

Neuroendocrine Responses to a Cold Pressor Stimulus in Polydipsic Hyponatremic and in Matched Schizophrenic Patients

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Schizophrenia, many believe, reflects an enhanced vulnerability to psychological stress. Controlled exposure to stressors, however, has produced inconclusive results, particularly with regards to neurohormones. Some of the variability may be attributable to the nature and psychological significance of the stimulus and failure to control physiologic confounds. In addition, it is possible that the heterogeneity of schizophrenia is an important factor. In a carefully designed study and in a controlled setting, we measured the neuroendocrine response of eight polydipsic hyponatremic (PHS), seven polydipsic normonatremic (PNS), and nine nonpolydipsic normonatremic (NNS) (ie normal water balance) schizophrenic in-patients as well as 12 healthy controls (HC) to two different stressors: one of which appears to influence neuroendocrine secretion through its psychological (cold pressor) and the other (upright posture) through its systemic actions. Subjects in the three psychiatric groups were stabilized and acclimated to the research setting, and all received saline to normalize plasma osmolality. Following the cold pressor, plasma adrenocorticotropin and cortisol levels showed a more prolonged rise in PHS patients relative to PNS patients. NNS patients, in contrast, exhibited blunted responses relative to both of the polydipsic groups and the HC. Peak vasopressin responses were also greater in PHS and blunted in NNS patients. Responses to the postural stimulus were similar across patient groups. These findings provide a mechanism for life threatening water intoxication in schizophrenia; help to reconcile conflicting findings of stress responsiveness in schizophrenia; and potentially identify a discrete patient subset with enhanced vulnerability to psychological stress.

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INTRODUCTION

The hypothesis that schizophrenia is characterized by an enhanced vulnerability to psychological stress has evolved over time but consistently has been attributed to hippocampal dysfunction (Mednick and Schulsinger, 1968; Gray, 1998; Walker and Diforio, 1997; Moghaddam, 2002; Myin-Germeys *et al*, 2005). Changes in hypothalamic–pituitary–adrenal (HPA) axis and arginine vasopressin (AVP: antidiuretic hormone) activity, stress sensitive markers of hippocampal function (Herman *et al*, 2002, 2005; Nettles *et al*, 2000; Onaka and Yagi, 1998), however, generally are normal or even blunted in schizophrenia (Albus *et al*, 1982;

Breier *et al*, 1988; Gispén-de Wied, 2000; Jansen *et al*, 1998, 2000; Kudoh *et al*, 1999; Marcelis *et al*, 2004). Previous reports of enhanced activity may instead be attributable to responses to specific stressors, systemic effects of the stimulus, severity, or lability of psychiatric symptoms, or to acute hospital admission, *per se* (Herman *et al*, 2005; Walder *et al*, 2000; Walsh *et al*, 2005).

The absence of clear evidence of enhanced neuroendocrine responses in schizophrenia also leaves open the possibility that only a subset of patients exhibit enhanced vulnerability. Recognition of this possibility has led others to seek evidence of HPA axis dysfunction in patients differentially exposed to factors thought to influence hippocampal function (Braehler *et al*, 2005). In this regard, hippocampal function varies along its longitudinal axis in both rodents (Bannerman *et al*, 2004; Moser and Moser, 1998; Risold and Swanson, 1996) and primates (Barbas and Blatt, 1995; Strange *et al*, 1999; Strange and Dolan, 2001), and structural findings are often more prominent in one or the other end of the hippocampus in patients (Narr *et al*,

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2004; Pegues *et al*, 2003; Szeszko *et al*, 2003; Velakoulis *et al*, 2001; Weiss *et al*, 2005). The ventral segment in rodents (analogous to anterior segment in primates (Rubin *et al*, 1966)) modifies neuroendocrine (Herman *et al*, 1995, 2005; Mueller *et al*, 2004; Nettles *et al*, 2000) and behavioral (Flores *et al*, 2005; Trivedi and Coover, 2004) responses to psychological but not physical stresses (but see Tuvnes *et al*, 2003). Indeed, an animal model of schizophrenia, in which the development of this segment is disrupted in the neonatal rat (Lipska, 2004), exhibits increased AVP and HPA axis responses to a psychological stimulus (Chrapusta *et al*, 2003; Mitchell and Goldman, 2004) as well as enhanced behavioral (Flores *et al*, 2005) responses to psychological stress. Thus one might predict that only patients with prominent anterior hippocampal pathology would exhibit increased vulnerability to psychological stress.

Diminished volume of the anterior hippocampus is particularly prominent in schizophrenic patients with polydipsia and hyponatremia (Elkashef *et al*, 1996; Luchins *et al*, 1997; Goldman *et al*, 2005). About 20% of schizophrenic patients drink excessive amounts of water ('primary polydipsia') and about 20% of these retain enough of this excess water to produce a dilutional hyponatremia (de Leon *et al*, 1994). Furthermore, polydipsic patients with and without hyponatremia display impaired HPA axis negative feedback (Goldman *et al*, 1993) that is consistent with hippocampal pathology (Goldman *et al*, 2006). In addition, hyponatremic polydipsic (but not normonatremic polydipsic) patients exhibit an unexplained increase in AVP secretion that contributes to their water retention (Goldman *et al*, 1988, 1996). Finally, this defect worsens with the stress of acute psychosis in the hyponatremic subset, culminating in episodes of life-threatening water intoxication (de Leon *et al*, 1994; Goldman *et al*, 1997).

Based on the (1) previous reports of blunted neuroendocrine responses to psychological stress in schizophrenia; (2) evidence that ventral/anterior hippocampal dysfunction enhances neuroendocrine responses to psychological stress; and (3) evidence that patients with water imbalance exhibit signs of anterior hippocampal pathology and enhanced neuroendocrine activity, summarized above, we tested the following hypotheses: AVP and HPA axis responses to psychological stress will be enhanced in (1) polydipsic hyponatremic (PHS) schizophrenic relative to polydipsic normonatremic (PNS) schizophrenic patients, (2) these two groups relative to healthy controls (HC), and (3) these three groups relative to nonpolydipsic normonatremic (NNS) schizophrenic patients (ie a sample representative of the majority of patients who have normal water balance). In addition, we predicted that the neuroendocrine responses to a systemic stimulus would not differ in these groups.

As psychological stress responses are heavily influenced by perceptions of reality, and reality testing is, by definition, distorted in schizophrenia, standardizing a psychological stimulus for this population is a daunting challenge. In fact, schizophrenic patients are often quite adept at simply ignoring psychological stimuli (Dawson and Nuechterlein, 1984; Jansen *et al*, 2000; Zahn and Pickar, 2005). Psychological stressors are stimuli that signal, but do not in themselves constitute, a potential threat to homeostasis. The recognized attributes of a psychological stressor

include aversiveness (Peters *et al*, 1998), and neuroendocrine components that both anticipate (Gaab *et al*, 2005) as well as habituate (Herman *et al*, 2005) to the stimulus. We chose the cold pressor (ie immersion of a limb in ice water) because it exhibits these attributes (al'Absi and Petersen, 2003; Bullinger *et al*, 1984; Ehrenreich *et al*, 1997; Farhadi *et al*, 2005; Gregg *et al*, 1999; Lovallo, 1975) yet is difficult to ignore. In fact, the response is independent of the subjects' attentiveness (Hodes *et al*, 1990) and is predicted by the duration of exposure to the stimulus (Lovallo, 1975). Equally important is that data suggest that the systemic actions of the cold pressor (ie cold, elevated blood pressure) have no effect or may actually blunt HPA secretion (Edelson and Robertson, 1986; Kendler *et al*, 1978; Wittert *et al*, 1992). For the systemic stimulus, we chose becoming upright after a period of recumbency (postural stimulus) because it lacks these attributes of a psychological stimulus (Hennig *et al*, 2000), and the neuroendocrine response is accounted for by its systemic effects (Hennig *et al*, 2000; Jacob *et al*, 1998).

PATIENTS AND METHODS

Subjects

Psychiatric subjects were recruited from psychiatric inpatient and outpatient facilities. Subjects were grouped by a two-step procedure before and following transfer to the Psychiatric Clinical Research Center at University of Illinois at Chicago (UIC). All had primary diagnoses of schizophrenia or schizoaffective disorder and were without major medical or neurological disorders, and were not taking corticosteroids or medications with recognized effects on AVP function (Goldman *et al*, 1997). Potential subjects with polydipsia and hyponatremia had unexplained plasma sodium levels ≤ 125 mEq/l, and multiple urine specific gravities < 1.008 (hyposthenuria); those with polydipsia and normonatremia had no plasma sodium levels ≤ 135 mEq/l, but a similar history of hyposthenuria; those with normal water balance had no sodium levels ≤ 135 mEq/l or urine-specific gravity levels < 1.015 . Informed witnessed written consent was obtained after the Institutional Review Boards of the University of Chicago (UC) and UIC approved the studies. Informed consent was assessed by determining the ability to repeat key elements of the consent form, performance on cognitive screening tasks, involvement of family and other patient advocates, and regular reassessment of willingness to continue with the research.

During the first 3 weeks of admission, subjects were acclimated to the research setting and psychotropic medication doses were optimized. Haloperidol was the preferred antipsychotic, but patients could receive olanzapine or risperidone if they had had adverse reactions to first generation agents. Mood instability was treated with valproic acid (see legend Table 1). Final patient assignment into PHS, PNS, or NNS schizophrenic groups was based on spot urine and plasma samples obtained three times weekly during the first 3 weeks of hospitalization. PHS had mean urine osmolalities mmol/kg (U_{osm}) < 300 mmol/kg and mean plasma osmolalities (Posm) < 285 mmol/kg and one or more Posms < 275 mmol/kg; PNS met the same criteria

Table 1 Clinical Parameters and Basal Laboratory Values in Three Schizophrenic Subject Groups and Controls

Measure ^a	Polydipsic hyponatremic (PHS, n = 8)	Polydipsic normonatremic (PNS, n = 7)	Nonpolydipsic normonatremic (NNS, n = 9)	Healthy controls (HC, n = 12)	Significant contrasts ^b (p < 0.05)
	n (%)	n (%)	n (%)		
Male	5 (63)	5 (78)	6 (67)	7 (58)	NS
Smoker	5 (63)	4 (57)	4 (44)	1 (8)	PHS+PNS > HC
Schizoaffective	1 (13)	1 (14)	2 (22)	—	NS
Atypical neuroleptic	3 (37)	2 (27)	1 (11)	—	NS
	Mean (SD)	Mean (SD)	Mean (SD)		
Age (years)	47.5 (6.4)	38.7 (10.5)	38.6 (10.9)	38.3 (10.3)	NS
Age of onset mental illness (years)	23.0 (9.1)	22.2 (3.8)	27.5 (10.3)	—	NS
0700 hours plasma osmolality (mosmol/kg) ^c	280.2 (5.7)	294.2 (5.7)	289.5 (2.8)	—	PHS < PNS
1430 hours urine osmolality (mosmol/kg) ^c	188.7 (182.1)	254.1 (97.6)	689.7 (369.0)	—	PHS+PNS < NNS
PANSS total ^d	58.4 (24.6)	63.9 (23.0)	57.2 (15.9)	—	NS
PANSS positive	13.4 (5.4)	16.7 (7.1)	15.5 (4.5)	—	NS
PANSS negative	17.7 (9.0)	15.1 (6.3)	14.1 (4.6)	—	NS
HAM-D	8.3 (2.6)	13.0 (13.1)	18.3 (13.1)	—	NS
GAF	34.4 (8.2)	41.4 (7.8)	38.5 (3.6)	—	NS
Dose antipsychotic (mg/day) ^e	491 (320)	482 (395)	645 (241)	—	NS
Plasma sodium (mmol/l)	130.2 (10.2)	138.3 (6.1)	138.9 (3.0)	138.8 (1.7)	PHS < PNS
Thyrotropin (mIU/l)	1.42 (0.48)	1.85 (1.4)	1.68 (.55)	1.48 (0.82)	NS
Plasma creatinine (mg/dl)	1.1 (0.4)	0.9 (0.3)	0.9 (0.2)	0.9 (0.2)	NS
Plasma potassium (mmol/l)	4.2 (0.5)	4.3 (0.3)	4.4 (0.2)	4.1 (0.2)	NS
Total plasma calcium (mmol/dl)	9.3 (0.5)	9.6 (0.4)	9.6 (0.4)	9.6 (0.3)	NS

^aTo maximize power, group comparisons were limited to three *a priori* orthogonal (Difference) contrasts: polydipsic hyponatremic vs polydipsic normonatremic patient groups (PHS vs PNS); two polydipsic patient groups vs healthy controls (PHS+PNS vs HC); and these three groups (or two polydipsic groups if data were not obtained or relevant in healthy controls) vs nonpolydipsic patient group (PHS+PNS+HC vs NNS, or PHS+PNS vs NNS). NS, none of three contrasts were significant.

^bDifferences in ordinal/nominal data with mixed effects ordinal linear regression (MIXOR). Differences in continuous variables were analyzed by analysis of variance.

^cMean values of spot urine and plasma samples obtained three times weekly during first 3 weeks of hospitalization.

^dPANSS denotes Positive and Negative Symptom Scale; HAM-D, Hamilton Depression Scale; GAF, Global Assessment of Functioning.

^eMilligrams of chlorpromazine equivalent per day estimated as 1 mg/day haloperidol or risperidone, or 2.5 mg/day of olanzapine = 50 mg chlorpromazine. Three hyponatremic polydipsic subjects, four polydipsic normonatremic subjects, and five nonpolydipsic subjects were receiving bupropion. Four hyponatremic polydipsic and four normonatremic polydipsic subjects, and one nonpolydipsic subject received valproic acid.

for mean Uosm, but their mean Posm > 285; NNS had mean Uosm > 500 mmol/kg and mean Posm > 285 mmol/kg. Measures of urine dilution have been shown to reliably predict fluid intake (Shutty *et al*, 1997). Thus all PHS patients had a history of moderately severe hyponatremia and polydipsia, but at the time of the study may only have been episodically hyponatremic/hypoosmolemic and moderately polydipsic (Shutty *et al*, 1997).

During the fifth week of admission, trained raters administered the Positive and Negative Symptom Scale (PANSS) (Kay *et al*, 1987), Hamilton Depression Scale (HAM-D) (Bech *et al*, 1986), and the Global Assessment of Functioning Scale (GAF) (Endicott *et al*, 1976). Patients underwent an MRI scan during the fourth week as part of a related study (Goldman *et al*, 2005). Psychiatric diagnosis was confirmed at discharge in a multidisciplinary conference and relied largely on the Structured Clinical Interview for DSM-IV (Spitzer *et al*, 1992).

HC were recruited by advertisements placed around the University communities as described previously (Goldman *et al*, 1996) and were studied as outpatients. Each was

administered the SCID-NP by a trained rater (Version 2.0) (First *et al*, 1996), and none had current or past axis I disorders, or medical conditions or were taking medication known to alter AVP function. We attempted to match groups on age and gender.

Procedures

Studies were performed in the evening because that is when HPA axis activity is most stable and hippocampal influences are thought to be most apparent (van Eekelen *et al*, 2003). In order to further stabilize basal HPA axis activity, subjects were acclimated to the Clinical Research Center before the study. On the day of the study, subjects refrained from smoking and eating after 1200 hours. After a light low-carbohydrate dinner at 1700 hours, subjects assumed a supine posture and two intravenous catheters were placed after which a baseline Posm was determined (Figure 1). Starting at 1800 hours, 0.45%, 0.9%, or 3.0% saline were infused at a rate calculated to produce a Posm of between 285 and 290 mmol/kg by 1900 hours. By normalizing Posm,

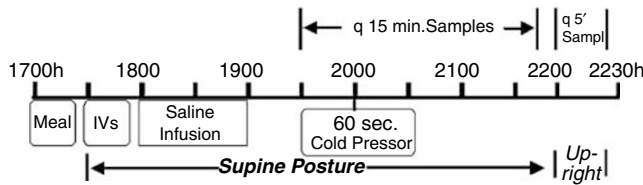


Figure 1 Time line of psychological (cold pressor) and systemic (postural) stressors. Study was conducted in the evening when HPA axis is most stable. Subjects assumed a supine posture before placement of intravenous catheters at 1730 hours. At 1800 hours subjects received a 1-h infusion of saline to normalize Posm. One hour later they submerged a hand in an ice water bath for 60 s. Blood samples and assessments were generally obtained at 15 min intervals from 30 min before until 105 min following the cold pressor. Four and one-half hours after becoming supine, subjects stood up, and samples were obtained at 5 min intervals for the next 20 min.

we controlled for the major factor that has been demonstrated to modulate AVP responses to non-osmotic stimuli in normals (Robertson, 2001) as well as in those with schizophrenia (Goldman *et al*, 1996, 1997).

Sampling and assessments began at 1930 hours and continued until 2220 hours. At each sampling time, blood was drawn, and vital signs (Dinamap XL Vital Signs Monitor; Critikon, Tampa, FL), and presence of nausea or light-headedness were recorded. At 2000 hours subjects placed their non-dominant hand in ice water up to the wrist, and were encouraged to keep it immersed for 1 min. Immediately afterward, they were asked how painful the stimulus felt on a scale of 1–4. For the postural stimulus, subjects stood up for 20 min after the 2200 hours sample was taken (Figure 1). Sampling and assessments generally occurred at 15-min intervals before and following the cold pressor, except that the last blood sample before the stimulus occurred at –10 min, and additional blood samples were obtained 5 and 10 min following the stimulus. For the postural stimulus, a baseline sample was obtained, plus three additional samples at 5-min intervals starting 10 min after subjects stood up (Figure 1).

Psychiatric subjects were studied during the fifth week of their admission after being acclimated to the research setting and stabilized on medication. All received the cold pressor. HC were studied on two outpatient visits. In randomly assigned order, they immersed their hands in the ice bath on one visit and in tepid water on the other. This enabled us to both characterize the normal response to the cold stressor and to refine the number of time points to include for each repeated measure analysis.

Laboratory

Collected blood samples were processed as described previously (Goldman *et al*, 1988). Posm, cortisol, and vasopressin were measured in the Clinical Research Center core labs at UIC (freezing point depression, Advanced Instruments 3D3, Needham, MA); UC (Chemiluminescent automated immunoassay, DPC, Immulite 1000, Los Angeles, CA); and Northwestern University (RIA) (Goldman *et al*, 1996), respectively. Plasma adrenocorticotropin (ACTH) was measured at UC endocrine laboratory (Chemiluminescent automated immunoassay, Nichols Advantage, San Clemente, CA).

Data Analysis

We took the natural log of cortisol and vasopressin levels to normalize the data. No other measures were transformed. A mixed effect linear regression model was chosen to analyze responses for each repeatedly sampled measure (Gibbons *et al*, 1993; Hedeker and Gibbons, 1996a). By modeling the shape of each subject's response, rather than measuring the deviation from the group norm, the accuracy and power of the analysis is greater than that of analysis of variance for repeated measures (Hedeker and Gibbons, 2006). In so doing, this approach also handles missing data, intra-subject correlation, and time-varying covariates in a more flexible and realistic manner.

For the cold pressor, the number of time points for each measure includes all values from –30 min until and including the time point where the group's responses to the cold pressor and control condition appeared to be identical (see Supplementary Figure 1). For the postural stimulus, all timed samples were included in the analyses. To characterize the difference in the pattern of each group's response, the shape of the response was decomposed into linear, quadratic, and cubic components. These three time trends and the intercept were entered as random effects in the model. Only the significance of the linear and quadratic trends and their interactions (reflecting differences in the slope and peak of the responses, respectively), however, were considered of interest. Power was maximized by limiting the statistical assessment to three *a priori* group contrasts in order to test if: (1) the PHS group's responses exceeded those of the PNS group; (2) these two polydipsic groups' responses exceeded those of the HC; and (3) these three groups responses exceed those of the NNS patients.

These three group (Difference) contrasts and their nine interactions with the time trends were entered as fixed effects. Significant main effects are only reported below when interaction terms were insignificant. To make the Results section easier to follow, we translate group by time trend interactions in the text ('higher peak response in the two polydipsic groups compared to the HC'), rather than just listing the specific contrast (eg 'quadratic time trend interaction with group contrast, which compared the two polydipsic groups to HC'). The two conditions (cold pressor, tepid water) in HC were analyzed in an analogous manner to that described above for the group analysis, but the actual contrasts and interactions are listed in the Results section as they are easier to grasp. All effects are reported as Z-scores. Covariates were examined by adding them as additional fixed effects to the model and assessing the significance of their contribution (also are expressed as Z-scores). Non-repeated measure analyses were conducted by analysis of variance or ordinal regression (Hedeker and Gibbons, 1996b) as indicated. Probabilities reflect two-tailed distributions even though we had directional hypotheses.

RESULTS

Cold Pressor and Tepid Water Conditions: HC

HC were studied on two occasions in order to both characterize the normal response to the cold stressor, as well as to refine the number of time points to include in the

repeated measures analyses. In randomly assigned order, controls immersed a hand in the ice bath on one visit and in tepid water on the other. Blood pressure increased following immersion in the ice bath (-10 to $+1$ min: 9.1 ± 13.6 mmHg) but did not change in the tepid water condition (-1.7 ± 5.0 mmHg; time by condition interaction: $Z = 2.35$, $p = 0.019$). Plasma ACTH rose before, as well as transiently following, the cold pressor but did not appear to change in the control (tepid water) condition (quadratic trend by condition interaction: $Z = 2.36$, $p = 0.018$, Supplementary Figure 1a). Plasma cortisol appeared to lag ACTH following the cold pressor before slowly returning to baseline, and also did not appear to change in the tepid water condition (Supplementary Figure 1b, quadratic trend by condition: $Z = 3.27$, $p = 0.001$). Plasma vasopressin (AVP) resembled the ACTH response, that is AVP rose before and transiently following the cold pressor, and did not appear to change in the tepid water condition (Supplementary Figure 1c, quadratic trend by condition: $Z = 1.96$, $p = 0.048$). Heart rate, Posm, expired air carbon monoxide, glucose, plasma sodium, and blood urea nitrogen did not change and were similar in both conditions (data not shown).

Cold Pressor: Three Patient Groups and HC

The patient population consisted mostly of middle-aged subjects with chronic and disabling mental illness (Table 1). The three patient groups resembled each other on demographic and clinical measures, though PHS patients appeared to be older ($t = 1.92$, $df = 9.7$, $p = 0.084$). Paranoia related items on the PANSS, a putative predictor of stress

reactivity (Lopes-Machado *et al*, 2002), did not differ between patient groups (data not shown). Treatment with atypical vs typical antipsychotic medications did not differ between groups. Water imbalance appeared to be somewhat less severe in the two polydipsic groups than in previous studies (Goldman *et al*, 1996) (Table 1). Only one of the control subjects, but many of the patients, smoked tobacco products (Table 1).

Acute Response to Cold Pressor

Before the cold pressor, measures known to modulate neuroendocrine responses were similar across the four groups (Table 2). Although Posm was lower at the start of the study in the PHS subjects, this was corrected by the saline infusion (Table 2). Subjective pain responses to the cold pressor were similar across all four groups. Blood pressure responses on the other hand were significantly blunted in the NNS group (ie normal water balance) relative to the other three groups ($t = 2.66$, $df = 21.3$, $p = 0.014$), who resembled each other on this measure. The two polydipsic groups kept their hands immersed in the ice bath for less time than the HC ($t = 2.75$, $df = 14.5$, $p = 0.015$), whereas immersion time was intermediate in the NNS group.

HPA Axis Responses to Cold Pressor

Basal ACTH levels (-30 min) were similar in the two polydipsic groups but higher than in HC (Figure 2a, $t = 3.27$, $df = 19.0$, $p = 0.001$), whereas levels were intermediate in the NNS group. PHS patients exhibited a more sustained rise in ACTH than PNS patients ($Z = 2.63$,

Table 2 Factors Known to Modulate Neuroendocrine Responses to Stress in Three Schizophrenic Subject Groups and Controls^a

Measure	Polydipsic hyponatremic (PHS)	Polydipsic normonatremic (PNS)	Nonpolydipsic normonatremic (NNS)	Healthy controls (HC)	Significant contrasts ($p < 0.05$)
<i>Before saline infusion</i>					
Plasma osmolality (mosmol/kg)	272.1 (17.0)	287.6 (2.1)	289.2 (3.2)	288.6 (4.4)	PHS < PNS
<i>Immediately before cold pressor^b</i>					
Plasma osmolality (mosmol/kg)	285.7 (8.7)	287.8 (1.7)	288.9 (2.7)	288.4 (1.7)	NS
Plasma sodium (mmol/liter)	139.3 (3.5)	139.2 (1.0)	138.3 (1.6)	139.4 (1.7)	NS
Plasma glucose (mg/dl)	93.1 (9.8)	90.8 (6.4)	94.0 (12.9)	87.7 (4.2)	NS
Mean arterial pressure (mmHg)	86.2 (17.5)	84.6 (13.5)	93.0 (14.5)	86.9 (8.0)	NS
Expired air CO (p.p.m.)	8.6 (10.0)	8.3 (6.4)	7.9 (8.9)	4.2 (1.3)	NS
<i>Immediately after cold pressor^c</i>					
Time in ice bath (s)	35.0 (22.8)	43.0 (17.7)	48.4 (16.9)	56.9 (10.7)	PHS+PNS < HC
Pain (1 = none to 4 = severe)	3.4 (1.2)	2.7 (1.4)	3.6 (0.5)	2.7 (1.0)	NS
Change in MAP (mmHg)	7.2 (15.5)	8.5 (16.7)	-0.5 (3.4)	8.7 (13.0)	PHS+PNS+HC > NNS

NS, nonsignificant.

^aData are mean (SD). See legend Table 1 for definition of the group effects, which were assessed.

^bMeasures reflect values 10 min before cold pressor. Expired air carbon monoxide (CO), an index of nicotine exposure, was determined by Micro-smokerlyzer, Bedford, Rochester, England.

^cPain reflects subject's report immediately following termination of the stimulus. Change in MAP (mean arterial pressure) is from -10 min to $+2$ min.

$p = 0.008$) (ie hypothesis 1). The response appeared to be greater in these two groups than in the HC, but the difference did not achieve significance ($p > 0.05$) (hypothesis 2). Both the slope ($Z = 2.22$, $p = 0.026$) and peak ($Z = 2.26$, $p = 0.021$) of the response were blunted in NNS patients relative to the other three groups (hypothesis 3). Figure 3a shows the same data as percent of the subjects' -30 min ACTH levels in order to facilitate visual comparison of the groups' response patterns. As reported previously (Lovallo, 1975), ACTH responses were predicted by the duration of hand immersion in the ice bath across subjects (covariate $Z = 2.30$, $p = 0.02$). Neither basal ACTH levels (-30 min), blood pressure, age, pre-infusion, or concurrent Posm, subjective pain, nor behavioral ratings were related to the ACTH responses.

Cortisol responses were strongly predicted by ACTH levels 15 min earlier ($Z = 6.02$, $p < 0.001$) but group differences did not appear to closely resemble those seen with ACTH. Thus peak levels were higher in PNS than PHS subjects, and HC responses resembled those of the two polydipsic groups. Statistical analysis, however, not only demonstrated that the peak response was lower in normonatremic patients compared to the other three groups (hypothesis 3: $Z = 3.69$, $p = 0.001$) (Figure 2b), but also that the rise was more sustained (ie greater slope) in the PHS than the PNS subjects (hypothesis 1: $Z = 3.49$, $p = 0.001$).

AVP Response to Cold Pressor

Basal AVP levels (-30 min) were diminished in the two polydipsic groups relative to HC (Supplementary Table 1, $t = 2.83$, $df = 13.6$, $p = 0.014$), and were intermediate in the NNS group to the two polydipsic groups and the controls. An anticipatory (ie prestimulus) linear rise in AVP was significant ($Z = 3.13$, $p = 0.001$) and statistically equivalent across groups. Peak response to the cold pressor was greater in PHS than PNS patients (hypothesis 1: $Z = 2.04$, $p = 0.040$), and again blunted (albeit marginally) in patients with normal water balance relative to the other three groups (hypothesis 3: Figure 2c, $Z = 1.77$, $p = 0.083$). The responses of the HC and the two polydipsic groups were not statistically different. Group differences can again be more easily appreciated by plotting AVP as percent of the subjects' AVP level at -30 min (Figure 3b). The AVP response was predicted by basal AVP levels (covariate $Z = 2.01$, $p = 0.044$), pre-infusion Posm (covariate $Z = 2.00$, $p = 0.044$), as well as concurrent ACTH levels ($Z = 3.83$, $p = 0.001$). Posm rose consistently in the two polydipsic groups following the cold pressor relative to HC (Figure 2d, $Z = 2.61$, $p = 0.009$), who resembled the NNS patients in this regard. The change, however, did not correlate with the AVP response. Age, subjective pain, duration of immersion in the ice bath, blood pressure, and behavioral ratings were also not related to the AVP responses.

Response to Postural Stimulus

Four and one-half hours after assuming a supine posture, and 120 min after the cold pressor stimulus (Figure 1), subjects stood up and remained upright for the last 20 min of the study. One HC became frankly orthostatic and her

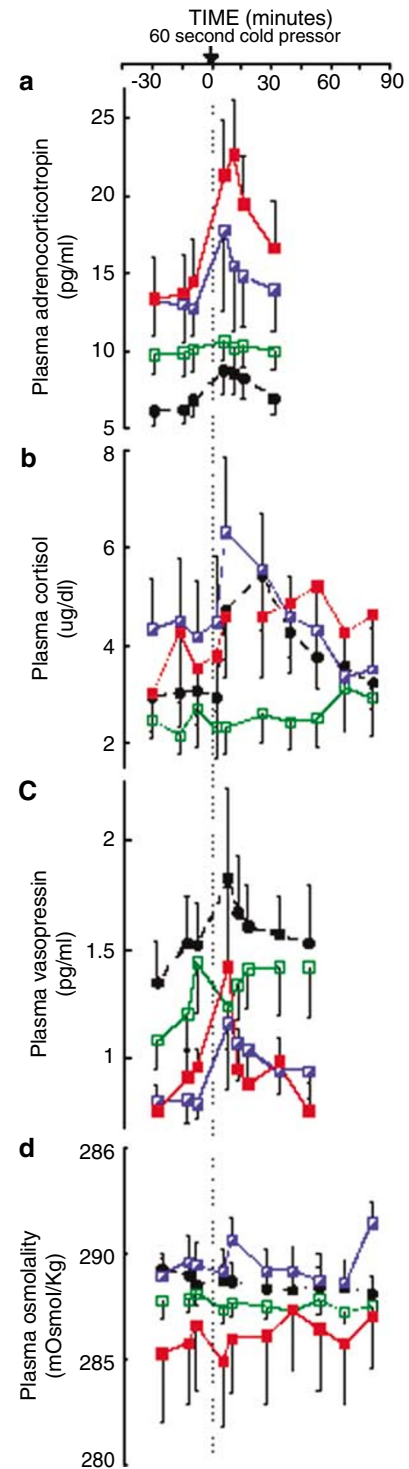


Figure 2 Effects of cold pressor on plasma hormones and related measures. Each symbol shows the mean (\pm SEM) for all subjects in a given group for each variable at a different time interval before and following the cold pressor. Groups consisted of schizophrenic patients who were polydipsic and hyponatremic (PHS) (■), polydipsic and normonatremic (PNS) (■), and nonpolydipsic and normonatremic (NNS) (□), and HC (●). Mixed effects linear regression demonstrated that hormonal responses were enhanced in PHS relative to PNS, and enhanced in both these groups and HC relative to NNS.

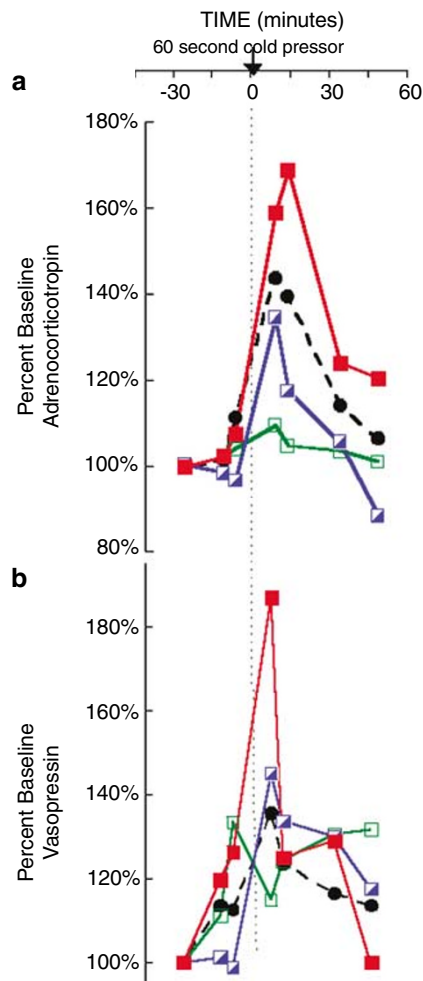


Figure 3 Percent increase in plasma ACTH and vasopressin. Each symbol shows the percent changes from baseline. See legend of Figure 2 for explanation of symbols.

data were excluded. No other subject reported nausea or lightheadedness at any time. Basal cardiovascular measures were similar across groups. Blood pressure remained stable upon arising and did not differ across groups (Supplementary Figure 2a). In contrast, heart rate increased more in those with polydipsia and hyponatremia than polydipsic patients without hyponatremia (Supplementary Figure 2b, $Z = 2.48$, $p = 0.013$), who in turn appeared to resemble the other two groups. The HC exhibited only a transient rise in ACTH, in contrast to the sustained increase seen in the two polydipsic groups (Supplementary Figure 2c, $Z = 2.13$, $p = 0.03$), as well as the NNS patients. In contrast, cortisol (Supplementary Figure 2d; $Z = 3.18$, $p = 0.001$) and AVP (Supplementary Figure 2e, $Z = 2.74$, $p = 0.006$) showed sustained and similar increases in all groups.

DISCUSSION

Divergent Neuroendocrine Responses to the Cold Pressor in Schizophrenia

Neuroendocrine responses to a cold pressor stimulus are enhanced in schizophrenic patients with polydipsia and

hyponatremia relative to those who are normonatremic, but are blunted in patients with normal water balance. Specifically, ACTH and AVP responses are relatively prolonged and show a greater rise, respectively, in hyponatremic compared to normonatremic polydipsic patients, whereas these same measures are blunted in NNS patients relative to both polydipsic patient groups as well as to healthy comparison subjects (Figures 2a, c, 3a and b). Although more group overlap was apparent with the plasma cortisol response (Figure 2b), group differences were analogous to those seen with ACTH and indeed were highly predicted by the ACTH response. The blunted neuroendocrine response to a psychological stimulus in the patients with normal water balance along with their preserved response to a systemic stimulus reproduces previous findings in persons with schizophrenia (Jansen *et al*, 2000) (but see Elman *et al*, 1998), but the findings in the PHS patients are completely novel. In contrast our predicted differences between the two polydipsic groups and HC were not apparent. Thus, although our first and third hypotheses were supported, our second hypothesis was not.

As the subject groups were matched and the procedures were conducted in a carefully monitored setting, we can confidently address the potential confounding role of recognized factors in these findings. Olanzapine and other atypical neuroleptics have recently been shown to inhibit basal cortisol secretion in normals and those with schizophrenia (Mann *et al*, 2006), however, exposure to atypicals did not differ across groups (Table 1), and if anything appeared more common in those with polydipsia. Other agents appear to have minimal or no effects on neuroendocrine function in schizophrenia (Goldman *et al*, 1996; Kaneda *et al*, 2002; Kawai *et al*, 2002; Goldman and Hussain, 2004) and exposure to these agents, in any case, was also similar across patient groups (Table 1). Likewise, psychiatric symptoms did not differ across patient groups, though we cannot exclude the possibility that undetected symptoms (eg repetitive behaviors) may have contributed (Luchins, 1990). Although hyponatremic subjects appeared to be older (Table 1), including age as a covariate did not influence the findings (Otte *et al*, 2005; Stout *et al*, 1999) nor would any effect be expected from this small an age difference (Otte *et al*, 2005; Stout *et al*, 1999). As expected, time of exposure to the cold pressor did predict the hormone response (Lovallo, 1975), but this too was similar across groups and was, in fact, nonsignificantly briefer in the hyponatremic polydipsic subjects (Table 2). Posm was normalized before the cold pressor (Table 2), so that concurrent hypoosmolemia/hyponatremia *per se* can be excluded. Pre-infusion osmolality was negatively correlated with the AVP response, consistent with the recognized blunting effects of chronic hyponatremia on AVP and ACTH responses, (Dohanics *et al*, 1991; Goldman *et al*, 1996; Robertson, 2001). Thus the role of long-standing hyponatremia can also be discounted, though inclusion of a non-psychiatric control group with hyponatremia would more definitively address this possibility. Polydipsia cannot account for the findings, as it too blunts AVP responses (Moses and Clayton, 1993) and in any event, the PNS patients' responses were blunted relative to those with hyponatremia (Figure 2a and b). Finally, the diminished

blood pressure response in NNS patients cannot explain their blunted neuroendocrine responses, as blood pressure change tends to be inversely correlated with the neuroendocrine responses (al'Absi and Petersen, 2003).

Responses Suggestive of Altered Response to Psychological Stress

Although more definitive conclusions require replication with other sets of stimuli, these data support the concept that responses to psychological, but not systemic stress, diverge in schizophrenia depending on the presence and severity of water imbalance. Thus, previous studies indicate the cold pressor has the attributes of a psychological stressor (eg aversiveness and productive of anticipatory responses), and that these tend to increase HPA activity (al'Absi and Petersen, 2003; Bullinger *et al*, 1984; Ehrenreich *et al*, 1997; Gregg *et al*, 1999), whereas its systemic effects (eg cold, hypertension) tend to decrease activity (Edelson and Robertson, 1986; Kendler *et al*, 1978; Wittert *et al*, 1992). Group responses to a systemic stimulus (Hennig *et al*, 2000; Jacob *et al*, 1998), which produced an equivalent HPA and perhaps greater AVP response (compare Figure 2 and Supplementary Figure 2), were similar. We must acknowledge, however, that although the effects of the systemic stimulus in normals is attributable to its systemic actions, we cannot exclude the possibility that psychological factors contribute to its actions in persons with schizophrenia.

Mechanism of Water Intoxication in Schizophrenia

Impaired water excretion that varies with the stress of acute psychosis was first demonstrated before the introduction of antipsychotic medications by Targowla (1923), and subsequently confirmed in medication naive patients by Hobson and English (1963) and Suzuki *et al* (1992). Barahal in 1938 first noted the association between acute psychosis and life-threatening water intoxication (also see de Leon *et al*, 1994). Subsequent studies in controlled experimental settings have demonstrated that both the basal impairments in water excretion (Goldman *et al*, 1988, 1996) and their aggravation during acute psychosis (Goldman *et al*, 1997) are attributable to unexplained increases in vasopressin activity that are not seen in other schizophrenic patients. Although effective treatments for hyponatremia have subsequently been introduced, reports of fatalities from water intoxication continue to accumulate (Farrell and Bower, 2003; Hayashi *et al*, 2005; Loas and Mercier-Guidez, 2002). The current study establishes a plausible mechanism for the previously unexplained rise in vasopressin activity in hyponatremic schizophrenics: an enhanced neuroendocrine response to psychological stress.

Model of Enhanced Stress Vulnerability

Although the mechanism is not apparent from these data, accumulating evidence indicates anterior hippocampal formation pathology may be responsible for this enhanced neuroendocrine activity. Thus this segment of the hippocampus normally constrains AVP and ACTH responses to

psychological stress (Herman *et al*, 1995, 2005; Mueller *et al*, 2004; Nettles *et al*, 2000); the findings in patients are recreated in an animal model of schizophrenia, which disrupts the neurodevelopment of this brain region (Mitchell and Goldman, 2004); and this subset of patients exhibits diminished anterior hippocampal volume (Goldman *et al*, 2005) as well as other impairments indicative of hippocampal-mediated neuroendocrine dysfunction (Goldman *et al*, 2006). Finally, our unpublished data demonstrate a significant association between anterior hippocampal volume and the cold pressor AVP response in hyponatremic polydipsic patients ($r = -0.825$, $p = 0.022$). More direct evidence is needed, however, before rejecting alternative explanations.

A hippocampal-mediated vulnerability to psychological stress has been proposed to underlie schizophrenia (Mednick and Schulsinger, 1968; Gray, 1998; Walker and Diforio, 1997; Moghaddam, 2002; Myin-Germeys *et al*, 2005), and thus PHS schizophrenic patients may facilitate our understanding of how hippocampal pathology increases stress vulnerability. This region of the hippocampus has been associated not only with schizophrenia and neuroendocrine responses to psychological stress but also with affective and behavioral stress responses (Flores *et al*, 2005; Trivedi and Coover, 2004), suggesting the neuroendocrine disorder could be a manifestation of the pathologic changes responsible for the severe mental illness.

Strengths, Caveats, and Limitations

The strengths of this study are the inclusion of the two matched control groups of schizophrenic patients; the two-stage selection process to characterize water balance in the patient groups; the acclimation of subjects to the research setting; the saline infusion to normalize Posm; the inclusion of a control (systemic) stimulus; the characterization of the normal cold pressor response in the HC; and the careful monitoring of possible confounding factors in a clinical research center.

There are, however, important caveats and limitations. Thus, although the sample size is similar to those of previously reported studies (Goldman *et al*, 1988, 1997), it is small, thereby suggesting findings may be difficult to reproduce or that undetected group differences (ie type II errors) could account for them. The cold pressor is a mixed physical and psychological stimulus, and although our data and that of others suggest that the neuroendocrine release is attributable to its psychological effects, we cannot exclude that group differences in systemic responses contribute to the results. Also we cannot exclude the possibility that the cold pressor altered the impact of the subsequent postural stimulus.

Finally, the findings do not address the mechanism of the blunted neuroendocrine responses in patients with normal water balance. Furthermore, although they replicate previous observations of diminished blood pressor response to cold pressor in these patients (Earle and Earle, 1955), the data (Table 2) appear inconsistent with the broad literature suggestive of pain insensitivity in schizophrenia (Singh *et al*, 2006). Further studies are required to resolve these issues.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)