Effect of Hypertonic Saline on the Corticotropin-Releasing Hormone and Arginine Vasopressin Content of the Rat Pituitary Neurointermediate Lobe

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Abstract

The changes in the levels of corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) in the neurointermediate lobe of the pituitary (NIL) following hypertonic saline administration were examined in rats. The plasma osmotic pressure in rats receiving 2% NaCl for 8 days was greatly increased. Plasma AVP concentration in rats receiving 2% NaCl for 8 days were significantly higher than in control rats (566% of the control level). Plasma corticosterone was significantly higher in the saline-treated rats than in controls, whereas plasma ACTH was not significantly different. The pituitary ACTH concentration was much higher in the saline-treated rats than in controls. CRH in the NIL was increased significantly by saline treatment (419% of the control concentration), whereas the CRH in the paraventricular nucleus and median eminence of control and saline-treated rats did not differ significantly. The AVP in the NIL fell greatly in saline treated rats. The extract from both control and saline-treated rats showed a major peak for immunoreactive CRH, with a retention time identical to that of rat CRH. However, the peak was much higher in the extract from saline-treated rats. The immunoreactive AVP peak was greatly reduced in saline-treated rats. These results suggest that hypertonic saline administration increases the CRH in the NIL and causes AVP hypersecretion and/or hyperfunction of magnocellular-NIL CRH might be responsible for pituitary-adrenal stimulation in saline-treated rats.
paraventricular nucleus (PVN) (Olschowka et al., 1982; Antoni et al., 1983; Swanson et al., 1983; Niimi et al., 1988). CRH has also been detected in the magnocellular cells of both the PVN and the supraoptic nucleus (SON) (Kawata et al., 1982; Burlet et al., 1983), and in the posterior pituitary (Kawata et al., 1983; Hashimoto et al., 1984; Suda et al., 1984; Jeandel et al., 1987). We previously reported that CRH immunoreactive nerve terminals are distributed around the blood vessels in the pig posterior lobe, particularly in the proximal adjacent to the intermediate lobe (Kawata et al., 1983) and that the CRH present in the neurointermediate lobe of the rat pituitary gland (NIL) is mainly composed of authentic rat CRH (1-41). (Hashimoto et al., 1984). We also observed that the CRH concentration increased while the arginine vasopressin (AVP) concentration decreased in the NIL in a lithium-induced diabetes insipidus rat model (Sugawara et al., 1988). Therefore, it has been assumed that osmotic changes affect not only AVP secretion but also the amount of CRH in the NIL. The present study examined the changes in CRH and AVP in the NIL after hypertonic (2%) saline administration.

Materials and Methods

Male Wistar rats weighing approximately 200 g were housed at room temperature and given food and water ad libitum. These rats were randomly divided into three groups. One group was given 2% NaCl for 2 days, another group was given 2% NaCl for 8 days, while the third group was given tap water as a control. All rats were sacrificed in the morning (10:00-12:00) on the same day by rapid decapitation which prevented excess exposure to stress. The truncal blood from each rat was collected in chilled glass tubes containing EDTA and centrifuged. Plasma samples were stored at −20°C to measure plasma AVP, ACTH and corticosterone. The PVN, ME, NIL and anterior pituitary were quickly dissected out, frozen on dry ice, and stored at −20°C. CRH and AVP were extracted from the PVN and ME by the previously reported method (Hashimoto et al., 1985). ACTH was extracted from the anterior pituitary in 0.1 N HCl with a glass homogenizer.

High performance liquid chromatography

Extracts from the NIL of 4 control and 4 saline-treated (8 days) rats were dissolved in 200 μl 0.1% trifluoroacetic acid (TFA). After centrifugation at 10,000 g for 10 min, the clear supernatant was injected onto a μ-Bondapack C18 column (3.9 mm × 15 cm; particle size 10 μm, Waters). The column was eluted with a gradient of increasing acetonitrile concentrations (0-20% for 5 min, 20-60% for 40 min and 60% for 5 min) in 0.1% TFA at a flow rate of 1.0 ml/min. The column effluent was monitored at 220 nm with an UV detector. Fractions eluted every 2 min were collected, evaporated to dryness under N2, and stored for CRH and AVP radioimmunoassays. Mixtures of synthetic AVP, oxytocin, 1-39 ACTH and CRH (purchased from Peptide Research Laboratory in Osaka) were chromatographed as markers to identify the peaks of the extracts.

Assays

The dried tissue extracts and HPLC-eluates were resuspended in a buffer (0.02 M phosphate buffer containing 0.14 M NaCl, 0.5% BSA, 25 mM EDTA and 1 mM ascorbic acid). The CRH concentration after resuspension in the buffer was measured by a specific rat CRH radioimmunoassay (Hashimoto et al., 1985). The AVP concentration was measured by the previously reported radioimmunoassay method (Ohno et al., 1981). The ACTH concentration in the anterior pituitary was measured by the previously reported radioimmunoassay method (Ohno et al., 1981). The ACTH concentration in the anterior pituitary was measured by the previously reported radioimmunoassay method (Hashimoto et al., 1976). Plasma AVP was extracted by the acetone-petroleum ether method and measured by the abovementioned radioimmunoassay method (Hashimoto et al., 1981). Plasma ACTH concentrations were measured by radioimmunoassay with commercially-available RIA kits (DPC, USA). Plasma corticosterone was measured by the previously reported method (Hattori et al., 1986).

Plasma osmotic pressure was determined with an osmometer (NIKKISO OSA-21, Japan). Tissue protein was measured with a Protein Assay Kit (Bio Rad, USA).
Statistics
The multiple range test was used for statistical analysis after analysis of variance.

Results

Body weight and plasma osmotic pressure
The body weight of the three groups of animals was measured on the first, sixth and eighth days of the experiment. On the eighth day, rats that had received 2% NaCl for 8 days, had a significantly lower body weight than the other 2 groups of rats, and the groups that had received 2% NaCl for 2 days had a lower body weight than the control rats (Fig. 1).

Plasma osmotic pressure in the control rats (293.0 ± 2.0 mOsm/kg H₂O, mean ± SEM) and the rats receiving 2% NaCl for two days (299.2 ± 4.5 mOsm/kg H₂O) did not differ significantly. However, plasma osmotic pressure in the rats receiving 2% NaCl for 8 days was greatly increased (389.8 ± 11.3 mOsm/kg H₂O).

Fig. 1. Effect of hypertonic saline (2% NaCl) administration on body weight. * p<0.05, ** p<0.01

Fig. 2. Effect of hypertonic saline administration on plasma arginine vasopressin (AVP), ACTH and corticosterone. * p<0.05, ** p<0.01
Plasma AVP, ACTH, corticosterone and pituitary ACTH concentrations

Plasma AVP in rats treated with saline for 8 days was significantly higher (566% of the control) than in control rats (Fig. 2). Plasma ACTH among in three groups did not differ significantly, but the plasma corticosterone was significantly higher in the rats given saline for 8 days than in the control group. Pituitary ACTH concentrations were also higher in the rats given saline than in the control group (Fig. 3).

CRH and AVP concentrations in the PVN, ME and NIL

In rats given saline for 8 days, the CRH concentrations of the PVN and ME did not differ from those in control rats, whereas CRH in the NIL was significantly increased to 419% of the control level (Fig. 4). AVP in the NIL in rats given hypertonic saline for 2 or 8 days fell to 53% and 5%, respectively, of the control group (Fig. 5). AVP in the ME of the rats given saline for 8 days declined to 32% of the control concentration, while AVP in the PVN in the 3 groups did not significantly differ.
HPLC of immunoreactive CRH and AVP in NIL extracts

The NIL extracts from control rats (n=4) showed a major peak of immunoreactive CRH with a retention time identical to that of rat CRH (Fig. 6). Two additional small peaks were eluted earlier. The NIL extract from rats given saline for 8 days (n=4) also showed a major peak at the position as rat CRH; however, the peak was greater than that seen in the control group.

The NIL extracts from control or saline-treated rats showed a peak of immunoreactive AVP on HPLC with the same retention time as synthetic AVP; however, the peak was greatly reduced in saline-treated rats.

Discussion

The present study has shown that the CRH and AVP in the NIL change after several days of hypertonic saline administration. AVP in the NIL was dramatically reduced after hypertonic saline administration, and in the ME it fell to one third of the concentration in control rats. The AVP concentration in the PVN of control and hypertonic saline-treated rats did not differ significantly. However, in situ hybridization methods showed that the AVP messenger RNA (AVP mRNA) content of the PVN and SON increased greatly after 8 days of hypertonic saline administration (data not shown). Plasma AVP increased greatly with hypertonic saline administration. These results indicate that the reduction in AVP in the NIL was due to the continuous hypersecretion of AVP because of osmotic stimulation.

CRH cells that project to the external layer of the ME to regulate ACTH secretion are concentrated in the parvocellular region of the PVN, and CRH neurons projecting to the NIL originate in the magnocells in the SON and/or PVN. Although CRH in the ME and the NIL did not show a similar change after hypertonic saline administration, the AVP concentration in the ME and the NIL changed in parallel. This is reasonable as most of the AVP immunoreactivity in the ME is found in the AVP neurons.

![Graph showing IR-AVP levels in ME, PVN, and NIL](image)

Fig. 5. Effect of hypertonic saline administration on immunoreactive AVP in the ME, PVN and NIL. * p<0.01
Control
2% NaCl, 8 days

Fig. 6. Reversed phase, high performance liquid chromatography of CRH (■) and AVP (○) immunoreactivities in extracts of neurointermediate lobes from control and hypertonic saline-administered rats. Extracts (195/200) from 4 rats were injected onto a μ Bondapak C₁₈ column (3.9×150 mm). The column was eluted at 1 ml/min after injecting the sample in 0.1% trifluoroacetic acid, with a gradient of acetoni-trile (0-20% for 5 min, 20-60% for 40 min and 60% for 5 min).

in the internal layer of the ME which project to the posterior pituitary.

CRH in the NIL greatly increased with hypertonic saline administration. CRH in the PVN and ME, as well as plasma ACTH in control and saline-treated rats did not differ, whereas plasma corticosterone were greatly increased after 8 days of hypertonic saline administration. The increase in plasma corticosteroid with normal or reduced plasma ACTH has been reported in certain cases of depression, anorexia nervosa and alcoholism (Holsboer et al., 1984; Rivier et al., 1984; Capagnini et al., 1986). It is assumed that a chronic excess of endogenous CRH results in continuous activation of the pituitary adrenal axis in these cases, although the feedback-suppressive effects of mild hypercortisolism normalized plasma ACTH. Therefore, "normal" plasma ACTH with the increased plasma corticosterone in hypertonic saline-treated rats suggests the existence of a balance between the forces of pituitary stimulation and the negative feedback effect exerted by increased corticosterone. The increase in plasma corticosterone might be caused by increased adrenal sensitivity to ACTH which had been caused by chronic pituitary ACTH stimulation until plasma ACTH normalization. The "normal" plasma ACTH might also be caused by the loss of the ACTH releasable pool, or down regulation of CRH and/or AVP receptors. However, the increase in anterior pituitary ACTH and plasma corticosterone suggests the existence of pituitary stimulation.

Pituitary stimulation might be caused by either chronic secretion of hypothalamic CRH, AVP or neurointermediate CRH. CRH concentrations in the PVN and ME did not show any significant change. Tissue CRH may not be a meaningful indicator of CRH function, as it is the result of CRH synthesis, release and altered turnover. It cannot be definitely concluded whether hypothalamic CRH hyperfunction occurred or not, as we did not determine the CRH mRNA concentration in the present study. It has recently been reported that salt loading resulted in an increase in CRH mRNA in the supraoptic nucleus (SON), but that there was no overall change in CRH mRNA in the PVN (Young, 1986; Lightman and Young, 1987). Therefore, it is unlikely that CRF secretion into the portal vessels is enhanced in salt load-
ing rats, and there may be hyperfunction of magnocellular-neurointermediate CRH neurons. AVP hypersecretion and/or hyperfunction of magnocellular-neurointermediate CRH neurons might be responsible for pituitary-adrenal stimulation in salt loading rats, although we cannot conclude this from the present results.

Hypertonic saline administration for 2 days did not increase plasma osmotic pressure but tended to increase plasma AVP, corticosterone and CRH in the NIL and decreased AVP in the NIL. However, these changes were small compared with the changes in 8 day saline administration. The results indicate that the increase in plasma osmotic pressure was balanced by the secretion of AVP from the NIL in 2 day saline administration.

The present study at least reveals that the amount of CRH in the NIL is increased by hypertonic saline administration. The function of the CRH found in the SON and NIL is not yet known. It is interesting that CRH and AVP change in different ways after hypertonic saline administration although CRH and AVP in the NIL are of the magnocellular origin in the PVN and/or SON. The present data appear to indicate that the transport of AVP and CRH from the magnocells to the posterior pituitary is stimulated and that AVP release into the general circulation is stimulated but CRH release is not. Kawata et al. (1983) reported that the distribution of the CRH-containing nerves in the posterior pituitary was found to be similar to that of the vasopressin-containing nerve terminals. These results might indicate that CRH in the NIL has a paracrine effect on the AVP secretion, although it is difficult to explain the mechanism of inverse changes in CRH and AVP content in the NIL. Very recently, when this manuscript was in preparation, Jessop et al. (1989) reported a similar result: that CRH in the NIL increased after salt loading. They also postulated that CRH in the NIL might have a local paracrine effect on AVP and/or oxytocin. However, it is still not known whether the increase in CRH in the NIL plays a role in vasopressin secretion or ACTH secretion in the present study, and the significance of the increased amount of CRH in the NIL remains to be clarified.

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References


