohypophysis. An in vitro experiment demonstrated AVP release from these axons (13) Increases in the synthesis and release rates of AVP and OXY (6, 23, 33, 36) are well-known effects of chronic osmotic stimulation on these neurones. Recent observations showed that the synthesis rate of CRF-41 produced by oxytocinergic neurones (5,35) is also increased (8, 21, 40) in response to osmotic stimulation. Thus, in our experiments, if there was any deficit in CRF-41 and AVP secretion by parvocellular neurones, magnocellular neurones could compensate or even overcompensate for such a deficit so the exposure to ACTH secretagogues of corticotropic cells was unchanged or even increased during osmotic stimulation. Exposure to excessive amounts of CRF-41 and AVP is known to down regulate their respective receptors on corticotropes (20,39) Moreover, AVP by itself has the ability to down regulate CRF-41 receptors of the pituitary (14), thus down regulation of CRF-41 receptors could have occurred even in the absence of an increased CRF-41 secretion. The decreased ACTH output of hypophysial fragments obtained from 2% saline-drinking rats to CRF-41 and AVP may indicate a down regulation of CRF-41 and AVP receptors which occurred during osmotic stimulation Therefore, we suggest that the decreased stress response in plasma ACTH concentration is a result, at least in part, of a decreased sensitivity of corticotropes to CRF-41 and AVP.

We have detected an increased basal corticosterone concentration in 2% saline-drinking rats compared to water-drinking controls. Although this increase was small, and in a similar experiment

previously performed in this laboratory the difference between basal corticosterone levels of W and S rats was statistically not significant, it probably reflects a mild, chronic stress caused by a persisting thirst as a result of 2% saline drinking. Since circulating glucocorticoids are known to inhibit ACTH secretion (18) it could be presumed that in our experiments the elevated basal level of corticosterone in S rats had an inhibitory effect on ACTH secretion caused by ether stress. A study by Vernikos *et al.* (38), however, demonstrated no deficit in ACTH response to an acute stress (IP saline) that was superimposed on a chronic stress (exposure to cold) They also found that a higher dose of prednisolone was required to inhibit stress response to ether in rats maintained at 4°C than in rats maintained at 22°C. They interpreted the failure of the elevated plasma corticosterone level caused by the chronic stress to decrease the ACTH response to an acute stress by a reduced effectiveness of the corticosteroid negative feedback. They have also found a slightly increased plasma level of ACTH. In our study the difference between basal levels of plasma ACTH between W and S rats was not statistically significant These results do not support the hypothesis that the elevated basal corticosterone level exhibited by S rats caused the diminished ACTH response to ether.

The diminished ACTH responses to ether stress exhibited by osmotically stimulated rats were followed by corticosterone responses that tended to be even higher (although statistically not significant) than the ones exhibited by water-drinking controls This observation demonstrates that in contrast to ACTH secretion corticosterone secretion was not interfered with by 2% saline drinking. Since the adrenal cortex is responsive to the log of the dose of ACTH, plasma corticosterone concentration rapidly reaches its maximum while plasma ACTH concentration is relatively low. In fact, about 70 pmol/l of plasma ACTH concentration is sufficient to elicit maximal corticosterone response (17) Hence, plasma ACTH level of 108.09 ± 17 32 pmol/l exhibited by S rats represented about the sufficient stimulus necessary to elicit maximal corticosterone response.

ACTH response to ether stress and ACTH secretion of pituitary fragments to CRF-41 stimulation of rats with osmotic stimulation and DEX treatment were blunted compared to W rats Pituitary fragments of S+D rats showed no response to AVP stimulation. These results are in agreement with previous observations (18) and demonstrate the ability of DEX to inhibit ACTH secretion also in hyperosmotic rats

While the prolactin response to stress has been well characterized, there is no agreement on its role in the regulation of salt-water balance. Prolactin has been reported to increase intestinal absorption of fluid and electrolytes (22) and osmotic stimulation has been shown to increase plasma prolactin concentration and prolactin binding in adrenal homogenates while decreasing binding in the kidney (26). Other studies demonstrated no change in plasma prolactin concentration in response to osmotic stimulation, suggesting no role for prolactin in the regulation of salt-water balance (27)

The decreased prolactin response to stress of osmotically stimulated rats could be a result of an increase in the release of substances of neural lobe origin with prolactin release-inhibiting ability. Oxytocin and AVP were reported to inhibit prolactin secretion (29). Release by neural lobe terminals of dopamine in response to electrical stimulation was reported to be increased in dehydrated rats compared to that in normosmotic rats (15) These mechanisms could account for the decrease in plasma prolactin concentrations of osmotically stimulated rats. Our results showing a decreased concentration of plasma prolactin in osmotically stimulated + DEX-treated rats are in agreement with those reported earlier (12)

In summary, our results indicate that chronic hypernatremia induced by 2% saline drinking interferes with the ACTH and prolactin responses to ether stress while the diminished ACTH response seems to be still sufficient to elicit maximal corticosterone response. A decreased sensitivity of corticotrope cells to CRF-41 and AVP could explain the impaired ACTH secretory response Further experiments may reveal the mechanism by which 2% saline drinking interferes with the ether stress-induced prolactin response.

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