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RELEASE OF HUMAN NEUROPHYSIN I DURING INSULIN-INDUCED HYPOGLYCEMIA IN DEPRESSED PATIENTS IS ABOLISHED AFTER RECOVERY WITH CLOMIPRAMINE TREATMENT

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SUMMARY

Release of human neurophysin I (hNp I) and neurophysin II (hNp II) during insulin-induced hypoglycemia was studied in 10 unipolar depressed women before and after 4 – 5 weeks of standard antidepressant drug treatment with daily intravenous infusions of clomipramine. Before treatment, a significant increase of hNp I but not of hNp II serum levels in response to hypoglycemia was observed. At retest during clomipramine administration, a marked clinical amelioration occurred in all patients as determined with the Hamilton Rating Scale for Depression; the hNp I response to insulin was abolished, but no effect on hNp II concentration could be demonstrated. No correlation was found between the degree of the depression score decrease and the amplitude of the inhibition of hNp I release or serum levels of clomipramine or its metabolite, desmethylclomipramine. The meaning of this difference in reactivity of the neurohypophyseal system in the course of depressive illness, based on the pharmacological and biochemical profiles of clomipramine action, is discussed.

INTRODUCTION

NEUROPHYSINS are polypeptides with molecular weight of about 10,000, which are synthesized within hypothalamic nuclei (mainly magnocellular supraoptic and paraventricular) as part of precursors (propressophysin and prooxyphysin) containing the sequences of the neurophysins, a recently identified peptide and the neurohypophyseal peptides, vasopressin (AVP) and oxytocin (OXY) (Seidah *et al.*, 1981; Land *et al.*, 1982). The radioimmunoassay (RIA) for neurophysins has been proposed and used as an index of neuropituitary function in different physiological and pathological conditions (Legros, 1975). Different forms of pituitary and circulating neurophysins have been described, but it is now generally accepted in human subjects that two types of immunoreactive neurophysins exist: one associated with AVP (hNp I) and one with OXY (nNp II) synthesis (Legros & Louis, 1973/74).

AVP dysfunction in depressive illness has been postulated on the basis that this peptide seems to be involved in different processes that are disturbed in affective disorders, such

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as memory, biological rhythms, REM sleep, pain perception, fluid and electrolyte balance, and neuroendocrine regulation (Gold *et al.*, 1978, 1980, 1981). Gold *et al.* (1981) reported that cerebrospinal fluid (CSF) AVP was significantly lower in drug-free depressed (non-psychotic) patients than in controls, and that, in bipolar patients, CSF AVP also was lower in the depressed (non-psychotic) phase compared with the manic phase. In the same study, plasma AVP response to hypertonic saline was found to be reduced in depression compared with mania. Lithium and carbamazepine, two drugs with both antimanic and antidepressant properties, significantly influenced the sensitivity of the AVP response to hyperosmolality (Gold *et al.*, 1983a,b).

Very few studies have been published about neurophysin levels in psychiatric disorders. We have shown that in bipolar depressed patients, CSF hNp I and hNp II levels are higher than in unipolar depressed or schizophrenic patients (Legros *et al.*, 1983a). A significant relationship between plasma hNp I and ACTH, and between AVP and cortisol was found in the 1600 hr post-dexamethasone sample in 11 non-suppressor but not in nine suppressor depressed patients, suggesting a putative role of vasopressin – neurophysinergic system in the early escape from dexamethasone suppression in some depressed patients (Legros *et al.*, 1983b).

Insulin-induced hypoglycemia increases plasma AVP in rats (Bayliss *et al.*, 1978) and humans (Bayliss & Heath, 1977), but the physiological mechanism remains unclear. The central nervous system (CNS) glucoprivation induced by administration of 2-deoxy-D-glucose stimulates thirst and AVP discharge in humans which is not related to changes in peripheral catecholamines, mean arterial pressure, plasma renin activity or osmolality (Thompson *et al.*, 1981). In the two experimental conditions, AVP release appears to be controlled by CNS neurotransmitter pathways whose functions are sensitive to variations in glucose metabolism.

We were interested in examining the effects of insulin-induced hypoglycemia on neurohypophyseal function in depressive illness. Our aim was to detect differences in basal concentration and release of hNp I and hNp II—as indexes of neuropituitary function—in patients before and after clomipramine treatment. The study was undertaken with the active collaboration of the Psychobiological Ward of the Department of Psychiatry, University of Vienna, and with the approval of the Human Research Committee.

METHODS

The specific experimental procedures have been described elsewhere (Langer *et al.*, 1980, 1981).

Ten unipolar depressed women, aged 50.6 ± 5.3 years old (mean ± S.D.) were included in the study. Seven of them were post-menopausal. Four smoked cigarettes socially (12 – 25 per day). They all met Research Diagnostic Criteria for major depressive disorder (Spitzer *et al.*, 1978) and were free of antidepressant medication for at least 3 weeks prior to the study. Other psychotropic drugs were withdrawn at entry, and oral diazepam (20 – 40 mg/day) was given during the first week after admission. A clinical evaluation, determination of the Hamilton depression score (HAM-D; first 16 items) and an initial insulin tolerance test (ITT) were carried out on each patient during this same first week. Clomipramine treatment was begun thereafter by intravenous infusions (dilution in 250 ml of 5% levulose) for 2 hr every evening until definite clinical improvement, but for no longer than 5 weeks (increasing dosage at weekly intervals; final dose range: 50 – 150 mg/day). The routine clinical tests and ITTs were repeated after 4 – 5 weeks of antidepressant treatment.

The ITT started at 0830 hr after an overnight fast. The intravenous catheter was inserted 45 min prior to the ITT. At time 0 min, regular insulin (0.1 U/kg) dissolved in 5 ml normal saline was injected intravenously as a bolus. Blood samples for analyses of glucose, hNp I and hNp II were drawn at times 0, 20, 30, 45 and 60 min. The blood was allowed to clot, centrifuged and the serum stored at −20°C.

hNp I and hNp II concentrations were determined using RIAs developed by Dax *et al.* (1979). Intraassay variability was 6.1% for hNp I and 3.3% for hNp II; interassay variability was 7 and 5.7% respectively; and sensitivity was 0.08 ng/ml and 0.2 ng/ml respectively. When the concentrations were below the sensitivity of the assays, they were assigned the lower limits of detection. Clomipramine and desmethylclomipramine concentrations were determined by high performance liquid chromatography.

Statistics

An integrated change score (ΔhNp I and ΔhNp II) was determined for each patient for the pre-treatment and post-treatment tests, calculated as the average of all post-insulin values minus the pre-insulin basal value. Integrated areas under the hNp I and hNp II response curves were calculated using net change from basal values by the trapezoid method. Statistical significance was assessed by single-sample *t*-test to compare basal value and the average of post-insulin levels before and after treatment, and by a paired *t*-test to compare glucose nadir, ΔhNp I, ΔhNp II and areas of neurophysin response before and after clomipramine treatment. A correlation matrix was established for HAM-D scores, selected ITT characteristics before and after recovery, and concentration of clomipramine and desmethylclomipramine (BMDP statistical software). All values are given as means ± (S.E.M.).

TABLE I. CLINICAL AND INSULIN TOLERANCE TEST CHARACTERISTICS BEFORE AND AFTER CLOMIPRAMINE TREATMENT (MEAN ± S.E.M.)

	Before treatment	After treatment	*p*
HAM-D	24.4 ± 1.7	6.7 ± 1.6	$< 10^{-5}$
Basal glucose (mg %)	79.3 ± 2.9	77.3 ± 1.5	NS
Glucose nadir (mg %)	30.1 ± 3.2	26.1 ± 4.8	NS
Basal hNp I (ng/ml)	0.23 ± 0.06	0.24 ± 0.07	NS
ΔhNp I (ng/ml)	0.09 ± 0.03	0.01 ± 0.04	< 0.05
hNp I AREA (ng/ml × min^{-1})	4.71 ± 1.64	0.79 ± 1.87	< 0.05
Basal hNp II (ng/ml)	0.40 ± 0.08	0.44 ± 0.09	NS
ΔhNp II (ng/ml)	0.00 ± 0.02	0.00 ± 0.03	NS
hNp II AREA (ng/ml × min^{-1})	0.24 ± 1.15	−0.22 ± 2.31	NS

RESULTS

On admission, the mean HAM-D score for all the patients was 24.4 ± 1.7 (range 14 − 30). During the initial ITT (Fig. 1), blood glucose fell consistently, reaching a minimum (glucose nadir = 30.1 ± 3.2 mg%) at 30 min. Mean serum hNp I concentration rose gradually during hypoglycemia from 0.23 ± 0.06 ng/ml to a peak of 0.42 ± 0.07 ng/ml at 45 min ($t = 2.86$; $p < 0.01$). Mean ΔhNp I was 0.09 ± 0.03 ng/ml and the area of hNp I response was 4.71 ± 1.64 ng/ml × min^{-1}. Serum hNp II levels did not change during the test, from an initial value of 0.40 ± 0.08 to a maximum of 0.42 ± 0.09 ng/ml at 45 min. Mean ΔhNp II was 0.00 ± 0.02 ng/ml and the hNp II area was 0.24 ± 1.15 ng/ml × min^{-1}.

After clomipramine treatment, all patients showed marked clinical improvement, as evidenced by the highly significant decrease of the HAM-D score to 6.7 ± 1.6 (range 1 − 14) ($t = 9.79$; $p < 10^{-5}$). During the second ITT (Fig. 2), the mean glucose nadir reached 26.1 ± 4.8 mg% at 30 min ($t = 0.47$; p = NS compared to the first ITT). No significant changes in basal glucose, hNp I or hNp II concentrations occurred compared to the values before treatment. The mean hNp I concentration rose very slightly from 0.24 ± 0.07 to 0.27 to 0.27 ± 0.08 ng/ml at 45 min. The mean ΔhNp I was significantly reduced to 0.01 ± 0.04 ng/ml, as was the hNp I area (0.79 ± 1.87 ng/ml × min^{-1}) ($t = 1.92$ and

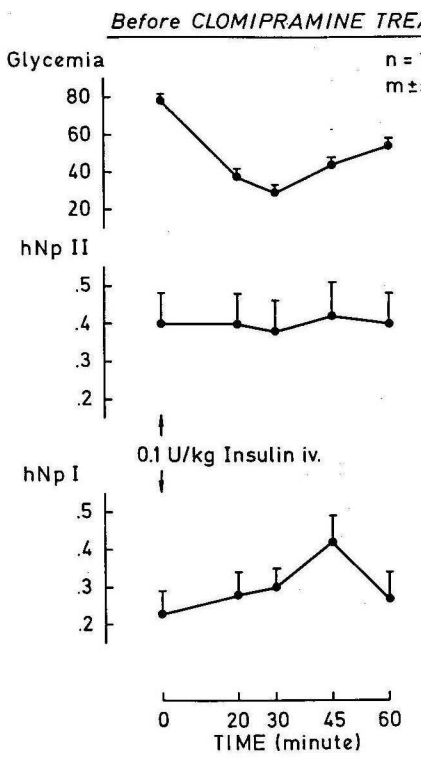

FIG. 1.

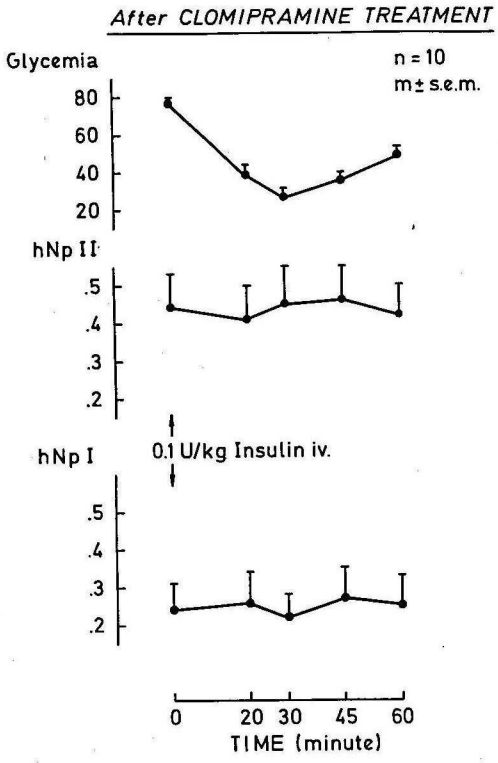

FIG. 2.

FIG. 1. Mean (± SEM) serum glucose (glycemia), hNp I and hNp II levels in ten unipolar depressed women during a 60 min insulin-induced hypoglycemia test, before clomipramine treatment. Glycemia is expressed in mg %, neurophysin values in ng/ml.

FIG. 2. Mean (± SEM) serum glucose (glycemia) hNp I and hNp II levels in the same patients during an identical insulin tolerance test, after 4 – 5 weeks of clomipramine treatment. Glycemia is expressed in mg %, neurophysin values in ng/ml.

1.90, respectively; $p < 0.05$ compared to first ITT). As before, serum hNp II levels remained constant during the ITT, with an initial value of 0.44 ± 0.09 ng/ml and a maximum of 0.46 ± 0.09 ng/ml at 45 min. Mean ΔhNp II was 0.00 ± 0.03 ng/ml and the hNp II area was -0.22 ± 2.31 ng/ml × min^{-1}.

Mean basal levels of clomipramine (CLOM) and desmethylclomipramine (DCLM) on the day of the ITT were 82.9 ± 24.6 and 52.2 ± 9.9 ng/ml, respectively.

As shown in Table 2, a correlation matrix was established for the variables which exhibited significant variation during the tests or from the first to the second ITT. A total of 55 intercorrelations between 11 variables was performed and consequently, two or three correlations might be significant at the 5% level by chance alone. Two significant relationships are interesting: one between glucose nadir and the HAM-D score before treatment ($r = 0.72$; $p < 0.05$) and the second between the level of DCLM and the HAM-D score after clomipramine administration ($r = -0.77$; $p < 0.01$). No correlations could

TABLE II. CORRELATION MATRIX OF CLINICAL (HAM-D & Δ) AND SELECTED ITT CHARACTERISTICS (GLUCOSE NADIR, hNp I AREA & Δ) PRE- AND POST-TREATMENT, AND CONCENTRATIONS OF CLOMIPRAMINE AND DESMETHYLCLOMIPRAMINE

	HAM-D_B	GLUC. N_B	hNp $AREA_B$	HAM-D_A	GLUC. N_A	hNp $AREA_A$	ΔHAM-D	ΔGLUC. N	ΔhNp I AREA	CLOM	DCLM
HAM-D_B	1										
GLUC. N_B	*0.72**	1									
hNp I $AREA_B$	−0.17	−0.24	1								
HAM-D_A	0.40	0.33	0.22	1							
GLUC. N_A	0.60	0.60	−0.59	0.17	1						
hNp I $AREA_A$	−0.33	−0.37	0.34	0.24	−0.35	1					
ΔHAM-D	−0.61	−0.42	0.35	0.49	−0.46	0.58	1				
ΔGLUC. N	−0.04	−0.43	−0.28	−0.17	0.47	−0.05	−0.13	1			
ΔhNp I AREA	−0.17	0.01	−0.50	0.04	0.41	*0.65**	0.20	0.33	1		
CLOM	−0.26	0.33	−0.51	−0.14	0.10	0.10	0.2	−0.52	0.50	1	
DCLM	*−0.68**	−0.42	−0.20	*−0.77***	−0.54	0.00	−0.01	−0.31	−0.16	0.42	1

*$p < 0.05$.
**$p < 0.01$.
B = before treatment; A = after treatment; GLUC. N = glucose nadir; CLOM = clomipramine; DCLM = desmethylclomipramine

be found among the magnitude of the depression score decrease, the degree of the inhibition of hNp I release, and serum levels of CLOM and DCLM.

DISCUSSION

As described for AVP in normal men (Bayliss & Heath, 1977), insulin-induced hypoglycemia increased serum hNp I concentrations in unipolar depressed women when tested before treatment. This vasopressin–neurophysinergic activation appeared to be controlled by CNS neurotransmitter pathways since Thompson *et al.* (1981) clearly demonstrated that the AVP release during CNS-glucoprivation induced by 2-deoxy-D-glucose was dissociated from all the possible peripheral mechanisms of control (osmolality, mean arterial pressure, peripheral β-adrenoreceptor stimulation, hyperreninemia, hypovolemia and decreased metabolic clearance). Similar studies in which glucoprivation has been induced in cervical cord-sectioned patients (Thompson & Campbell, 1978) also suggested a CNS control of glucopenia-induced AVP release, but the nature of the neurotransmitter is still unknown.

Clomipramine treatment by daily intravenous infusions was followed by a clear clinical improvement in our patients and, simultaneously, the neurohypophyseal system appeared to be less reactive to hypoglycemia, as hNp I release during the second ITT was significantly reduced. Since no correlations were observed among the degree of clinical improvement, the decrease in serum hNp I response, and the concentrations of clomipramine and desmethylclomipramine, it is difficult to ascertain whether the inhibition of hNp I release was the consequence of recovery from depression rather than a direct pharmacological effect of clomipramine. However, some points may bear on this question, as follows.

First, the basal values of serum hNp I and hNp II were not affected by clomipramine administration and/or clinical recovery. The effect on neurohypophyseal peptide release was shown only under the dynamic condition of hypoglycemia. Second, based on the main pharmacological profile of clomipramine—inhibition of serotonin uptake; inhibition of noradrenaline uptake through its major metabolite, desmethylclomipramine (Iversen & MacKay, 1979); blockade of H_2-histamine brain receptors (Kanof & Greengard, 1978); weak anticholinergic (Fjalland *et al.*, 1977) and antidopaminergic properties (Iversen & MacKay, 1979)—some possible neurotransmitter alterations can be offered to try to explain our data.

Very few studies have been published about a serotoninergic regulation of AVP release, and it generally has been considered absent (Dahlström & Fuxe, 1965; Bhargava *et al.*, 1972). Our current data support a potential role for an inhibitory serotoninergic control of hNp I release during hypoglycemia after clomipramine treatment, as a consequence of an increase in synaptic transmission of serotoninergic neurons.

Numerous reports have evaluated the effect of noradrenaline on AVP release with contradictory results (Bhargava *et al.*, 1972; Hoffman *et al.*, 1977). Kimura *et al.* (1981) found that intraventricular (icv) administration of noradrenaline provoked an inhibition of AVP release in dogs. Noradrenaline inhibits AVP release from the hypothalamo-neurohypophyseal system (HNS) in culture and attenuates acetylcholine-stimulated AVP release in a concentration-dependent manner; these effects are blocked by α-antagonists

but not by β-blocking agents (Armstrong *et al.*, 1982). Since desmethylclomipramine levels were not negligible in our patients, an α-noradrenergic effect and/or a β-noradrenergic desensitization could have occurred, with the treatment resulting in an inhibition of AVP release during hypoglycemia.

Histamine induces antidiuresis after icv administration in some mammals (Bhargava *et al.*, 1973) and stimulates supra-optic neurons when microelectrophoretically applied in rat brain (Haas *et al.*, 1975). The blockade of H_2-histamine receptors therefore could abolish neurohypophyseal activation. Kanof & Greengard (1978) showed that a large number of antidepressant drugs, particularly clomipramine, share the ability of blocking these receptors in mammalian brain.

Acetylcholine stimulates AVP release as evidenced by the first reports of Pickford (1939). Successive experimental models demonstrated the cholinergic activation of the neurohypophyseal system (Dyball, 1968; Bhargava *et al.*, 1972). Both muscarinic and nicotinic receptors are present in the magnocellular system (Barker *et al.*, 1971), but they appear to be mainly nicotinic, as the nicotinic blocking agents inhibit acetylcholine-stimulated AVP release in cultured HNS, whereas atropine (muscarinic antagonist) does not diminish the same response (Sladek & Joynt, 1979). Furthermore, nicotine is a potent stimulus for simultaneous release of AVP and neurophysin in man (Husain *et al.*, 1975). The anticholinergic properties of clomipramine, even if weak, could be involved in the modulation of hNp I release.

Another aspect of our study deserves some discussion. Insulin-induced hypoglycemia may be considered as a non-specific stress (Bayliss & Heath, 1977); the release of AVP, which is a major potentiator of corticotrophin releasing factor (CRF) bioactivity (Gillies *et al.*, 1982; Liu *et al.*, 1983), in the hypophyseal portal system simultaneously with its release into the peripheral blood might be the initiating step in the activation of the adenohypophyseal – adrenal cortical axis. It appears, though, that clomipramine could exercise an 'anti-stress' action probably by affecting some of the neurotransmitters outlined above, but presumably not by a serotoninergic mechanism since serotonin has been shown to facilitate CRF release (Jones *et al.*, 1976) and insulin stress increased hypothalamic serotonin metabolism (Yehuda & Meyer, 1984). Interestingly, the anxiolytic diazepam, given to all patients on admission, was not sufficient to abolish the hNp I response to hypoglycemia. Moreover this mechanism could explain why basal hNp I levels were not affected by clomipramine but the hypoglycemia-induced release of hNp I was abolished.

Keeping in mind the restriction mentioned above on the significance of results from the correlation matrix, two last aspects of the data are to be considered in this study. The positive relationship between the HAM-D score and the glucose nadir before treatment is in accordance with previous reports of a relative insulin resistance in depressed patients (Mueller *et al.*, 1969) and we could observe, even if not significant, a decrease of the glucose nadir after recovery. The inverse relationship between the desmethylclomipramine levels and the HAM-D score before treatment is impossible to interpret and must be the result of chance; the inverse correlation with the HAM-D score after treatment must be taken into account only with great circumspection, as there is no relationship with the ΔHAM-D.

Finally, our findings demonstrate that the neurohypophyseal activation observed during ITT in unipolar depressed patients is abolished after recovery with clomipramine treatment. This effect seems to be the consequence of the interaction of clomipramine—and/or its metabolite, desmethylclomipramine—probably with cholinergic pathways, less clearly with noradrenergic, serotoninergic or histaminic systems.

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