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Adrenocorticotropin and cortisol response to lysine vasopressin in relation to the outcome of the dexamethasone suppression test in major depressive disorder

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ABSTRACT — The pathophysiology behind the abnormalities of the hypothalamic pituitary adrenal cortex axis found in patients with major depressive disorder was studied by the use of the vasopressin test. The response of plasma adrenocorticotropin (ACTH) and cortisol to the injection of 10 IU lysine-vasopressin (LVP) was investigated in 18 patients meeting the DSM-III criteria for major depressive episode. The response was correlated to the outcome of the dexamethasone suppression test (DST) with the use of two different cut-off points, 139 nmol/l and 200 nmol/l respectively.

The results show that no significant difference was found in ACTH or cortisol response between patients having a normal or abnormal DST. The results do not seem to support the hypothesis that the abnormalities of the hypothalamic pituitary adrenal cortex axis involve a hypersecretion of corticotropin-releasing factor (CRF) and a subsequent desensitization of the corticotrophs to CRF-stimulated ACTH release.

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The most extensively reported neuroendocrine abnormality found in about 50% of the patients with major depressive disorder involves state dependent changes of the hypothalamic pitutary adrenal cortex (HPA) axis; hyperactivity during exacerbation of depression that disappears in remission. Failure to suppress cortisol secretion after administration of dexamethasone has been the most widely used laboratory aid during recent years in psychiatry to reveal this abnormality. Other manifestations of HPA axis derangement have included an altered circadian rhythm of cortisol and a hypersecretion of cortisol with increased numbers of secretory episodes (1-3).

The pathophysiology underlying the hyperactivity on the HPA axis has not yet been established. It is, however, generally believed that the abnormality is localized in the pituitary-hypothalamic region, but an indication of an increased sensitivity of the adrenal cortex to ACTH stimulation has also been found (4). Under normal conditions, the release of adrenocorticotropin (ACTH) from the corticotroph cells of the anterior pituitary gland is controlled by the hypothalamus through the secretion of various neuropeptides, of which corticotropin-releasing factor (CRF) serves as the primary regulator. Other regulatory factors include vasopressin, angiotensin II, vaso-active intestinal polypeptide (VIP), catecholamines, prostaglandins, ß-endorphin and glucocorticoids.

Recently, we have hypothesized that the abnormalities in the HPA axis in depressed patients involve a hypersecretion of CRF, which induces a desensitization of the corticotrophs to CRF-stimulated ACTH release (5). A support for this hypothesis is that continuous 24-h infusion of ovine-CRF in normal man provides a 24-h cortisol secretory pattern similar to that in depression (6). Furthermore, the concentration of CRF-like immunoreactivity in cerebrospinal fluid but not in plasma was significantly increased in depression compared to normals (7,8). However, the ACTH and cortisol response to an intravenous test dose of human-CRF (h-CRF) or ovine-CRF in depression is unclear. Both a blunted ACTH response and a normal ACTH and cortisol response to ovine-CRF and h-CRF have been reported in depression (9,10).

Arginine-vasopressin (AVP) stimulates ACTH secretion and potentiates the effect of CRF at the pituitary level (11). The two effects are probably mediated by different types of vasopressin receptors (12). Consequently, if the abnormalities in the HPA axis involve a hypersecretion of CRF, then the administration of vasopressin to patients with depression may help to elucidate the pathophysiology of this disorder. The present report describes the effect of vasopressin on ACTH and cortisol release in patients with acute major depressive disorder with normal or abnormal response to dexamethasone.

Material and methods

Subjects

Eighteen (four men, 14 women) depressed patients meeting the DSM-III criteria for major depressive episode from the psychiatric emergency ward at St. Göran's Hospital in Stockholm during a 10 month period were invited to participate in the study. They all gave verbal, informed consent. Exclusion criteria were: 1) present abuse of alcohol or drugs; 2) schizophrenia or any severe psychiatric disease other than depression; 3) severe somatic disease; 4) pregnancy. Seventeen of the patients were considered unipolar and one had a bipolar affective disorder. Sixteen patients had experienced one or more earlier depressive episodes. The length of the present depression ranged from 2 to 50 weeks with an average of 13 weeks. Eight patients were admitted as inpatients while 10 were treated as outpatients. The mean age was 44 ± 13 years (range 19-67), and the mean body-weight was 63 ± 17.5 kg (range 47-119).

All patients who had psychoactive drugs were allowed to continue their medication. Two patients had been on antidepressants for several months without beneficial effect: maprotilin 75 mg/d and lofepramin 140 mg/d. Three patients were on neuroleptic drugs: fluphenacine 0.75 mg/d, flupentixol 2 mg/d and one patient on a combination alimemazine 20 mg/d and propiomazine 25 mg/d, however taken only when needed. Twelve patients were taking various benzodiazepines, for the most only in small doses and taken only when needed: oxazepam 10-25 mg/d, nitrazepam 5 mg/d, flunitrazepam 2 mg/d, diazepam 5-30 mg/d (one patient took diazepam in doses up to 30 mg/d, the others only 5 mg/d) and lorazepam 3 mg/d. No patient had been treated with electroconvulsive therapy during the present depression before the vasopressin study.

In total, 13 patients were taking psychoactive drugs, whereas five were not. Of these 13 patients, eight were on benzodiazepines alone. Three of the patients not taking psychoactive drugs had been completely free of medication for at least 3 months prior to the onset of the study.

Clinical assessment

A physical and psychiatric examination was performed during the first day of the study. The psychiatric examination included clinical rating using the Comprehensive Psychopathological Rating Scale (CPRS) for depressive illness (13). The CPRS global scores (Gls) (range 0-3) were used, where score 3 stands for the most severe state. The sum of 22 items (CPRS 22) as recently described (14) and the sum of ten items (CPRS 10) (15) were also used. The ratings were performed by two psychiatrists and the mean of the two raters score was used.

Endocrine measurements

The *vasopressin test* was performed in the morning after fasting from 10 p.m. the evening before. An indwelling catheter was inserted in a forearm vein between 8-9 a.m. Thirty minutes later 10 IU lysine-vasopressin (LVP) (Postacton® Ferring Co, Malmö,

Sweden) was given as an intramuscular injection in musculus quadriceps femoris. The mean vasopressin dose was 0.158 IU/kg body weight (range 0.084-0.213 IU/kg).

Blood-samples were drawn once prior to the injection as well as 30, 60 and 120 min after. The blood samples were rapidly centrifuged and frozen.

A *dexamethasone suppression test (DST)* was performed the following day. At 10 p.m. the subjects received 1 mg dexamethasone (Decadron® MSD Co, Bromma, Sweden) orally and blood samples were drawn at 8 a.m., 15 p.m. and 22 p.m. the following day.

The DST was considered abnormal if serum cortisol was not suppressed below a) 139 mmol/l (= 5.0 microgram/dl)-*low cut-off point*, b) 200 nmol/l (= 7.3 microgram/dl)-*high cut-off point*.

Assays

The blood samples were analyzed by radioimmunoassay for ACTH (SILAB, Malmö, Sweden) and cortisol (Farmos Diagnostica, Turku, Finland).

The intra-assay variation for ACTH was 5%, inter-assay variation 8% and lower limit of detection 5 pmol/l. The reference area was 5-30 pmol/l (morning level). The corresponding figures for the cortisol determinations were 2.4%, 4-5%, 20 nmol/l and 170-660 nmol/l (morning level).

Statistical methods

Several measures describing the ACTH and cortisol levels were defined. *ACTH max;* the highest ACTH level after vasopressin administration. *Δ ACTH;* the difference between the highest ACTH after vasopressin administration and the basal level. The corresponding calculations were made for the cortisol levels. The *total area* is the total area under the ACTH or cortisol serum curve and the *net area* is the area under the curve above the baseline level. In general, data were non-normally distributed. Thus, statistical analyses were performed using the Wilcoxon rank-sum test (two uncorrelated groups) and calculating the Spearman rank correlation coefficient (r). The statistical power of the Wilcoxon rank sum test has been reported to be approximately equal to or even greater than the Student's test when data show a non-normal distribution (16). Data are presented as mean ± SEM, and the level of significance was $P < 0.05$. In addition P values < 0.10 are reported as well.

Ethical judgement

The investigation was approved by the Ethics Committee of the Karolinska Institute, Stockholm, Sweden.

Results

Ten patients showed a normal DST response (Group A), five patients showed an abnormal response using the high cut-off point (Group C) and additionally three (Group B) showed an abnormal response using the low cut off point for the DST. The median levels of ACTH were for Group A 20 pmol/l (baseline) and 29 pmol/l (peak level after stimulation) for group B 16 pmol/l (baseline) and 26 pmol/l (peak level after stimulation) and for Group C 19 pmol/l (baseline) and 27 pmol/l (peak level after stimulation). The median levels of cortisol were for Group A 478 nmol/l (baseline) and 853 nmol/l (peak level after stimulation), for Group B 331 nmol/l (baseline) and 824 nmol/l (peak level after stimulation) and for Group C 495 nmol/l (baseline) and 904 nmol/l (peak level after stimulation).

The ACTH and cortisol levels before and after vasopressin administration are displayed in Fig. 1. Depending on the low or high DST cut-off points patients in Group A or in Group A+B were classified as suppressors and compared with the nonsuppressors in Group B+C or in Group C, respectively. There were no significant differences between the groups in ACTH or cortisol levels at baseline, ACTH max, cortisol max, Δ ACTH or Δ cortisol. Similar results were obtained when the total or net areas under the ACTH or cortisol serum curve 0-120 or 30-120 min were compared.

The Spearman rank correlation matrix (not shown) revealed that baseline cortisol was positively correlated to cortisol max ($r = 0.66$, $P < 0.003$). A significant positive correlation was also found between Δ ACTH and Δ cortisol ($r = 0.53$, $P < 0.04$) in the whole group of patients. The correlation coefficients between Δ ACTH and Δ cortisol in Group A, B and C were $r = 0.94$, $r = -1.0$ and r

in ACTH or cortisol levels before or after vasopressin administration between the patients divided according to their DST response. This result suggests a dissociation between the DST and the vasopressin test in depression.

Discussion

A methodological problem in this study is the lack of a drug wash-out period. The reason for not using drug-free patients were mainly three: (1) The problem of sampling a sufficient number of drug-free patients over a reasonable period of time, (2) The ethical problem of denying a severely depressed patient continued antidepressive medication already prescribed by the referring physician, (3) The withdrawal of drug therapy may induce secondary changes in different endocrine systems for various lengths of time e.g. a sustained decrease of 5-hydroxytryptamine in blood for at least 2 weeks following withdrawal of antidepressants has been reported (17). Because of the lack of a wash-out period in this investigation the possible influence of drugs was examined. A consistent influence of drugs on the results was not found.

In the present study two cut-off points for the outcome of the dexamethasone test were used. The reason is that it has been demonstrated by a number of investigators during recent years that the specificity and the sensitivity of the dexamethasone test are dependent of several factors, e.g. dose, criterion levels, sample timing, and clinical factors such as hospitalization, diet and age (3). Thus, results from one study showed that going from a criterion for non-suppression of cortisol $>$ 82 nmol/l, to $>$ 165 nmol/l, decreased the sensitivity from 48 to 37% but increased the specificity from 81 to 90% and the predictive value from 72 to 79%. An increase of the number of post-dexamethasone cortisol determinations from two points to six points increased the sensitivity from 31 to 44% (18). The results from the present study showed that the conclusion was similar regardless if the low or high cut-off point was used.

Results from previous studies indicate that CRF is the most important physiological stimulator of ACTH secretion in vivo. CRF binding sites have been localized in the rat anterior pituitary (19,20) and the number of binding-sites has been shown to be regulated by glucocortiocoids (21,22). The adenylate cyclase system is probably involved in the mechanism of action of CRF on the anterior pituitary (23).

Vasopressin plays a direct role in the regulation of ACTH secretion and receptors for AVP in pituitary cell membranes, clearly distinct from those that bind CRF, have been demonstrated (24). It has also been shown that the vasopressin receptor, as other receptors for the releasing hormones, can undergo homologous down-regulation according to a process apparently linked to the desensitization of cell response (25).

CRF and vasopressin have been shown to exert a synergistic effect on ACTH release in man. The combination of CRF and LVP administration in man induced greater ACTH release than the sum of the responses to CRF and LVP alone. The synergistic effect of CRF plus LVP concerned *only* ACTH release, while cortisol release after CRF plus LVP was equivalent to the sum of the maximal increments of this hormone after CRF and LVP alone, possibly reflecting that the maximum stimulatory capacity of the adrenal cortex had been reached (26). The potentiation of CRF activity by vasopressin is well documented from numerous experiments in animals.

Glucocorticoids are well-known inhibitors of ACTH secretion and dexamethasone reduces the ACTH response to CRF and vasopressin. The site of action of glucocorticoids in the corticotrophs is still unknown, but most likely is at a step subsequent to cyclic AMP formation (27).

It has recently been shown that while neither CRF nor LVP were able to produce a marked elevation of cortisol and ACTH in healthy humans pretreated with dexamethasone a substantial escape from the inhibition of plasma ACTH and cortisol response was found when both peptides were administered in combination (28).

We have recently proposed that the mechanism behind the abnormalities in the hypothalamic pituitary adrenal cortex axis seen in patients with major depressive disorder and revealed by an abnormal dexamethasone test involves a hypersecretion of CRF which in turn induces a desensitization of the corticotrophs to CRF-stimulated ACTH release (5). The results from the present study indicate that there is no difference in ACTH and cortisol re-

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