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Abnormal response to metabolic stress in schizophrenia: marker of vulnerability or acquired sensitization?

M. MARCELIS, E. CAVALIER, J. GIELEN, P. DELESPAUL AND J. VAN OS*

Department of Psychiatry and Neuropsychology, South Limburg Mental Health Research Network, EURON, Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands; Service de Chimie médicale, University Hospital Liège, Liège, Belgium; Division of Psychological Medicine, Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK

ABSTRACT

Background. Previous work suggests that individuals with schizophrenia display an altered homovanillic acid (HVA) response to metabolic stress. The present study replicated and extended this paradigm, including individuals with elevated genetic risk for schizophrenia.

Method. Patients with psychosis ($n=50$), non-psychotic first-degree relatives of patients with psychosis ($n=51$) and controls without psychosis ($n=50$) underwent, in randomized order, double-blind administration of placebo and the glucose analogue 2-deoxy-D-glucose (2DG), which induces a mild, transient clinical state of glucoprivation. Plasma HVA and cortisol were assessed twice before the start of the 2DG/placebo infusion (baseline values), as well as four times post infusion. Data were analysed using multi-level random regression techniques.

Results. During the stress condition, significant increases in plasma HVA and cortisol were found. The increase in plasma HVA level during the stress condition was significantly stronger in patients than in controls, whereas this was not the case in relatives *v.* controls. The increase in plasma cortisol during the stress condition was significantly less in patients than controls, but no significant difference in the increase of plasma cortisol during stress was found in the comparison between relatives and controls.

Conclusions. Patients with psychosis, but not their non-psychotic first-degree relatives, show an altered neurobiological response to metabolic stress, suggesting that this dysregulation is not a genetically transmitted vulnerability, but an illness-related effect, possibly reflecting acquired sensitization of neuroendocrine systems by repeated environmental stressors or repeated stimulation with agonistic drugs.

INTRODUCTION

Altered stress-sensitivity in schizophrenia may reflect an acquired response, related to progressive sensitisation of stress-related neuroendocrine systems, a vulnerability that is transmitted genetically and can also be demonstrated in the healthy carriers of the genotype, as implied by the stress–vulnerability model, or a combination of the two (Zubin & Spring, 1977). Genetic transmission of sensitivity to an environmental risk factor is a form of gene–environment interaction, which is thought to constitute a potent causal mechanism for psychotic illness (Tienari *et al.* 1985; Cannon *et al.* 1993), but remains difficult to investigate (Kendler & Eaves, 1986). One of the factors bedevilling observational gene–environment research paradigms is that it is often difficult to separate environmental from

* Address for correspondence: Professor Jim van Os, Department of Psychiatry and Neuropsychology (DRT 10), Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands.
(Email: j.vanos@sp.unimaas.nl)

genetic effects. For example, an 'environmental' measure of life events in an observational study may be confounded by genetic effects that influence psychological traits that predispose to life event exposure (Kendler *et al.* 1993).

Experimental study designs may be helpful in circumventing the problem of genetic 'noise' that hamper studies with observational measures of the environment (Van Os & Sham, 2002). One such experimental design is the metabolic stress paradigm. Metabolic stress can be induced by intravenous infusion of the glucose analogue 2-deoxy-D-glucose (2DG). This glucose analogue inhibits intracellular glucose metabolism and produces a mild, transient state of intracellular hypoglycaemia (Welle *et al.* 1980). The effect of this stressor on catecholamine and neuroendocrine systems, can be assessed by repeatedly measuring homovanillic acid (HVA) and cortisol in plasma over time (Breier, 1989; Breier *et al.* 1993). Individuals with schizophrenia have been found to have an increased HVA-response to metabolic stress as compared to healthy controls (Breier *et al.* 1993), suggestive of an abnormal regulation of dopaminergic (DA) and/or noradrenergic (NA) systems during stress conditions. In addition, the 'fast' DA/NA mediated response to stress may be dissociated from the 'slow' hypothalamic–pituitary–adrenal (HPA) response under similar conditions (Breier *et al.* 1993).

First-degree relatives share, on average, 50% of their genes with their patient relative and have repeatedly been found to have intermediary values of, for example, cognitive abnormalities (Cannon *et al.* 1994; Krabbendam *et al.* 2001) and cerebral structural abnormalities (Sharma *et al.* 1997; Cannon *et al.* 1998). Given the fact that the shared environment has no substantial influence on familial resemblance in studies of schizophrenia liability (Kety *et al.* 1994; Cardno *et al.* 1999), being a first-degree relative can be considered a useful proxy measure of genetic risk in studies on schizophrenia.

In the present study, we dealt with the question whether altered response to metabolic stress is associated with the clinical phenotype of schizophrenia or with the genetic vulnerability for the disease. We investigated this issue by applying the 2DG paradigm (Breier *et al.* 1993) to patients with psychotic illness, non-psychotic first-degree relatives and healthy controls. We tested for the presence of gene–environment interaction by using first-degree relative status as a proxy measure of genetic risk, and the experimental metabolic stress paradigm as a measure of the environment. We predicted that (1) patients would have an abnormal metabolic stress response and (2) that first-degree relatives of patients would have values intermediary to those of patients and controls.

METHOD

Subjects

This study is part of the Maastricht Psychosis Study (Krabbendam *et al.* 2001; Myin-Germeys *et al.* 2002). Patients with a lifetime history of psychosis according to the RDC criteria (Spitzer *et al.* 1978) were recruited at the community mental health centre in Maastricht, The Netherlands. All patients were in remission or in partial remission as defined as not in need of in-patient treatment. Non-psychotic first-degree relatives were recruited through the participating patients, as well as through local relatives' associations. Relatives were free from a lifetime history of psychosis. The study population originated from 67 families with at least one patient with psychosis. The total sample comprised 50 patients and 51 relatives. Of the 50 healthy relatives, there were 6 mothers, 7 fathers, 20 sisters, 17 brothers and one son. Of the 67 families, 41 families contributed one case or one relative, 2 families contributed two and three relatives respectively, and 24 families contributed at least one case and one relative.

Unrelated healthy controls were sampled from the general population, using a mailing procedure to randomly selected households in the local catchment area. Controls were excluded whenever they had a personal or family history of psychosis or other major psychiatric disorder requiring hospital admission.

Other inclusion criteria for all participants were: aged 18–55 years, and being in good health as determined by a physical examination, electrocardiography and routine laboratory investigations. Individuals with neurological, endocrine, cardiovascular and/or other serious medical disorders were excluded, as well as individuals with a history of severe head trauma with loss of consciousness. In addition, individuals who used alcohol in excess of five units

Table 1. *Demographic characteristics of study participants*

	Patients (S.D.) ($n=50$)	Relatives (S.D.) ($n=51$)	Controls (S.D.) ($n=50$)	F (df)	p
Gender (ratio male/female)	26/24	26/25	25/25		
Age (years)	31·2 (7·5)	37·2 (11·3)	35·0 (8·9)	5·3 (2, 148)	0·01
Educational achievement	3·6 (1·4)	4·6 (1·7)	4·4 (1·6)	5·64 (2, 148)	0·004
BPRS	39·1 (10·3)	28·4 (5·2)	25·6 (2·3)	54·8 (2, 146)	0·001

per day were also excluded, as well as patients who used illicit drugs on a weekly basis.

Clinical and diagnostic procedures

Patients ($n=50$), relatives ($n=51$) and controls ($n=50$) were interviewed with the expanded version of the Brief Psychiatric Rating Scale (BPRS; Lukoff *et al.* 1986), the PANNS (Kay *et al.* 1987, 1988), and case note and other historical material was additionally screened for symptoms listed in the OCCPI (McGuffin *et al.* 1991). Where necessary, additional information was derived from interviews with the responsible medical officer. Based on the combined information, the computerized program OPCRIT (McGuffin *et al.* 1991) yielded Research Diagnostic Criteria diagnoses. There were 40 patients (80%) with a diagnosis of schizophrenia and 10 patients received a diagnosis of schizo-affective disorder (20%). In addition, six relatives had a (lifetime) diagnosis of major depression.

Groups were frequency matched on age and sex. There was a slight difference in educational level between the groups (see Table 1). The mean age of first psychotic symptoms was 22·0 years (S.D. = 5·7). The mean total score of the patients on the BPRS was 39·1 (S.D. = 10·3). Patient scores were significantly higher compared to relatives and controls, with no significant difference between the latter two groups ($F=54·8$, df = 2, 146, $p<0·001$). Of the 50 patients, 47 were on antipsychotic medication, 17 used benzodiazepines, 9 antidepressants and 3 lithium. In the group of relatives, one person used an antipsychotic (pipamperon), 2 used antidepressants and 4 benzodiazepines. In the patient group, 39 persons were cigarette smokers *v.* 11 non-smokers. In the relatives, these figures were 21 *v.* 30, and in the controls 14 and 36 respectively. The group differences with regard to smoking habits were statistically significant ($\chi^2=26·90$, df = 2, $p<0·001$).

2-Deoxy-glucose protocol

All subjects underwent double-blind administration of the glucose analogue 2DG and placebo, in randomized order. The 2DG doses were 50 mg/kg mixed in 100 ml of isotonic saline. Placebo was a comparable volume of isotonic saline (NaCl 0·9%). Both conditions (2DG/placebo) were given within 1 week, with at least 2 days in between. Subjects had to fast from midnight prior to both test days, and were only allowed to drink water *ad libitum*. In addition, they were not allowed to take alcohol in excess of 2 standard units the day before the test, and subjects restricted cigarette smoking on the morning of the test day to no more than 2 cigarettes, and not within 1·5 h before the start of the test. During the test, subjects were lying on a bed and remained resting there from 08:45 to 12:30 hours. At 08:45 hours, an intravenous catheter was inserted in the antecubital fossa and kept patent with a slow drip of isotonic saline. At 09:50 hours two baseline venous blood samples were taken 10 min and 0 min prior to infusion. At 0 min, 2DG or placebo was infused over a period of 20 min. Four more blood samples were taken at 60, 90, 120 and 150 min after infusion.

Blood was collected in tubes containing 0·5 ml of an EDTA (40 mg/ml) and $Na_2S_2O_5$ (20 mg/ml) solution, gently mixed and immediately placed on ice. Subsequently, plasma was obtained by centrifugation (15 min at 3000 rev/min) in a refrigerated centrifuge (5 C) and immediately stored at −80 C until assaying. A 515 Waters isocratic HPLC was used for assaying HVA and 3-methoxy-4-hydroxyphenylglycol (MHPG), with a Symmetryshield RP18 25 cm column for the separation of the compounds. Intra-assay variability was 3% for MHPG and 5% for HVA. Inter-assay variability was 7% for MHPG and 9% for HVA. Cortisol levels were determined from plasma

Table 2. *Effects of 2-deoxyglucose (2DG) on plasma HVA (ng/ml) in psychotic patients, non-psychotic first-degree relatives and healthy controls*

	Time1 Mean (S.D.)	Time2 Mean (S.D.)	Time3 Mean (S.D.)	Time4 Mean (S.D.)	Time5 Mean (S.D.)	Time6 Mean (S.D.)
Controls ($n=50$)						
Placebo	10·2 (7·0)	9·9 (7·0)	9·2 (6·7)	8·3 (5·6)	8·0 (5·4)	7·8 (4·9)
2DG	9·0 (3·9)	8·7 (4·2)	8·8 (4·1)	9·4 (4·3)	9·8 (4·3)	9·8 (4·7)
Relatives ($n=51$)						
Placebo	9·0 (5·8)	8·7 (5·5)	7·8 (4·8)	7·8 (4·8)	7·4 (4·3)	7·4 (4·1)
2DG	8·3 (4·0)	8·1 (3·8)	8·4 (3·1)	8·9 (3·3)	9·4 (3·5)	9·9 (3·6)
Patients ($n=50$)						
Placebo	10·1 (4·6)	9·7 (4·2)	8·4 (4·2)	8·0 (4·0)	7·6 (3·4)	7·4 (3·2)
2DG	9·5 (4·4)	9·4 (4·1)	9·0 (3·6)	9·9 (4·2)	10·5 (4·7)	11·3 (5·4)

by radio-immunoassay with an intra-assay and inter-assay variability of 8% and 10% respectively.

Statistical analyses

The data were analysed with the STATA computer program, version 7 (StataCorp, 2001). Group was treated as two 2-level dummy variables in which patients and relatives were compared with the control group (reference). The condition variable also reflected two levels, the placebo condition (reference), and the 2DG condition. HVA and cortisol were sampled on six occasions (time 1–6). This time variable (time 1–6) was divided into the variables timeA (time 1–2) and timeB (time 3–6) reflecting the two pre- and the four post-infusion measurement occasions. TimeB served as the variable of interest, timeA as covariate to control for baseline values. The mean HVA/cortisol level of the two pre-infusion samples for each person and each condition were used to construct a baseline HVA variable (HVA_base) and baseline cortisol variable (CORT_base).

The association between metabolic stress, on the one hand, and plasma HVA and cortisol (the continuous outcome variables), on the other, was investigated. Group, condition and timeB, as well as the two- and three-way interactions between these variables were used as the explanatory variables. The baseline variables (HVA_base in HVA models and CORT_base in cortisol models), as well as the timeA variable were used as covariates in all models. In addition, age, sex, cigarette smoking/non-smoking and mean alcohol intake per week were used as covariates in separate analyses to adjust for their *a priori* hypothesized confounding effects. Interaction terms were evaluated by Likelihood Ratio test. As the average measure of HVA or cortisol is assumed to vary across persons, two observations will be more similar if they are from the same person. Our design of repeated measures within the same person therefore compromised statistical independence of the observations. In order to deal with this issue, multilevel random regression models were fitted (Goldstein, 1987). In multilevel regression, dependency of observations within persons is taken into account by estimating a within-person as well as a between-person level variance. Thus, the model used had two levels: measurement occasions (level 1) clustered within subjects (level 2). We did not introduce a third level to take account of familial clustering because the majority of patients and relatives were independent (41 out of 67 families contributed one case or one relative). Effect sizes of explanatory variables were expressed as regression coefficients (β) from the multilevel models.

RESULTS

Plasma HVA

Mean levels of HVA (ng/ml) at the six measurement points during the stress and placebo conditions are presented in Table 2. There was a significant effect of condition on HVA ($\beta=1{\cdot}51$, $p<0{\cdot}001$). In addition, a significant condition × timeB interaction was found (LRS = 327·22, $p<0{\cdot}001$), indicating an increase in HVA over time in the 2DG condition. When group was added to the statistical model, two three-way interactions with condition and timeB were fitted using the two dummy variables (patients

Table 3. *Effects of 2-deoxyglucose (2DG) on plasma cortisol (ng/μl) in psychotic patients, non-psychotic first-degree relatives and healthy control*

	Time1 Mean (S.D.)	Time2 Mean (S.D.)	Time3 Mean (S.D.)	Time4 Mean (S.D.)	Time5 Mean (S.D.)	Time6 Mean (S.D.)
Controls ($n=50$)						
Placebo	12·8 (5·4)	11·8 (5·0)	8·2 (4·0)	7·3 (4·0)	6·8 (3·3)	7·5 (4·2)
2DG	13·9 (8·2)	12·8 (7·6)	21·9 (6·9)	25·3 (6·6)	26·9 (7·6)	27·2 (9·9)
Relatives ($n=51$)						
Placebo	15·0 (12·8)	14·2 (12·9)	10·2 (8·7)	9·4 (8·9)	9·2 (8·8)	9·3 (8·6)
2DG	13·6 (5·8)	12·4 (5·6)	21·2 (5·1)	24·7 (6·1)	26·0 (7·0)	26·5 (7·9)
Patients ($n=50$)						
Placebo	12·5 (5·0)	11·6 (4·6)	9·1 (3·7)	8·7 (3·5)	8·6 (3·2)	8·9 (3·5)
2DG	12·4 (4·6)	11·2 (4·5)	19·1 (5·6)	23·0 (5·2)	24·2 (5·1)	24·3 (6·1)

v. controls and relatives *v.* controls). A significant group × condition × timeB interaction was found for patients ($\beta=0{\cdot}24$, $p<0{\cdot}039$), indicating that the increase in HVA in the stress condition as compared to the placebo condition was stronger in the patient than in the control group. Adjustment for age, sex, alcohol intake and cigarette smoking did not change the results ($\beta=0{\cdot}24$, $p<0{\cdot}044$). The three-way interaction comparing relatives with controls was not significant ($\beta=-0{\cdot}06$, $p<0{\cdot}588$).

Plasma cortisol

Mean levels of cortisol (ng/μl) at the six measurement points during both conditions are presented in Table 3. There was a significant overall effect of condition on plasma cortisol ($\beta=10{\cdot}45$, $p<0{\cdot}001$). In addition, there was a significant condition × timeB interaction (LRS = 874·14, $p<0{\cdot}001$), indicating a significant increase in cortisol in the 2DG condition as compared to the placebo condition. This increase in cortisol in the stress condition varied with group. Stratified analyses yielded a negative group × condition × timeB interaction for patients ($\beta=-0{\cdot}84$, $p<0{\cdot}013$), indicating that the increase in cortisol in the stress condition as compared to the placebo condition was significantly less strong in patients than in controls. Adjustment for age, sex, weekly alcohol intake and cigarette smoking did not change the results ($\beta=-0{\cdot}85$, $p<0{\cdot}011$). There was no evidence for a significant difference in cortisol increase during stress between relatives and controls (group × condition × time interaction: $\beta=-0{\cdot}097$, $p<0{\cdot}772$).

DISCUSSION

The present study investigated whether changes in plasma HVA and cortisol levels in response to a metabolic stressor were related to a proxy measure of genetic vulnerability for schizophrenia. In contrast with the hypothesis, patients with psychosis, but not the non-psychotic first-degree relatives, were found to have an altered stress response as compared to controls. The plasma HVA levels during the stress condition were significantly increased in patients *v.* controls, whereas the increased plasma cortisol levels were significantly blunted in patients *v.* controls. In the relatives, the increases in both plasma HVA and cortisol levels were not significantly different from those in controls.

Methodological considerations

The glucose-analogue 2DG causes intracellular hypoglycaemia by competing with glucose-6-phospate during the early stage of glycolysis, and inhibiting intracellular glucose utilization. This stress paradigm has been found to affect DA metabolism strongly and to cause substantial plasma elevations of cortisol levels, as well as significant effects on physiologic variables (blood pressure, heart rate, temperature) (Breier, 1989; Breier *et al.* 1993). Glucose deprivation has profound effects in the brain, for which glucose is the most important source of energy. A PET study (Ilman *et al.* 1999) showed that pharmacological doses of 2DG lead to widespread activation of cortical and subcortical blood flow in healthy volunteers.

Much of the plasma HVA originates from central and peripheral NA systems. Even under

fasting conditions, around 75% of plasma HVA derives from the NA neuronal metabolism (Kopin *et al.* 1988). Nevertheless, plasma HVA is assumed to also reflect central DA activity (Amin *et al.* 1992). Evidence supporting this hypothesis comes from studies showing similar correlations between plasma and CSF HVA on the one hand, and psychotic-like symptoms on the other hand in schizotypal personality disorder (Siever *et al.* 1991, 1993). Additional evidence has been derived from studies showing correlations between plasma (and CSF) HVA and the clinical response to antipsychotic medication (Pickar *et al.* 1987; Davila *et al.* 1988; Sharma *et al.* 1989). Furthermore, a strategy that has been described to improve the interpretation of plasma HVA levels, is the enhancement of the relative contribution of the central nervous system to plasma HVA by treatment with the peripheral MAO inhibitor debrisoquin (Miller *et al.* 1997). The finding of, for example, correlations between symptom severity and plasma HVA level has been confirmed in studies of patients on debrisoquin (Maas *et al.* 1988; Pickar *et al.* 1988), supporting the view of a central origin of plasma HVA.

Other lines of research, however, suggest that the peripheral HVA levels are likely to be under central regulatory control, without specifically reflecting central DA activity. In fact, there is evidence for hypothalamic involvement (Yoshimatsu *et al.* 1987) in the regulation of HVA outflow by the sympathetic nervous system. Taken together, it seems reasonable to assume that plasma HVA level changes during conditions of stress, whether or not directly reflecting central DA activity, are regulated by central factors. Moreover, the main goal of the present study was to investigate whether alterations in the general neurobiological stress-response during metabolic perturbation may be an indicator of genetic risk for schizophrenia (i.e. whether it is an endophenotype). From this perspective, we did not attempt to distinguish differential contributions of central and peripheral DA and NA systems.

Contrary to relatives and controls, almost all patients were medicated with an antipsychotic drug, influencing both dopamine and cortisol levels, although chronic use of antipsychotics has not been found to affect cortisol levels (Meador-Woodruff & Greden, 1988). The fact that the patients showed a significantly stronger increase in plasma HVA during the 2DG condition as compared to controls indicates that (chronic) antipsychotic treatment does not preclude stress-induced increases in dopamine function, which is in agreement with other reports (Breier, 1989; Breier *et al.* 1993). Moreover, in a prior study (Breier *et al.* 1993), medicated and non-medicated groups did not differ significantly in the HVA and cortisol response to metabolic stress. In addition, a blunted cortisol response following physiological stress has also been described in non-medicated patients (Breier *et al.* 1988).

Benzodiazepines have been shown to diminish effects of stress on cortisol (Breier *et al.* 1991). In this study, 17 patients used a benzodiazepine. We cannot fully exclude the possibility that the tendency to blunted cortisol responses in the patient group has been influenced by benzodiazepine use. However, when the individuals using this type of medication were *post hoc* excluded from the analyses, the results remained the same, which argues against a medication-related effect.

Plasma HVA concentrations are sensitive to various confounding factors, such as diet, exercise, smoking and diurnal variation (Amin & Friedhoff, 1997). Nevertheless, all these factors were controlled for in the present study at both the selection and the analysis stage. The HVA data were collected on two separate days within 1 week, with at least 2 days in between. Amin *et al.* (1998) have shown that plasma catecholamine metabolites in normal subjects have good reproducibility.

Findings

The finding of increased HVA response during metabolic stress in patients with schizophrenia replicates prior studies with smaller sample sizes (Breier, 1989; Breier *et al.* 1993). In addition, the present study is the first in investigating the effects of metabolic stress in non-psychotic first-degree relatives. The results indicate that an altered stress response as depicted by increased HVA levels is an illness-related finding and not associated with a proxy measure of genetic vulnerability for the disorder. In other words, the dysregulation in the DA–NA mediated stress response may reflect a disease effect rather than a transmitted vulnerability, presumably

associated with the development of psychotic symptoms in schizophrenia. In a recently postulated framework for linking the psychological and biological aspects in psychosis (Kapur, 2003), it was suggested that a dysregulated, hyperdopaminergic state may lead to stimulus-independent release of dopamine which may take over the normal process of contextually driven salience attribution and leads to aberrant assignment of salience to external objects and internal representations. Hallucinations and delusions may consequently arise from cognitive explanations for these altered experiences.

Furthermore, it has been suggested that in chronic schizophrenia progressively enhanced susceptibility to psychotic state and relapse occurs. Sensitization of the endogenous mesolimbic dopaminergic system, triggered by environmental stressors or repeated stimulation with agonistic drugs, may be the underlying mechanisms in this acquired susceptibility (Brake *et al.* 1997; Glenthoj *et al.* 1997; Ujike, 2002). In the present study, the deviant DA/NA-mediated response in patients may reflect the effect of acquired sensitization, whereas first-degree relatives may either have escaped exposure to environmental factors that induce this kindling phenomenon, or may not have a sufficiently elevated level of vulnerability to develop the kindling phenomenon in the first place.

There was a blunted cortisol response to metabolic stress in patients compared to controls. In relatives, the increase in plasma cortisol level during the 2-DG condition did not significantly differ from that in controls. These results suggest that an altered neuroendocrine stress response (dysfunctioning hypothalamic pituitary–adrenal axis) is associated with the clinical state of schizophrenia. There is other evidence for diminished cortisol changes in patients with schizophrenia in response to different types of stressors. For instance, in a study using lumbar puncture as the stressor (Breier *et al.* 1988), patients with schizophrenia did not show an increase in plasma cortisol concentration, whereas depressed patients and controls did. In addition, a blunted cortisol response in schizophrenia has been found during a psychological stress task (Albus *et al.* 1982; Jansen *et al.* 1998, 2000). On the other hand, a number of studies using the metabolic stress paradigm failed to show significant differences between patients and controls with regard to cortisol changes (Breier & Buchanan, 1992; Breier *et al.* 1993; Elman *et al.* 1998). Inconsistencies in the results as regards cortisol response to metabolic stress may be due to methodological issues (sample size, statistical procedures) or chance. The important positive conclusion that can nevertheless be drawn is that the DA/NA mediated response to metabolic stress is dissociated from the HPA/cortisol response, an independent replication of the findings reported by Breier (Breier *et al.* 1993).

In conclusion, increased HVA response to metabolic stress, dissociated from the concurrent cortisol response, may reflect dysfunctional adaptation of a centrally regulated stress response to certain environmental demands placed on the individual. This abnormality may put individuals at risk of developing psychotic symptoms. Although our experimental design allowed precise control of dosage and timing of the environmental exposure, the ecological validity is necessarily low. Future research should focus on the possible natural environmental stressors that impact on this biological vulnerability in patients.

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