Hypothalamic–pituitary–adrenal axis up-regulation in rats submitted to pituitary stalk compression

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Abstract

The present study investigated the hypothalamic–pituitary–adrenal (HPA) axis activity in response to stress in adult male rats submitted to pituitary stalk compression (PSC) or sham operation. Animals received water or oral salt loading (2% NaCl) for one or eight days before the day of the experiment. On the 14th day post-surgery rats were killed under basal conditions or after 15 min immobilization stress. In the PSC group urine output increased significantly and plasma vasopressin (AVP) levels failed to respond to osmotic stimuli. Short-term salt load induced a significant increase in AVP levels in the sham-operated group. The PSC group presented higher adrenocorticotrophin (ACTH) and corticosterone levels compared with sham-operated rats, both in water intake and salt load conditions. Immobilization stress induced a similar increase

in plasma ACTH and corticosterone concentrations in sham-operated and PSC groups under water intake. However, long-term salt load blunted the ACTH and corticosterone responses to immobilization stress in sham-operated rats. PSC rats submitted to short- and long-term salt loading presented no changes in ACTH and corticosterone levels after immobilization. Immobilization stress caused neither AVP responses nor plasma osmolality changes in sham and PSC groups. There was no difference in median eminence AVP content among all groups. In conclusion, the high ACTH and corticosterone levels found in PSC rats under water intake and salt loading conditions suggest an up-regulation of the HPA axis, with a preserved adaptive mechanism to chronic stress.

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Introduction

Adrenocorticotrophin (ACTH) secretion is mainly regulated by corticotrophin-releasing hormone (CRH), which is synthesized in the hypothalamic paraventricular nucleus (PVN). Vasopressin (AVP) also acts as a mediator of ACTH secretion, potentiating the effects of CRH (Aguilera 1998). Although AVP is a weak ACTH secretagogue, it has an important role in the adaptive mechanism to chronic stress (De Goeij *et al.* 1991, Bartanusz *et al.* 1993, Aguilera 1994). Adrenalectomy and chronic osmotic stimuli, such as long-term salt loading, increase the AVP:CRH mRNA expression and secretion in a subset of parvocellular neurons (Kiss *et al.* 1984, Sawchenko *et al.* 1984, Holmes *et al.* 1986). The role of magnocellular AVP in the hypothalamic–pituitary–adrenal (HPA) axis response to stress is, however, controversial. Previous reports demonstrated that the pituitary–adrenal response to exogenous CRH in patients with central diabetes insipidus (DI) is not only preserved, but is also potentiated by hyperosmolality (Mazza *et al.* 1994, Elias

et al. 1997) indicating that increased plasma osmolality may participate in pituitary–adrenal regulation involving mediators other than magnocellular AVP.

Moreover, in rats submitted to pituitary stalk compression (PSC), an experimental model of central DI, plasma concentrations of ACTH and corticosterone are increased under basal conditions (Makara 1993). In subsequent studies, an increased AVP content in the median eminence and reduced levels of CRH and AVP mRNA in parvocellular neurons have been shown in rats submitted to PSC (Makara *et al.* 1995, 1996). In contrast, Dohanics *et al.* (1992), using another PSC experimental model, reported no changes in AVP content in the median eminence and a retrograde degeneration of the magnocellular neurons. Therefore, there are still controversies regarding the PSC experimental model and studies employing this model under different stress stimuli are lacking. In the present study we investigated the HPA axis activity in rats submitted to PSC during water intake or after short- or long-term salt loading, under basal conditions and after immobilization stress conditions.

Materials and Methods

Animals

Male Wistar rats weighing 260–300 g were housed in individual cages in a temperature-controlled room ($23 \pm 2^\circ\text{C}$) with a 12 h light:12 h darkness cycle. Standard laboratory chow and fluid were available *ad libitum* except during test sessions. All animal protocols were carried out in accordance with the Ethical Committee for Animal Use of the School of Medicine of Ribeirao Preto, University of Sao Paulo.

Pituitary stalk compression and experimental protocol

The rats were anaesthetized with sodium thiopental (Abbott Laboratories; 50 mg/kg body weight) by i.p. injection, placed in a Kopf stereotaxic frame with nose down 3.5 mm, and submitted to PSC or sham-operation, according to the modified technique of Dohanics *et al.* (1992). The skull was opened 3.8 mm posterior to bregma and, through a 3×3 mm aperture, a triangular-shaped wire was lowered in the coronal plane until it touched the floor of the skull for stalk compressed rats or to a depth of 9 mm from the brain surface for sham-operated rats.

Water intake and urine output were monitored daily. PSC rats with sustained urine output greater than 40 ml/day (mean ± 2 S.D. from controls) were considered to have had successful surgery. Rats received water or were salt-loaded orally with saline (2% NaCl) for one day (short-term salt load) or eight days (long-term salt load) before the day of experiment. On the 14th day post-surgery, between 0800 and 1000 h, rats were killed by decapitation under basal conditions or after 15 min immobilization stress induced by restraint in a metal cylinder. Trunk blood was collected into ice-cold plastic heparinized tubes and centrifuged immediately after experimental procedures. Plasma was stored at -20°C until hormone assay. The median eminence was surgically removed from the brain, homogenized in 200 μl 0.1 M acetic acid and assayed for AVP.

Assays

Plasma osmolality (pOsm) was determined by depression to the freezing point (Advanced Digimatec 3 DII, Advanced Instruments, MA, USA). ACTH, corticosterone and AVP levels were measured by specific RIA after previous extraction from plasma using silicic acid, ethanol and petroleum ether respectively (Lopez-Jimenez *et al.* 1989, Elias *et al.* 1997). The recovery rates were greater than 87% for all methods. The assay sensitivity was 15.7 pg/ml for ACTH, 0.4 $\mu\text{g}/\text{dl}$ for corticosterone, and 0.6 pg/ml for AVP. The intra- and interassay coefficients of variation were, respectively, 4.3 and 16% for ACTH, 8 and 19% for corticosterone, and 2.7 and 17% for AVP.

Figure 1 Time course of urine output (ml/24 h) in sham-operated rats (○; $n=29$) and PSC rats (●; $n=21$). Dotted line: mean $\pm$ s.d. from controls.

All samples from a single experiment were assayed in duplicate in the same assay.

Statistical analysis

All data are presented as the arithmetic mean $\pm$ S.E.M. Data between groups were compared using the Mann–Whitney test, and the significance of differences among multiple groups was determined by the Kruskal–Wallis test followed by Dunn's test. Significance was assumed when $P < 0.05$. Statistical analysis was performed with GraphPad Prism3 (GraphPad Software, San Diego, CA, USA).

Results

Weight gain was similar in sham-operated and PSC rats throughout the study. PSC rats had a significant increase in water intake ($n=21$; 75.4 ± 2.1 ml/24 h, $P < 0.0001$) as well as urine output (64.1 ± 3.2 ml/24 h, $P < 0.0001$) compared with sham-operated rats ($n=29$; 17.3 ± 1.0 and 15.0 ± 0.7 ml/24 h respectively). A triphasic urine output pattern was observed in PSC rats (Fig. 1). One day after surgery, urine output increased abruptly in PSC rats (90 ± 17 ml/24 h), declining on the second day (40 ± 7 ml/24 h), before reaching a plateau (around 60 ml/24 h) from the 3rd to the 14th day.

Plasma osmolality and vasopressin

Plasma osmolality (pmOsm/kg) and plasma AVP (pg/ml) levels (means $\pm$ S.E.M.) were similar in sham-operated ($n=12$; 288 ± 2 and 3.0 ± 0.3 respectively) and PSC ($n=7$; 293 ± 2 and 2.0 ± 0.4 respectively) rats under water intake. Short- and long-term salt load induced a similar and significant increase in pOsm in the PSC group ($n=7$; 306 ± 5 and 319 ± 7). These values were higher than those observed in the sham-operated group

Figure 2 Plasma AVP concentrations from sham-operated (open bars) and PSC (solid bars) rats under water intake or short-term (S1) and long-term (S8) salt load. Data represent the means and S.E.M. of values for *n* rats (shown within bars). **P* < 0.05 compared with PSC.

(*n* = 12; 293 ± 1 and 301 ± 3 respectively, *P* < 0.05). No significant increment was observed in plasma AVP levels in PSC rats after both salt load protocols. The sham-operated group had higher AVP levels after both salt load protocols compared with the PSC group (Fig. 2). Immobilization stress did not alter plasma osmolality or plasma AVP concentrations in sham-operated and PSC rats. There was also no difference among groups in the median eminence AVP content.

ACTH and corticosterone plasma levels

Plasma ACTH (pg/ml) and corticosterone (μg/dl) values (means ± S.E.M.) were higher in PSC (*n* = 8; 88.0 ± 4.4 and 7.6 ± 1.4 respectively) than in sham-operated (*n* = 12; 64.4 ± 6.5 and 2.7 ± 0.6 respectively, *P* < 0.05) rats during water intake. PSC rats (*n* = 7) also had higher ACTH and corticosterone levels compared with the sham-operated group (*n* = 10–13) after short-term salt load (146.6 ± 16.7 and 28.6 ± 4.0 vs 64.6 ± 6.7 and 3.7 ± 0.9 respectively, *P* < 0.05) and after long-term salt load (121.3 ± 13.5 and 11.4 ± 1.7 vs 79.8 ± 9.6 and 6.5 ± 1.4 respectively, *P* < 0.05). Plasma ACTH and corticosterone levels were increased after short-term but not after chronic salt loading compared with water intake in PSC rats (Fig. 3). Immobilization stress induced a similar increase in plasma ACTH and corticosterone concentrations in sham-operated (*n* = 9; 471.1 ± 92.1 and 21.0 ± 1.9) and PSC (*n* = 9; 351.6 ± 57.4 and 20.8 ± 3.0) groups under water intake. However, long-term salt load blunted the ACTH and corticosterone responses to immobilization stress in the sham-operated group (*n* = 5; 123.9 ± 32.8 and 16.6 ± 2.4). PSC rats submitted to short and long-term salt loading presented no changes in ACTH and corticosterone levels after immobilization (Fig. 3).

Discussion

Clinical studies on central DI in humans have been carried out for several decades. Trauma, either accidental or

post-neurosurgery, is one of the most frequent causes of central DI in humans and is due to pituitary stalk lesion and destruction of the neurohypophysis (Baylis 2001). On the other hand, inherited central DI, a rare autosomal dominant disease, is caused by progressive destruction of magnocellular neurons due to the accumulation of the mutated prepro-hormone AVP-neurophysin II in the supraoptic nucleus (Ito *et al.* 1997). In the Brattleboro rat, an experimental model of inherited DI, a deficiency in magnocellular neurons has been described (Pickering & North 1982, Schmale & Richter 1984). Other experimental models of central DI have been created, some of them based on pituitary stalk transection (Pu *et al.* 1995, Huang & Dellmann 1996). However, this surgical procedure induces not only central DI but also anterior pituitary failure (Makara *et al.* 1979), therefore studies involving the activity of the HPA axis cannot be performed. To overcome this difficulty, the pituitary stalk compression model of central DI has been used (Dohanics *et al.* 1992, Makara 1993). Both authors used similar techniques, which allowed the characterization of the relationship between magnocellular AVP and the HPA axis. In the present study, we induced central DI using a surgical procedure slightly modified from Dohanics *et al.* (1992), since we have used a different rat strain (Wistar). Urine output has been used to confirm successful surgery; however, there is no consensus on the definition of polyuria in rats submitted to experimental DI (Makara 1993, Makara *et al.* 1995). Thus, we considered the mean + 2 S.D. of urine output values from control rats as a cut-off for polyuria. The increased urine output with no plasma AVP response to osmotic stimuli confirmed that neurohypophyseal deficiency was achieved in the PSC group.

PSC rats showed higher corticosterone and ACTH levels under water intake, and after short-term and long-term salt loading compared with the sham-operated group, suggesting that the DI experimental model induced an up-regulation of the HPA axis under basal conditions. AVP and oxytocin, which have been shown to accumulate in the median eminence in rats submitted to PSC (Makara *et al.* 1996), could play a role in mediating this up-regulation. However, in the present study, in agreement with Dohanics and colleagues' findings (1992), we did not observe a difference in median eminence AVP content between sham-operated and PSC rats. The different surgical techniques used could have contributed to this discrepancy. In addition, we cannot rule out other factors such as angiotensin II and catecholamines as possible mediators of the increased activity of the HPA axis (Lightman & Young 1987, Harbuz *et al.* 1991, Aguilera *et al.* 1995). Moreover, central atrial natriuretic peptide (ANP), which is a putative counterregulator of the HPA axis (Franci *et al.* 1992, Jessop 1999), could contribute to the up-regulation of this axis in PSC rats. Indeed, we previously reported that DI patients presented lower

Figure 3 Plasma ACTH and corticosterone concentrations in sham-operated (left panel) and PSC (right panel) rats, under basal conditions (open bars) or after immobilization stress (solid bars), while under water, short-term (S1) or long-term (S8) salt load. Data represent the means and S.E.M. of values for *n* rats (shown within/above bars). *a*, $P < 0.05$ compared with basal sham-operated; *b*, $P < 0.05$ compared with PSC under water and S8; *c*, $P < 0.05$ compared with immobilized PSC or sham-operated, under water.

plasma ANP concentrations than control subjects under basal conditions, and also after isotonic and hypertonic saline infusion (Elias *et al.* 1997). The lower ANP secretion in central DI may be due to the effects of neurohypophyseal hormones on ANP regulation (Kamoi *et al.* 1990, Shimizu *et al.* 1991).

We also performed a stress-induced stimulation of the HPA axis using a 15-min immobilization, a potent HPA axis stimulus. Sham-operated and PSC rats showed a significant and similar increase in ACTH and corticosterone levels when submitted to immobilization under water intake, confirming the preserved HPA axis response to this paradigm in PSC rats. On the other hand, PSC rats submitted to short- and long-term salt loading presented no changes in ACTH and corticosterone levels after immobilization compared with the basal condition. Short-term osmotic stimulus probably elicits a maximum HPA axis response in PSC rats, thus any further increase in ACTH and corticosterone cannot be attained by a novel stimulus. Additionally, immobilization resulted in no plasma AVP change in sham rats under freely available water intake and salt loading compared with basal conditions, indicating that magnocellular AVP does not play a significant role in the HPA axis response to this stress paradigm. This result is supported by the report that immobilization results in an increase in both CRH and AVP mRNAs in the parvocellular neurons of the hypothalamic paraventricular nucleus but do not change magnocellular AVP mRNA levels (Herman *et al.* 1995, Ma & Lightman 1998, Aubry *et al.* 1999).

In the present study, both sham-operated and PSC groups presented blunted plasma ACTH and corticosterone responses to immobilization under long-term salt load. The prolonged osmotic stimulus has previously been shown to inhibit the pituitary–adrenal response to immobilization stress (Jessop *et al.* 1990, Chowdrey *et al.* 1991, Elias *et al.* 2002). This effect could not be ascribed solely to the negative feedback due to the elevated basal corticosterone levels, since adrenalectomized rats submitted to a prolonged osmotic stimulus have also been shown to have a decreased ACTH response to stress (Chowdrey *et al.* 1991, Aguilera *et al.* 1993). Thus, non-glucocorticoid dependent factors (Jessop 1999) induced by prolonged salt loading, acting as inhibitors, may contribute to the blunted HPA axis activity. The adaptation of the HPA axis response to a superimposed novel stress observed in rats under chronic osmotic stimulus is in contrast with the facilitation of this axis under other chronic stressors (Aguilera 1998), but it is similar to the decrease of the absolute response of corticosterone observed in rats submitted to repeated environmental stress (Imaki *et al.* 1992). Prolonged osmotic stimulation has been shown to induce a decrease in CRH mRNA in parvocellular neurons of the PVN (Aguilera *et al.* 1993) and a reduced binding of AVP to the anterior pituitary (Aguilera 1994). These changes might contribute to the blunted pituitary–adrenal response to immobilization as an adaptive mechanism in an attempt to preserve electrolyte homeostasis. Therefore, in PSC rats, despite the HPA axis up-regulation under basal conditions, the inhibitory effect of long-term salt

loading on the immobilization response suggests that the chronic adaptive mechanisms are preserved in this DI experimental model.

In conclusion, the central DI obtained in rats by compressing the pituitary stalk, described in this study, may be used as a model of central DI secondary to central surgical procedures in humans. The lack of AVP response to osmotic stimuli associated with increased urine output supports neurohypophyseal hormone deficiency. The preserved ACTH and corticosterone response to immobilization stress indicates the integrity of the HPA axis in PSC rats. However, the higher basal ACTH and corticosterone levels found under water intake and after osmotic stimulus suggest that the HPA axis is up-regulated in this experimental model. Moreover, the blunted ACTH and corticosterone responses to a superimposed stress indicate a preserved adaptive mechanism to a chronic osmotic stimulus.

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