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In this issue: This study provides convincing evidence for the importance of genetic factors in determining natural and surgical menopause. *(See page 1875.)*

Also in this issue: The data indicate that the elevated plasma levels of free metanephrines in patients with pheochromocytoma are derived from catecholamines produced and metabolized within tumors. *(See page 2175.)*

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Glucocorticoids Suppress Corticotropin-Releasing Hormone and Vasopressin Expression in Human Hypothalamic Neurons*

ZEYNEL A. ERKUT, CHRIS POOL, AND DICK F. SWAAB

Netherlands Institute for Brain Research, Graduate School Neurosciences, Amsterdam, The Netherlands

ABSTRACT

Glucocorticoids are widely used in clinical practice in a variety of immune-mediated and neoplastic diseases, mostly for their immunosuppressive, leukopenic, antiedematous, or malignancy-suppressive actions. However, their usage is limited because of serious and sometimes life-threatening side-effects.

Endogenous glucocorticoids are secreted by the adrenal cortex under the control of the hypothalamus and the pituitary gland. This hypothalamo-pituitary-adrenal axis, in turn, is under the negative feedback control of glucocorticoids. Although the suppression of adrenocortical and pituitary gland functions by glucocorticoids has been shown in humans, a feedback effect at the level of the hypothalamus, as shown in rat, has not been reported to date. The present study shows for the first time that glucocorticoids suppress both CRH and vasopressin (AVP) in the human hypothalamus. We studied immunocytochemically the postmortem hypothalami of nine corticosteroid-exposed subjects and eight controls. The number of CRH-expressing cells in the hypothalamic paraventricular nucleus of glucocorticoid-exposed patients was only 3.3% of that in the controls, and the total immunoreactivities for AVP were 31% and 33% of that in the controls in the supraoptic nucleus and the paraventricular nucleus, respectively, whereas the immunoreactivity for oxytocin did not differ between the two groups.

Suppression of hypothalamic CRH and AVP neurons by glucocorticoids may have important consequences for neuroendocrinological mechanisms such as the disturbance of water balance during the treatment as well as the immunological processes in the brain and the pathogenesis of the withdrawal syndrome after discontinuation of corticosteroid treatment. In addition, as both AVP and CRH neurons also project to other brain structures and influence memory, mood, and behavior, their suppression by glucocorticoids may be responsible for at least part of the central nervous system side-effects of glucocorticoids. (*J Clin Endocrinol Metab* **83:** 2066–2073, 1998)

GLUCOCORTICOIDS are widely used in a broad spectrum of neoplastic (1–3) and immune-mediated diseases (3–6), because of their acute leukopenic, antineoplastic, antiedematous, and immunosuppressive effects. However, their actions on the human central nervous system (CNS) and especially on the hypothalamus are equivocal.

Endogenous corticosteroid secretion from the adrenal cortex is mainly under the control of ACTH of the pituitary gland. ACTH secretion, in turn, is controlled to a great extend by the hypothalamic CRH and vasopressin (AVP). AVP and CRH also potentiate each other's effect on ACTH release (7).

The hypothalamo-pituitary-adrenal (HPA) axis is the major system involved in the maintenance of homeostasis in stress. In immune-mediated diseases it is presumed that the endogenous increase in glucocorticoid production is essential to quell the symptoms and to overcome the disease attack (8–12). However, the overproduction of endogenous glucocorticoids is apparently often not sufficient to prevent and quell the symptoms of the disease, and administration of high doses of exogenous glucocorticoids is then needed to suppress the ongoing disease process.

Glucocorticoid therapy potentially has numerous central and systemic side-effects that can become hazardous and even life-threatening. The central side-effects include mood changes such as depression, euphoria, fatigue, and insomnia as well as impairment of memory and cognition, psychosis, and convulsions (13–17). Systemic side-effects commonly involve endocrine systems causing, for example, diabetes mellitus, Cushingoid signs, osteoporosis, disturbance in water balance, and the suppression of the endogenous HPA axis (1, 18–24). However, the mechanisms involved in the induction of such side-effects are not yet clearly understood.

Animal experimental studies showed glucocorticoid suppression of the adrenal cortex, pituitary corticotropic cells, and hypothalamic CRH and AVP neurons (25–31). Although glucocorticoids are also shown to suppress the adrenal and pituitary in humans (21–23), their effects on the hypothalamic level are controversial. Endocrine studies on blood and cerebrospinal fluid, estimating alterations in CRH and AVP levels after corticosteroid treatment, gave conflicting results (13), and the only study performed on human hypothalamic tissue unexpectedly showed no change in CRH or AVP content after corticosteroid treatment (32).

In the present study, performed on postmortem hypothalami of patients who had been exposed to glucocorticoids during the last premortem periods, we show for the first time that both hypothalamic CRH and AVP expressions are strongly suppressed by glucocorticoids in humans.

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Address all correspondence and requests for reprints to: Dr. Zeynel A. Erkut, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands. E-mail: e.zeynel@nih.knaw.nl.

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Materials and Methods

Hypothalami of 17 subjects were examined for CRH and AVP immunoreactivity, using a double labeling immunocytochemical (ICC) method. Briefly, the formalin-fixed and paraffin-embedded postmortem tissue was cut into 6-μm thick serial sections, and to every 50th section the double ICC staining protocol for CRH and AVP was applied as described previously (33). In this protocol, the monoclonal rat antibody against CRH (PFU-83) (34) was used (provided by Dr. F. J. H. Tilders, Free University, Amsterdam, The Netherlands). The rabbit antiserum Truus (29.1.1986), after being adsorbed with oxytocin (OXT) to remove cross-reactivity with OXT, was used to detect AVP in its processed form. In addition, selected representative hypothalamic sections from all subjects were stained for OXT and the AVP precursor glycopeptide using monoclonal mouse anti-OXT (A-I-28, provided by Dr. A. Hou-Yu, Columbia University, New York, NY) (35) and polyclonal rabbit anti-glycopeptide against a synthetic human glycopeptide fragment representing residues 22–39 (Boris, provided by Dr. W. G. North, Dartmouth Medical School, Lebanon, NH) (36), respectively.

Eight of the subjects, who were free of glucocorticoid therapy during the premortem period and died from different diseases, served as controls. From the seven patients who formed the glucocorticoid exposed group, one was exposed to high levels of endogenous glucocorticoids as the result of an adrenal tumor, and six had been exposed to exogenous glucocorticoid administration at different doses and durations during the premortem period until their death. Two subjects, who were receiving glucocorticoid treatment until 2 weeks (no. 16) and 2 months (no. 17) before death, were evaluated separately. The patients in the glucocorticoid-exposed group had no common condition or medication other than glucocorticoids, and none had a pituitary disorder as an indication for corticosteroid therapy. Clinico-pathological information on the subjects as well as the dose and duration of glucocorticoids are summarized in Table 1.

Evaluation of CRH-immunopositive neurons

After the double labeling ICC protocol, the total CRH cell number in the paraventricular nucleus (PVN) was estimated by counting only those CRH-immunoreactive cell profiles that presented with a nucleolus, followed by multiplication of the count by the sample frequency of the sections through the hypothalamus (49 ± 0.4).

Evaluation of AVP-immunoreactive neurons

To study the total volume occupied by the AVP-immunoreactive cells, the intensity of AVP immunoreactivity in the cells, and the total AVP immunoreactivity in the PVN and the supraoptic nucleus (SON), every 100th section throughout the hypothalamus was stained for

TABLE 1. Clinicopathological data of the subjects

Patient no.	Sex	Age (yr)	ctd	pmd	fxp	brw	Diagnosis, clinicopathological information, summary of the steroid medication
Controls							
1 (90–901)	m	30	18:00	4 h 50	46	1325	Fallot's tetralogy, bacterial endocarditis
2 (87–260)	m	37	09:25	36 h	46	1510	Alcohol and benzodiazepine intoxication, cerebral edema
3 (86–403)	f	53	14:00	24 h	17	1410	Chronic myeloid leukemia with dura mater metastasis
4 (92–046)	f	54	ND	13 h	ND	1080	Traffic accident
5 (92–047)	m	54	ND	14 h	31	1410	Bronchogenic carcinoma
6 (90–060)	m	68	15:30	7 h	47	1365	Coronary by-pass, myocardial infarction
7 (94–191)	m	78	12:15	8 h 25	24	1442	Metastatic prostate carcinoma, renal insufficiency, death due to cardiac arrhythmia
8 (93–019)	m	78	12:10	52 h 50	70	1340	Bronchopneumonia, cardiopulmonary insufficiency
Mean		56.5		20 h 10	40.1	1360.3	
± SEM		6.2		5 h 55	6.7	45.2	
Corticosteroid group							
9 (83–173)	f	46	06:10	5 h 50	33	1360	Metastatic adrenal carcinoma causing high levels of adrenal steroids [urinary 17-ketosteroids, 4164 μmol/24 h (normal, 21–52); 17-hydroxy-corticosteroid, 381 μmol/24 h (normal, 10–52); plasma cortisol, 0.69 μmol/L at 1000 h and 0.77 μmol/L at 1500 h (normal, 0.14–0.55);] perioperative corticosteroid supplement
10 (95–026)	m	62	10:15	6 h 35	35	1350	Metastatic adenocarcinoma; prednisone, 30 mg/day for last 18 days
11 (93–133)	m	64	06:00	8 h 10	30	1450	Chronic myeloid leukemia; prednisone, 60–80 mg/day for last 5 months
12 (95–120)	m	65	01:15	4 h 45	28	1500	Basal cell carcinoma, asthma bronchial; chronic low dose beclomethason inhalation and 200 μg/day for last 7 days
13 (93–094)	f	67	ND	<17 h	79	1340	Lung carcinoma with metastasis and thrombocytopenia; prednisone 60 mg/day for last 7 days
14 (93–095)	m	75	12:00	53 h	618	1280	Metastatic prostate carcinoma with pneumonia, lung edema, and heart failure; prednisone, 30 mg/day last 2 days
15 (92–156)	f	76	09:25	<8 h	269	1225	Ovarium adenocarcinoma with metastasis; prednisone, minimal 60 mg/day for last 8 days
Mean		65.0		14 h 5	156.0	1357.9	
±SEM		3.8		6 h 40	83.7	35.5	
P		0.488		0.298	0.406	0.685	
Subjects lately exposed to corticosteroids							
16 (86–354)	f	33	ND	18–41 h	20	1035	Metastatic lung carcinoma. Dexamethasone up to 9 mg/day, mostly 0.5–1.5 mg/day for >1 month, gradually stopped 14 days before death
17 (95–132)	f	72	13:50	9 h 10	34	1075	Cardiac failure with respirator insufficiency, cachexia, dehydration; chronic prednisone use of 5 mg/day, doses of 5–30 mg/day last 4 months, stopped 2 months before death

The variables age, pmd, fxt, and brw were tested between the control and corticosteroid-exposed group, and the *P* values given were determined by the Mann-Whitney U test. brw, Brain weight in grams; ctd, clock time of death; f, female; fxp, fixation period in days; m, male; ND, not determined; pmd, postmortem delay of abduction in hours. Antiinflammatory and Na^+-retaining potentials of prednisone/prednisolone (PRED) and dexamethasone (DEX), relative to cortisol; PRED, 4 and 0.8; DEX, 25 and 0.1, respectively.

AVP alone and colored by 3,3'-diaminobenzidine. In these sections, estimates of the total volume of AVP cells and the total amount of AVP immunoreactivity in these cells were determined with the help of a computerized image analysis system (IBAS, Kontron, Zurich, Switzerland). The PVN or SON, after being presented to IBAS in ×2.5 objective magnification, were automatically loaded into the image memory as 12 pieces of 2560 × 1536 pixel images by the ×10 objective and the computer-controlled scanning stage. After the original ×2.5 magnification image was reconstructed by computerized pasting of these 12 images in a 4 × 3 image plate, the area of interest was manually outlined, and the artifacts were deleted from the mask area when needed. Both the 2560 × 1536 pixel images and the manual outlines as well as the areas of deleted artifacts were stored on disk. IBAS calculated for every image a mask of the immunoreactive cell profiles. For every outlined area, the following parameters were automatically calculated: 1) the optical density of each mask and the mean optical density of all the masks in each section, 2) the total area of the masked structures (summation of the area covered by every single mask in each section), and 3) the structure-area weighted mean optical density (the mean integrated staining per section, which is calculated by multiplying the mean optical density of the mask by the total area of the mask in each section). The final and the main parameter, which is the total integrated immunoreactivity in the PVN or SON of the patient, was calculated by multiplying the mean integrated staining per section by the sample frequency.

Evaluation of OXT-immunoreactive neurons

To test possible unpredictable influences of technical parameters such as postmortem delay and fixation period, three sections through the PVN of each subject, with a sampling frequency of 78 ± 2.7, were stained for OXT and digitally evaluated in the manner described above for AVP.

The results of CRH cell count as well as AVP and OXT staining were compared between the glucocorticoid-exposed subjects and controls using a two-tailed Mann-Whitney U test, taking $P < 0.05$ as the level of significance.

Results

The variables age, sex, postmortem delay between the time of death and the brain autopsy, fixation period of the brain tissue, and brain weight of the glucocorticoid-exposed group and controls did not show a statistical difference ($P \geq 0.3$; see Table 1).

Suppression of CRH and AVP

Immunoreactivity for CRH in the PVN and for AVP in the PVN and the SON was strongly diminished in the glucocorticoid-exposed group compared to that in the controls. The decreased immunoreactivity for both peptides was also observed in the median eminence (Fig. 1).

CRH. The number of CRH-immunoreactive cells in the group of patients exposed to glucocorticoids was only 3.3% of that in the controls (mean ± SEM, 204 ± 90 *vs.* 6221 ± 2700; $P = 0.001$; Fig. 2A). The ratio of AVP-coexpressing CRH cells to the total CRH cells was 60% in the control group, which was similar to the values from previous studies (33, 37, 38). Because of the very low cell counts (less than five detectable cells in most subjects), it was not considered relevant to determine the colocalization ratio of the glucocorticoid-exposed group. The estimated CRH cell number in the subject who stopped glucocorticoid medication 2 weeks before death (no. 16) was 305, which is close to the mean value in the glucocorticoid-exposed group, whereas that in the subject who stopped medication 2 months before death (no. 17) was 2772, *i.e.* in the range of the mean of the controls.

AVP. The glucocorticoid-exposed group had decreased values for all three parameters compared to the control group (see *Materials and Methods* for detailed descriptions of the parameters). The mean staining intensity of the AVP-immunoreactive cells in the glucocorticoid-exposed group was 61% of the control value in the PVN (0.125 ± 0.024 *vs.* 0.204 ± 0.013 arbitrary units; $P = 0.021$) and 65% of that in the SON (0.130 ± 0.025 *vs.* 0.201 ± 0.017; $P = 0.083$; Fig. 2C). The total volume occupied by AVP-immunoreactive structures in the glucocorticoid-exposed group was 51% of the control value in the PVN ($73.80 \pm 17.94 \times 10^6$ *vs.* $144.34 \pm 19.80 \times 10^6$ μm^3; $P = 0.015$) and 37% in the SON ($79.32 \pm 26.48 \times 10^6$ *vs.* $211.65 \pm 33.40 \times 10^6$ μm^3; $P = 0.011$). Finally, the total integrated immunoreactivity for AVP in the glucocorticoid-exposed group was 33% of the control value in the PVN ($10.03 \pm 2.81 \times 10^6$ *vs.* $30.40 \pm 5.50 \times 10^6$ arbitrary units; $P = 0.008$) and 31% of that in the SON ($14.15 \pm 6.30 \times 10^6$ *vs.* $44.93 \pm 8.93 \times 10^6$ arbitrary units; $P = 0.011$; Fig. 2B). The mean AVP staining intensity for subject 16, who stopped dexamethasone treatment 14 days before death, was 0.332 in the PVN and 0.322 in the SON, and the total integrated immunoreactivity was 63.06×10^6 in the PVN and 124.26×10^6 in the SON; both of these were the highest values of all subjects. The mean staining intensity for subject 17, who stopped prednisone treatment 2 months before death was 0.115 in the PVN and 0.124 in the SON, whereas the total integrated immunoreactivity was 10.12×10^6 in the PVN and 7.86×10^6 in the SON, both in the range of values in corticosteroid-exposed subjects.

AVP staining was also evaluated as a ratio to the unchanged OXT staining (see below), and similar results were obtained. The mean staining intensity for AVP relative to the mean staining intensity for OXT was 0.70 ± 0.04 for controls and 0.42 ± 0.08 for the corticosteroid-exposed subjects ($P = 0.011$). Correlations between the absolute and relative staining intensities for AVP were $r = 0.89$ for controls ($P = 0.003$) and $r = 0.97$ for the corticosteroid-exposed subjects ($P < 0.001$).

Nonsuppression of AVP precursor glycoprotein and OXT

Both groups showed strong positive immunoreactivity for the AVP precursor glycopeptide, and the staining did not reveal any difference between the groups. The mean staining intensity for OXT was similar in the two groups (0.29 ± 0.01 for controls *vs.* 0.30 ± 0.01 for the corticosteroid-exposed group; $P > 0.5$; Figs. 2D and 3).

Effects of long postmortem delay, fixation period, and clinics on the immunoreactivity

Two hypothalami from the corticosteroid-exposed group had very long fixation periods (no. 14 and 15; 618 and 269 days) and one subject from each group had long postmortem delay (no. 8 and no. 14; 52 h and 50 min, and 53 h). To test the possible effects of a very long postmortem delay or fixation period on the staining of the brain tissue,

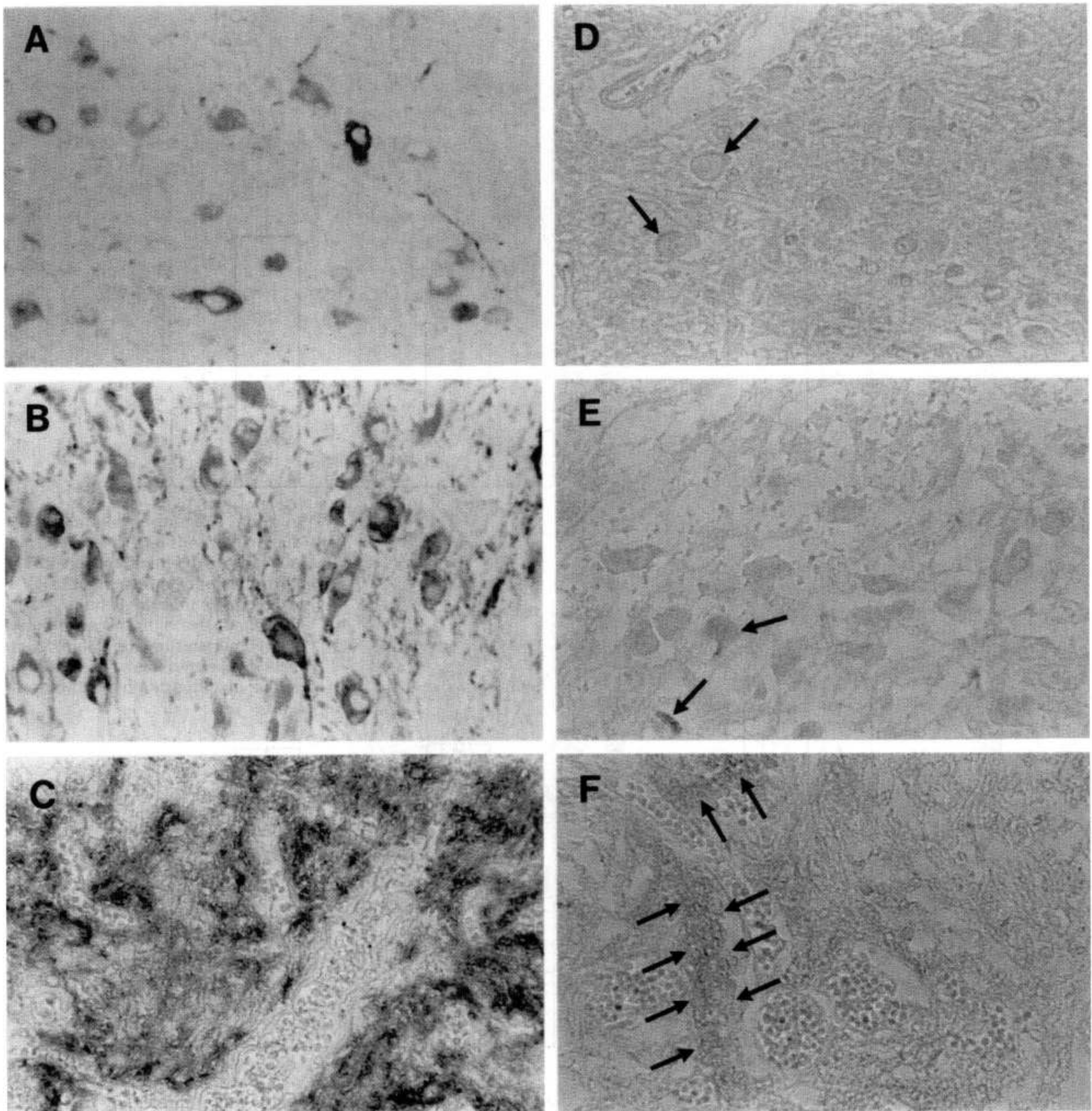

FIG. 1. Representative sections showing the immunocytochemical stainings of controls (A–C, patient 5) and corticosteroid-treated subjects (D–F, patients 12–14). PVN (A and D) and SON (B and E) show the immunoreactivity for AVP. Median eminence (C and F) shows costaining for AVP and CRH. *Arrows* show the positive immunoreactivity in the neurons and nerve terminals in the median eminence of corticosteroid-exposed patients (×300). Note the decreased staining of AVP and CRH in the corticosteroid-treated patients.

sections from one subject with a 120-h postmortem delay (no. 87-069) and from one with a 607-day fixation period (no. 95-083) were also included in our staining protocol. As strong immunoreactivity was obtained for AVP, OXT, and CRH, differences in postmortem delay or fixation period were not likely to account for the diminished CRH and AVP staining in corticosteroid-treated subjects. Any cause other than glucocorticoids for the variability in AVP immunoreactivity in the PVN and SON could not be found in the clinical records.

Discussion

Suppression of CRH and AVP by glucocorticoids

This study shows that CRH and AVP expression in hypothalamic neurons is strongly affected by glucocorticoids, indicating that a negative feedback inhibition by glucocorticoids of these hypothalamic neuropeptides also takes place in humans. The unchanged staining of OXT in both groups indicates that the suppressive effect of glucocorticoids on hypothalamic neuropeptides is selective, which is in accor-

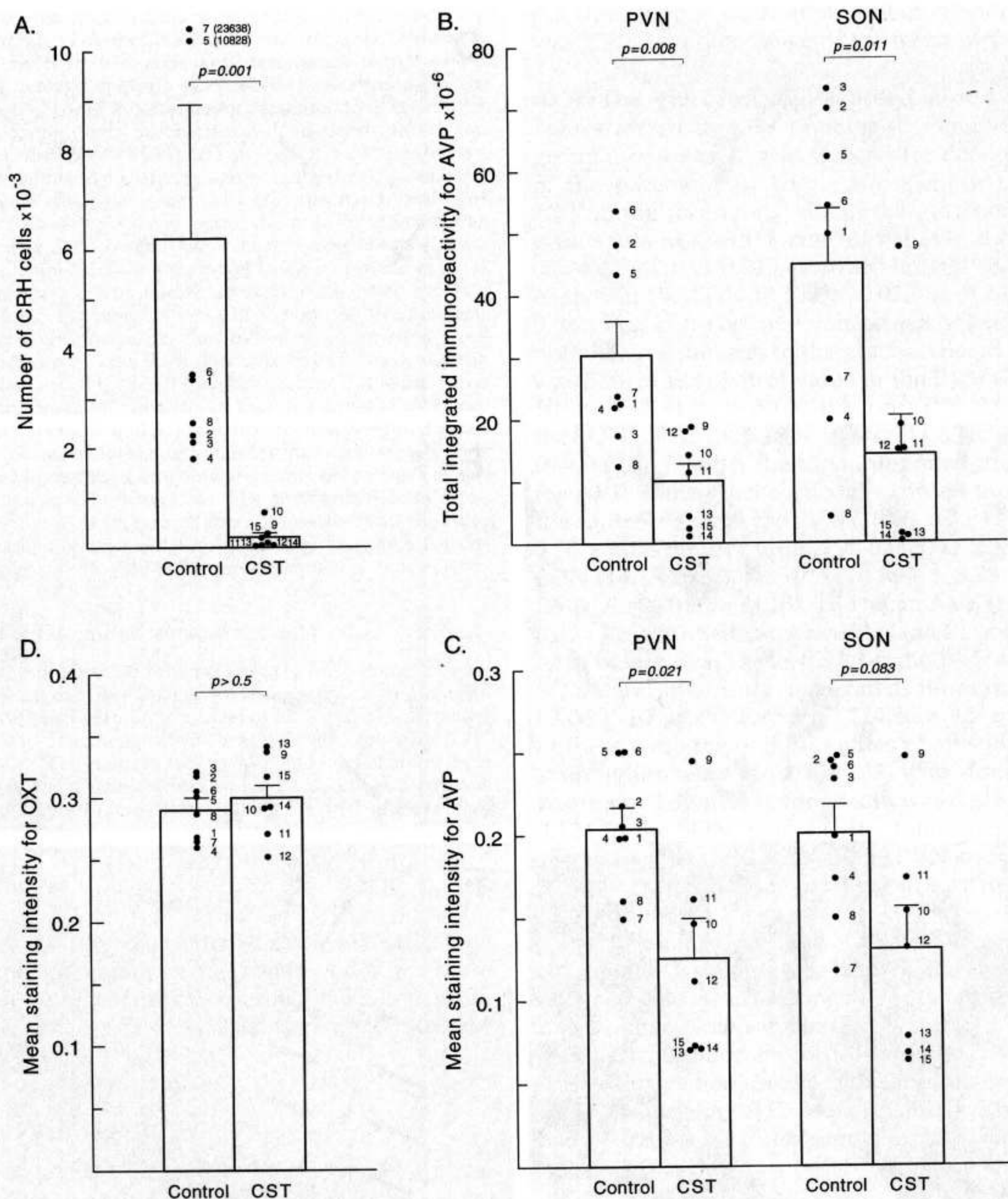

FIG. 2. Estimated number of CRH-immunoreactive cells in the hypothalamic PVN (A), the total integrated immunoreactivity for AVP (B), the mean staining intensity of AVP-immunoreactive cells in the PVN and SON (C), and the mean staining intensity for OXT in the PVN of the controls and the corticosteroid-exposed subjects (D; CST). The numbers of the plotted data refer to the numbers of subjects in Table 1. The *bars* and *error lines* represent the mean and SEM, and the *P* values are according to the Mann-Whitney U test.

dance with the animal experimental literature (26, 27). The staining for the AVP precursor was also strong in both the glucocorticoid-exposed subjects and the controls. In a pilot study we observed a strong *in situ* signal for AVP messenger ribonucleic acid in the PVN and SON of corticosteroid-exposed subjects with very low processed AVP expression (unpublished results). These two observations indicate that the reduced vasopressin immunoreactivity may be due to a suppressive effect of corticosteroids on the processing of the AVP precursor into AVP, rather than on the transcription stage of AVP synthesis. It should be noted that theoretically the diminished hypothalamic immunoreactivity for processed AVP can be due to a rapid and massive excretion of this peptide; however, this possibility is not in accordance with the suppressive effect of glucocorticoids on human plasma AVP levels (39).

The suppression of both hypothalamic CRH and AVP by glucocorticoids has been shown by *in vitro* and *in vivo* animal studies. In our study, we observed in humans a dramatic decrease in the expression of both CRH and AVP in the PVN and SON. This is in contrast with the results of a previous study (32) in which homogenized hypothalamic parts were studied by RIA and immunoradiometric assay, showing no difference in AVP and CRH contents

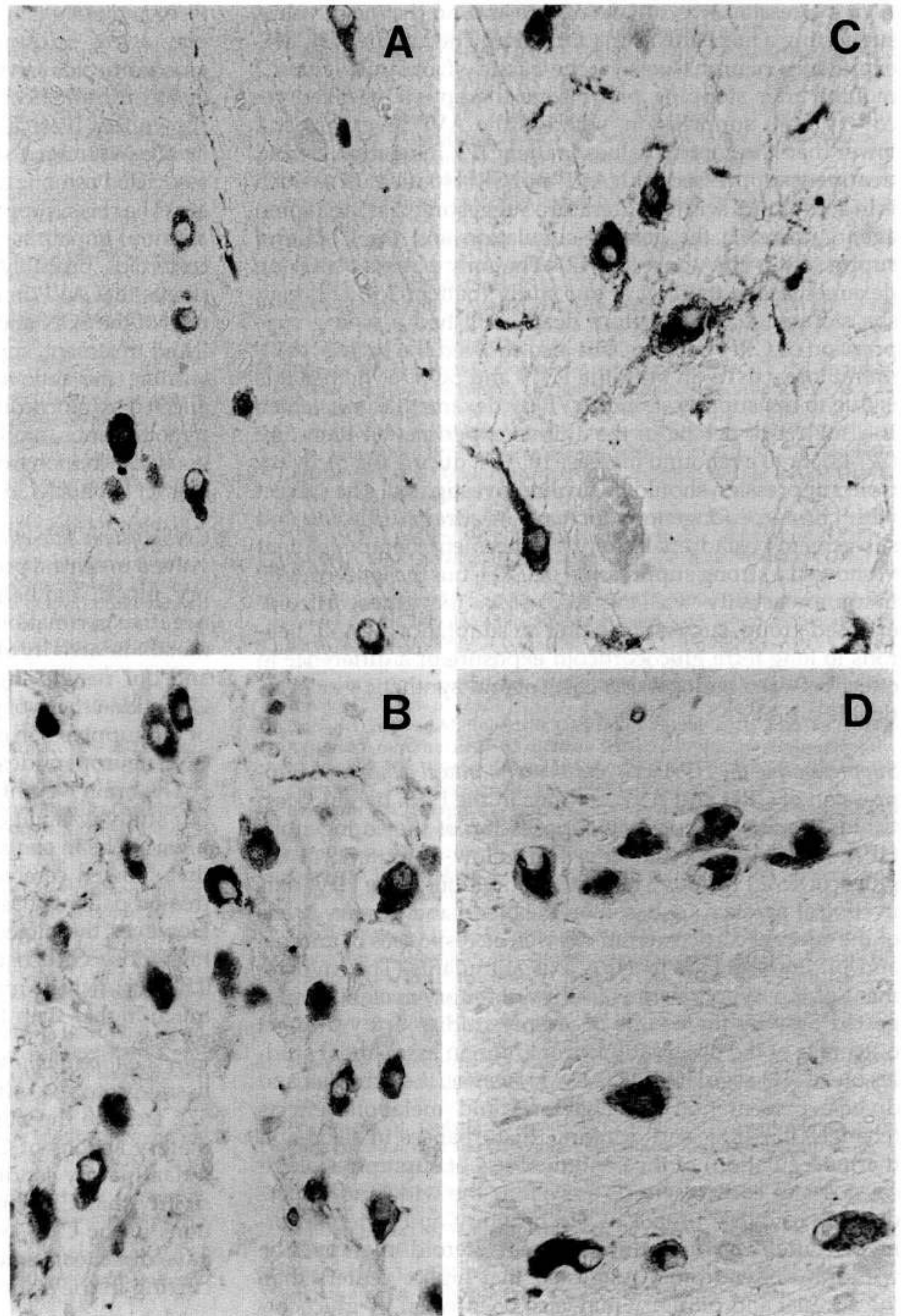

FIG. 3. Immunocytochemical staining for OXT (A and C) and the AVP precursor glycopeptide (B and D) are shown in the PVN of representative sections from controls (A and B, patient 2) and corticosteroid-exposed subjects (C and D, patients 12 and 14; magnification, ×300). No difference between controls and corticosteroid-treated patients was found.

between the corticosteroid-exposed subjects and controls while showing high levels of CRH and AVP in parts of the hypothalamus where neither immunoreactivity nor messenger ribonucleic acid for these peptides has ever been histologically demonstrated in humans, such as the dorsal and posterior hypothalamic areas. In addition, in the same study, levels of CRH were reported in the PVN, the major source of CRH, similar to those in other parts of the hypothalamus where CRH has only been found in trace amounts, such as the lateral hypothalamus and the mammillary bodies (40).

Basis and consequences of glucocorticoid suppression of hypothalamic CRH and AVP

Details of glucocorticoid suppression in our study. Prednisone, at various doses and durations of use, had a clear suppressive effect on CRH and AVP in all subjects studied. The suppressive effect of prednisone on both CRH and AVP expression was already obvious on the second day of administration (patient 14). On the other hand, after the highest cumulative dose of corticosteroid administration in our study, which is 60–80 mg/day prednisone for the last 5 months (patient 11),

AVP expression was still detectable around the mean value, suggesting a mechanism of adaptation. The number of CRH-expressing neurons was in the range of control values 2 months after stopping prednisone therapy, suggesting recovery from suppression, whereas the AVP level was still lower than the control values (patient 17). Inhalation steroid treatment suppressed both AVP and CRH (patient 12), which is in accordance with the literature suggesting that inhalation agents do reach the general circulation and the CNS and suppress the HPA axis (41, 42). The only patient receiving dexamethasone therapy in our study (patient 16), although she stopped 2 weeks before death, still had a strong suppression of CRH neurons, but also showed the highest AVP immunoreactivity in both the PVN and SON. Whether this is due to nonsuppression of AVP by dexamethasone, which is a subject of debate in the animal experimental literature (27, 43), or to a rebound increase in AVP during the recovery from suppression should be further investigated. The subject with chronic endogenous increase in adrenal steroids and subsequent postadrenalectomy replacement therapy (patient 9) showed a strong suppression of CRH, but the level of AVP immunoreactivity was the highest in the glucocorticoid-exposed group, suggesting either an adaptation of AVP neurons to long term glucocorticoid exposure or a difference in effect between endogenous cortisol and synthetic glucocorticoids on AVP.

Suppression of the HPA axis and the withdrawal syndrome. Suppression of CRH and AVP neurons in the PVN by glucocorticoids results in an overall suppression of the endogenous HPA axis by glucocorticoids. The follow-up criteria of the glucocorticoid negative feedback inhibition of the HPA axis in clinical practice are based on the blood and urinary levels of the adrenal corticosteroids as well as responses of adrenal and pituitary glands to HPA axis stimulants. The involvement of the hypothalamus in this inhibitory cycle was suggested only by the results of animal studies or by indirect outcomes of the observations from human assessment studies on ACTH regulation (13, 44). However, the complex and diverse presentation of behavioral and metabolic effects (changes in eating and drinking, disturbances in sleep and thermoregulation) of the continued use of supraphysiological doses of corticosteroids as well as the wide and unpredictable recovery profile of the pituitary and adrenal hormones after the discontinuation of steroid therapy, the withdrawal syndrome, suggested that higher centers than the adrenal and pituitary may also be affected by glucocorticoid suppression. Our results showing the suppression of hypothalamic CRH and AVP thus can be considered in the explanation of at least part of the central symptoms of glucocorticoid treatment and the withdrawal syndrome.

Suppression of vasopressinergic systems and disturbance in water balance. An important new aspect of our findings is the very prominent decrease in AVP immunoreactivity in the SON and PVN in three of the corticosteroid-exposed subjects. Recent studies in experimental animals demonstrated expression of glucocorticoid receptors not only on parvocellular neurons that may project to the median eminence and participate in HPA axis regulation, but also on magnocellular AVP neurons in the PVN and SON (45), that project to the posterior pituitary and play a role in osmoregulation. It is generally accepted that glucocorticoids can influence osmoregulation by a limited mineralocorticoid effect on the kidney. However, it has also been shown that plasma AVP is strongly suppressed by glucocorticoids in humans, suggesting another route in glucocorticoid-associated osmotic dysregulation (39). The existence of such an effect has been supported by data from animal studies showing a central impairment of osmoregulation by an excess of corticosteroids, probably mediated by AVP (46). Our study, which shows that AVP immunoreactivity in the magnocellular neurons of the SON and PVN was greatly decreased by glucocorticoid treatment, supports this view. However, we cannot determine the ratio of parvo- and magnocellular AVP cells affected by glucocorticoids in our study, because in the human hypothalamus, unlike the rat hypothalamus, there is a continuous distribution from small to large neurons, and there are no district subnuclei in the PVN with a particular function (47).

CNS system side-effects. AVP and CRH are known to be involved in central processes, playing a role in cognition, memory, mood, and behavior (48–50). A recent report showed a negative correlation between circulating cortisol levels and metabolic activity of the hypothalamus (51). This observation and our results suggest, therefore, that at least part of the CNS side-effects of glucocorticoid therapy may be the result of the suppression of centrally acting CRH and AVP or other CNS neuropeptides, as has been discussed by others (6, 13).

The brain weight of the corticosteroid-exposed patients in our study was not different from that of the controls, which seems to be in contrast with the findings of a previous study that showed cerebral atrophy in chronically corticosteroid-treated patients based on computed tomography scans (52). However, the subjects in that study had been exposed to very high dose corticosteroids for very long periods (between 0.5–5 yr), which made the cumulative corticosteroid doses much higher than those given to the subjects in our study.

Effects on the immunoregulatory role of CRH. CRH may have a tissue-protective effect and an immunoregulatory role (53–57). Despite the ongoing debate on the pre- or antiinflammatory actions of CRH in different tissues and in different inflammatory conditions (56, 57), we must consider the possibility that CRH may have direct immunomodulatory actions in the CNS, and suppression of CRH, therefore, may have direct consequences on the inflammatory processes occurring in the brain during certain CNS pathologies.

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