EFFECTS OF APPREHENSION OF LUMBAR PUNCTURE PROCEDURE ON SALIVARY CORTISOL, PLASMA VASOPRESSIN AND OSMOLALITY IN MAN

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SUMMARY

Evidence has accumulated that arginine-vasopressin (AVP) may play a role in the stress response, but little attention has been paid to this hormone during stress. The present study, therefore, investigated both plasma osmolality and salivary cortisol and levels of AVP in patients undergoing a lumbar puncture procedure for evaluation of nuclear disc prolapse. It was found that the emotional stress of the procedure significantly increased plasma levels of AVP and osmolality together with an increase in salivary cortisol. Correlational data indicated a significant positive relationship between the cortisol response and the changes in plasma osmolality. Interrelationships between osmolality, stress, vasopressin and glucocorticoids are discussed.

KEY WORDS—Cortisol, osmolality, stress, vasopressin

The psychoneuroendocrine response to stressful events typically involves the release of ACTH (adrenocorticotropic hormone) from the anterior pituitary gland. In addition, evidence has accumulated that peptides are released from the posterior pituitary in response to stress. In particular, a number of studies have reported a rapid release of oxytocin in rats subjected to a variety of stressful procedures. The release of antidiuretic hormone, arginine vasopressin (AVP), is thought to be influenced by stress, especially at the level of the neurohypophyseal portal system. The role of AVP in mediating the adrenal axis response to various perturbations is unclear, however, and data from animal experiments suggest that the nature of the stressor determines the hypotalamic response.

Little attention has been paid to the physiological changes that elicit or accompany alterations in plasma concentrations of 'stress hormones' such as ACTH or AVP. As AVP responds to changes in osmolality, and vice versa, osmolality may be affected by stress as well. Parrott et al.1,6 found that the rise in cortisol induced by isolation stress in sheep was associated with a transient depression of plasma levels of AVP together with an apparent redistribution of body water. Keil and Severs7 found that ether and acceleration stressors rapidly reduced plasma AVP concentrations in dehydrated rats, but not in normally hydrated rats. Furthermore, osmotic stress can stimulate ACTH release in both man8 and in the dog.9 Taken together, it is interesting to consider changes in water metabolism in studies on the relationship between stress and AVP.

Experimental data on the stress response of AVP in humans are scarce. Auböck and Konzett10 found no effect of cognitive stress on plasma AVP levels in man. A major problem of human studies is that several existing laboratory-based stress paradigms have significant shortcomings for assessing the

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neurobiological correlates of stress. Therefore, a 'real' emotional stressor was chosen in the present study to address the following questions: Firstly, does the stress related to the lumbar puncture (LP) procedure affect plasma levels of AVP in relation to changes in salivary cortisol levels? Secondly, if so, does the emotional stressor induce changes in plasma osmolality as well?

The LP procedure was selected to examine stress effects because most subjects experience LP as a strong emotional stressor. According to a retested control group design, patients undergoing LP for evaluation of lumbar disc pathology were studied immediately before the LP and 24 hours later at the same time. Plasma and saliva were obtained from control subjects at corresponding times. Salivary cortisol was studied because saliva can be collected easily and without stress, and salivary cortisol has been found to be a sensitive parameter in neuroendocrine studies of stress.11

SUBJECTS AND METHODS

Subjects and procedure

Fifteen patients (8 males and 7 females, mean age: 45.6 ± 9.0 yr, ranging from 30–60 yr) underwent lumbar puncture for myelographic evaluation of lumbar disc pathology. All LPs were performed between 11.60h and 12.30h (day 1) in an identical manner. Patients using tranquilizers or analgesics because of low back pain were excluded from the study. Immediately before, the LP saliva was collected with an absorbent cotton roll in the mouth (firstly) and blood was collected with the patient in a half-upright position (secondly). Saliva and blood were collected again at the same time the next day (24 hours later; day 2). Cortisol was assessed in saliva in order to avoid the biasing effect on cortisol secretion by the venupuncture.

Patients were matched with healthy volunteers for age and sex. The mean age of the volunteers was 45.9 ± 10.4, ranging from 28–61 yr. Saliva and venous blood were collected from the volunteers at the same times as for the controls.

Patients and control subjects were instructed to eat a standard breakfast before 9.00 am and not to smoke or drink coffee or tea after 9.00 am. None of the patients or controls had a history of endocrine or renal disease. The study was approved by the medical ethical council of the University Hospital and all subjects gave their informed consent.

AVP radioimmunoassay

All blood samples were collected into chilled, heparin-treated plastic tubes and centrifuged at 4°C (3200 rpm) for 30 minutes. After removal of plasma aliquots for the determination of osmolality, the remainder was stored at −20°C until AVP was measured as described previously by Ten Haaf et al.12 AVP was extracted from the plasma with activated Vycor (Corning Glass Works, New York) and resuspended in Veronal buffer, pH 8, containing 5 mg human serum albumin per millilitre, for the subsequent determination of AVP by radioimmunoassay with a specific, C-terminal directed antiserum. The cross-reactivity with oxytocin was <0.01% and with lysine VP about 10%. The intra-assay coefficient of variation was 13.5%. Each sample was measured in triplicate and all samples were assayed within the same assay. The lower detection limit of the assay was 0.2 fmol/ml. Osmolality was measured by freezing point depression (Gonotec Osmomat 030). Each sample was measured in triplicate immediately after plasma separation; the osmometer was calibrated before each assay. The coefficient of variation was 0.18%.

Salivary cortisol

Saliva was collected by holding an absorbent cotton roll in the mouth for 1–2 minutes. The roll was then placed in a capped plastic vial ('Sativette', Sarstedt B.V.). The samples were stored at −20°C until analysis. Salivary cortisol was determined in duplicate by direct radioimmunoassay15 by Dr. J. Sulon of the Steroid Laboratory, University Hospital Liège, Belgium. The lower detection limit of the assay was 0.69 nmol/l, with an intra-assay coefficient of variation of 4.4%. All samples were assayed in the same assay.

Experiments in our laboratory have shown that the use of a commercially available cotton roll (Sarstedt B.V.) as a means for collecting saliva produced identical salivary cortisol levels as non-absorbed pure saliva collected in an open tube (Sulon, personal communications). In addition, there is no need for a specific aliquot of saliva. The cotton roll can be placed anywhere in the mouth. The wet cotton roll is placed within an inner tube of a capped plastic vial. The bottom of the inner tube has a small prepunctured hole. Centrifugation of the plastic vial produces a clear salivary centrifugate in the outer part of the vial.
Statistical analysis

As the AVP and cortisol data deviated markedly from a Gaussian distribution, ranks over all observations were calculated and used for an analysis of variance with the factors 'group' (patient versus control) and 'day' (day 1 versus day 2), with repeated measures on the last factor. Spearman rank correlation coefficients (two-tailed) were calculated between individual cortisol levels (day 1–day 2) and the difference between plasma AVP levels and osmolality. A probability level of less than 0.05 was considered to be significant.

RESULTS

Cortisol

A repeated-measures analysis of variance revealed a group * day interaction $F(1,28) = 8.13$, $P < 0.01$. Because of the within-subjects design of the study, separate repeated-measures analyses for each subgroup were performed in order to evaluate the significant interaction term. Results revealed an overall 'day' effect for the patients $F(1,14) = 18.31$, $P < 0.001$, with higher cortisol levels on the first day. In contrast, there was no 'day' effect in the control group $F(1,14) < 1$, ns (see also Table 1).

Table 1—Mean (±SEM) salivary cortisol (nmol/l) and plasma AVP (fmol/ml) concentrations and osmolality (mosm/kg water) of the patient and control groups on both days

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>Patients</th>
<th>Controls</th>
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<tr>
<td></td>
<td>day 1</td>
<td>day 2</td>
<td>day 1</td>
<td>day 2</td>
</tr>
<tr>
<td>Cortisol</td>
<td>4.42 (0.58)</td>
<td>2.64 (0.27)</td>
<td>3.86 (0.63)</td>
<td>3.52 (0.45)</td>
</tr>
<tr>
<td>AVP</td>
<td>0.84 (0.29)</td>
<td>0.48 (0.12)</td>
<td>0.49 (0.12)</td>
<td>0.59 (0.12)</td>
</tr>
<tr>
<td>Osm</td>
<td>291.7 (1.3)</td>
<td>288.1 (1.0)</td>
<td>286.8 (0.9)</td>
<td>288.7 (1.0)</td>
</tr>
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Plasma AVP and osmolality

Repeated-measures ANOVA demonstrated a group * day interaction for both parameters; for AVP: $F(1,28) = 7.8$, $P < 0.01$, and for plasma osmolality: $F(1,28) = 20.82$, $P < 0.001$. Further subgroup analysis indicated an overall 'day' effect for both parameters in the patient group: for AVP: $F(1,14) = 5.81$, $P < 0.05$, for plasma osmolality: $F(1,14) = 14.66$, $P < 0.01$. Both parameters were higher on the first day. There was no overall 'day' effect for AVP in the control group ($F(1,14) = 2.39$, ns), indicating no significant difference in the mean AVP levels between the two days (see also Fig. 1). There was a positive relationship between salivary cortisol levels and changes in plasma osmolality ($R_s = 0.43$, $P < 0.05$), whereas a positive trend between cortisol levels and changes in AVP levels was not statistically significant ($R_s = 0.33$, $P < 0.1$). There was no significant correlation between the changes in AVP levels and plasma osmolality ($R_s = 0.22$, ns).

Fig. 1—Mean (±SEM) plasma AVP concentrations (fmol/ml) and osmolality (mosm/kg water) of the patient and control groups on both days. □ = Patients; ■ = Controls
DISCUSSION

The role of AVP in the stress response may be mediated by different mechanisms. Results from animal studies demonstrate a direct action of AVP at the pituitary to stimulate ACTH release. AVP can also influence CRF release at the hypothalamic level. Part of the difficulty in determining the role played by AVP in stress responses is caused by the fact that AVP levels in the neurohypophysis and the median eminence are under different control mechanisms, and locally high levels of AVP in the hypophyseal portal blood may be achieved without greatly affecting peripheral concentrations.

There are few data about the relationship between peripheral levels of AVP, osmolality and stress. Parrott et al. found that isolation stress in sheep decreased both plasma AVP levels and osmolality, an effect that was attenuated with more prolonged periods of isolation. The present data are at variance with those of Parrott and co-workers, and indicate that the acute stress associated with LP not only increased plasma levels of AVP, but also plasma osmolality.

The finding that plasma AVP levels were increased by an acute emotional stressor is surprising, as Auböck and Konzett did not find an effect of mental stress on plasma AVP levels in man. In addition, Edelson and Robertson found that the anticipation of a painful procedure decreased AVP in man without affecting osmolality or plasma cortisol. Further evidence obtained from animal studies supports a stress-induced decrease in plasma levels of AVP. A possible explanation for this discrepancy is that the stress-induced negative feedback of glucocorticoids on AVP release may only occur after a more prolonged period of increased cortisol secretion, and that acutely increased AVP levels may result from an interaction with the median eminence. Initial high levels of AVP may even contribute to the stress-induced cortisol response. Alternatively, it is possible that the stress-related changes in catecholamines may stimulate a baroreceptor mediated release of AVP, or that corticoids affect plasma volume.

It has been found that ischaemic pain may increase plasma AVP levels in man. Although it might be possible that back pain could also affect AVP release, this factor does not seem to be of relevance, as there were no significant differences between patient and controls on the second day for any of the biochemical parameters. Moreover, patients using analgesics or tranquillizers were excluded from the study.

The finding of a positive relationship between the cortisol response and the changes in plasma osmolality is new and raises interesting questions, such as whether emotional stress has a direct effect on water metabolism and renal function (mineralocorticoid action of cortisol) and whether stress acts primarily on the release of AVP. Perhaps the rise in osmolality caused the release of AVP rather than the stress of LP procedure.

Stress-induced changes in glucose release should also be considered, as the lack of a direct correlation between plasma AVP and osmolality changes does not support a stress-associated stimulus-response relationship between these two parameters. Particularly, hypoglycaemia can stimulate AVP release in man.

It is possible that the stress-related changes in plasma osmolality were detected because of the increased reliability of the assay (an intra-assay CV of 0.18% instead of 1.2% obtained with single determinations). Because of the small stress-related increase in osmolality of about 3 to 4 mosmoles, an intra-assay CV of less than 0.3% (about 1 mosmole) is necessary to detect these subtle changes.

In summary, the increase in salivary cortisol suggests that the LP procedure is an effective acute emotional stressor in man, and that it increases plasma AVP and osmolality. The precise physiological mechanism, which could conceivably also involve the stress-related effects of atrial natriuretic hormone or mineralocorticoids, remains to be elucidated. Because the parameters under study were only assessed at two times, the present data have to be considered as preliminary results. It will be important for future studies to examine the chronological relationship between cortisol, AVP and water metabolism before, during and after stress in order to elucidate the physiological significance of these mechanisms.

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REFERENCES

1. Lang, R. E., Heil, J. W. E., Ganten, D., Hermann, K., Unger, Th. and Rascher, W. Oxytocin unlike


