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Effects of Acute Metabolic Stress on Striatal Dopamine Release in Healthy Volunteers

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Several lines of evidence indicate that a variety of metabolic stressors, including acute glucose deprivation are associated with dopamine release. Pharmacologic doses of the glucose analogue, 2-deoxyglucose (2DG) cause acute glucoprivation and are associated with enhanced dopamine turnover in preclinical studies. In this study, we utilized [^{11}C]raclopride PET to examine 2DG-induced striatal dopamine release in healthy volunteers. Six healthy volunteers underwent PET scans involving assessment of 2DG-induced (40 mg/kg) decrements in striatal binding of the D_2/D_3 receptor radioligand [^{11}C]raclopride. Decreases in [^{11}C]raclopride specific binding reflect 2DG-induced changes in synaptic dopamine. Specific binding significantly decreased following 2DG administration, reflecting enhanced synaptic dopamine concentrations ($p = .02$). The administration of 2DG is associated with significant striatal dopamine release in healthy volunteers. Implications of these data for investigations of the role of stress in psychiatric disorders are discussed.

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Several lines of evidence suggest that dopamine is associated with mechanisms underlying the neurobiologic response to stress. In preclinical studies, increased dopamine turnover and stress-induced striatal dopamine release have been associated with a variety of stress paradigms including restraint, foot and tail shock, and exhaustion (Heyes et al. 1988; Dunn 1988; Abercrombie et al. 1989; Carlson et al. 1991; Keefe et al. 1993; Chrapusta et al. 1997). In humans, plasma concentrations of the dopamine metabolite, homovanillic acid (HVA) have been found to be increased with "examination stress" (Rauste-von Wright and Frankenhaeuser 1989) and physical activity (Kendler et al. 1983), though not with other stressors, including continuous arithmetic addition (Sumiyoshi et al. 1998). Using a video game paradigm, Koepp et al. (1998) demonstrated striatal dopamine release with psychological stress in healthy volunteers.

Glucose deprivation provides a method to measure the effects of metabolic stress on neurophysiology, including dopamine release. In preclinical studies, hypoglycemia was associated with varied neurophysiological effects, including increased cerebral blood flow (CBF) (Bryan et al. 1994) and increased striatal concentrations of conjugated HVA (Cottet-Emard and Peyrin 1982). Several human volunteer studies have similarly found insulin-induced hypoglycemia to be associated with increased plasma HVA (Woolf et al. 1983) and increased CBF (Della Porta et al. 1964; Neil et al. 1987; Kerr et al. 1993; Tallroth et al. 1993). No studies, to date, have assessed the effects of acute glucose deprivation on cerebral dopamine function with human subjects, in

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part because of methodological limitations with regard to directly assessing in vivo dopamine turnover in human brain as well as reliably and safely inducing metabolic stress in human volunteers.

2-Deoxyglucose (2DG) administration provides a useful alternate paradigm with which to study the effects of glucoprivation. 2DG is a glucose analog that is actively transported into cells via the same cellular mechanisms as those used to absorb glucose. In the cell, 2DG is initially metabolized through a common glucose pathway and is phosphorylated by hexokinase to 2-deoxyglucose-6 phosphate (2DG-6-P). 2DG-6-P is not further metabolized, however, and accumulates intracellularly. High concentrations of 2DG-6-P then inhibit glucose-6-phosphate isomerase, blocking glucose oxidation and stimulating a hypoglycemic-like response (Wick et al. 1957; Tower 1958; Horton et al. 1973).

Preclinical and clinical studies show 2DG to increase cerebral blood flow (CBF) to multiple cortical and subcortical regions (Breier et al. 1993a; Elman et al. 1999). Pharmacologic doses of 2DG produce a consistent stress response in healthy volunteers, including robust elevations of plasma cortisol, ACTH, and epinephrine levels, as well as behavioral concomitants of heightened anxiety (Goldstein et al. 1992; Breier et al. 1992; Elman et al. 1998). Further, 2DG-induced elevations in dopamine have been indirectly demonstrated in healthy volunteers by increased plasma HVA (Breier et al. 1993b).

In several recent studies, we and others have employed a PET technique to assess the effects of pharmacological agents (Breier et al. 1997, 1998; Smith et al. 1998) or mental stress (Koepp et al. 1998) on striatal dopamine release in vivo. This method involves utilizing the dopamine D_2/D_3 radioligand [^{11}C]raclopride to determine changes in specific binding, reflecting increases in striatal dopamine release induced by a pharmacological or psychological intervention. This technique was validated using pharmacological agents that affect dopamine release (e.g., amphetamine) in nonhuman primates (Breier et al. 1997).

The purpose of this pilot study was to use this [^{11}C]raclopride/PET displacement method to measure 2DG-induced striatal dopamine release in healthy volunteers. We hypothesized that 2DG would induce significant striatal dopamine release.

MATERIALS AND METHODS

Subjects

Six healthy male volunteers (mean age = 33.2 years, SD = 5.1) participated in this 2DG/[^{11}C]raclopride study. The healthy volunteers were recruited from the NIH healthy volunteer office and gave consent to this Institutional Review Board (IRB)-approved protocol. Volunteers were found to be free of psychiatric disorders on clinical examination and on a Structured Clinical Interview (Spitzer et al. 1990; First et al. 1997). Subjects were in good health and underwent a medical evaluation that included screening blood work and an EKG. A structural MRI was obtained with each subject to rule out anatomic abnormalities.

Clinical Protocol and Pharmacological Infusions

A bolus of 40 mg/kg of 2DG was administered forty minutes after commencement of [^{11}C]raclopride infusion. The dose of 2DG was selected based on previous clinical studies that demonstrated this dose produces a consistent stress response including increased cortisol and ACTH, as well as being safe and well tolerated (Breier 1989; Elman and Breier 1997, Elman et al. 1998, 1999). Behavioral responses were examined in healthy volunteers with a self-reporting anxiety visual analog scale consisting of a demarcated line. Subjects made a vertical intersecting mark at a point on the line to indicate the degree of anxiety experienced. The rating instrument was explained by a research psychiatrist and self-ratings were done for baseline (before the start of the PET scan), peak behavioral effects, and 60 minutes after completion of the scan (120 minutes after administration). Subjects rated their peak 2DG-induced anxiety following completion of the scan.

PET Scanning Protocol

Studies were conducted on a General Electric Advance scanner at the NIH Clinical Center. Acquisitions were done with the interplane septa retracted and a wide axial acceptance angle. Each scan yielded 35 planes 4.25 mm apart. The effective resolution of the reconstructed images was 6 mm both axially and in-plane. Transmission scans were performed using two rotating ^{68}Ge sources and were used for attenuation correction.

Subjects were positioned in the scanner such that acquired planes would be parallel to the orbital-meatal line. Head movement was minimized with individually fitted thermoplastic masks. Patches were applied over the orbits to reduce incoming light. [^{11}C]raclopride (3.3 to 8.0 mCi) was administered as a bolus followed by a constant infusion over 100 min. The bolus dose was 57% of the total amount administered. Beginning with the [^{11}C]raclopride bolus, 27 scans were acquired over the 100 min. period.

By infusing the [^{11}C]raclopride, near-equilibrium conditions can be reached before administration of a pharmacologic agent, allowing a direct measurement of the binding potential from the ratio of striatum/cerebellum-1. In previous studies in monkeys, equivalent specific binding values were found using the conventional bolus methods and the bolus/infusion technique (Carson et al. 1997). The use of the bolus/infusion para-

digm allows the measurement of baseline binding and change in dopamine concentration in a single scan without the need for intrascan blood sampling (Carson et al. 1997). In addition, this paradigm facilitates interpretation of post-2DG changes in the curve (Endres et al. 1997).

Image Data Processing and Statistical Analysis

Image processing was performed with MIRAGE software developed by the NIH PET center and all analysis was done by a single individual. Images corresponding to 0 to 5 minutes of raclopride infusion were added together to form a single "sum" image. Volumes of interest (VOIs) were drawn over the cerebellum and on the left and right striatum (caudate and putamen combined). After visual inspection, these VOI's were overlaid onto their corresponding position in each of the 31 individual scans and samples (mean pixel values) were generated for each VOI. Left and right striatal VOI's were averaged to a single striatal value. As noted, specific binding was calculated as follows: striatum/cerebellum-1.

Ratio data from five consecutive scans 20–40 minutes after the [^{11}C]raclopride bolus injection and immediately prior to 2DG administration ("baseline"), and five consecutive scans 75 to 100 minutes post-[^{11}C]raclopride bolus injection ("post-2DG") were averaged. The effects of 2DG on raclopride binding were assessed using paired t-tests to compare baseline and post-2DG specific binding.

The effects of 2DG on anxiety self-ratings were assessed using a single factor, repeated measures ANOVA that assessed the effects of time on rating scores. Simple uncorrected t-tests were used for post-hoc analyses between individual time points.

RESULTS

2DG administration induced a significant decrease in [^{11}C]raclopride specific binding from 2.78 ± 0.28 (baseline) to 2.63 ± 0.34 (post-2DG) ($t = 3.31$, $df = 5$, $p = .02$) (Figure 1). Average percent change in specific binding between baseline and post-2DG was 5.49%.

There was a significant time effect for self-ratings of anxiety on the visual analog scale ($F = 9.32$, $df = 2$, $p < .01$) (Figure 2). Post-hoc t-tests showed ratings during drug to be significantly greater than either before or a lengthy period after 2DG administration.

Changes in specific binding were not significantly correlated with the increase in anxiety self-ratings on the visual analog scale (Spearman $r = 0.23$, $p = .33$). 2DG-induced decrements in specific binding did not correlate with subject age (Spearman $r = -0.32$, $p = .54$) and were not related to baseline binding (Spearman $r = -0.31$, $p = .54$).

DISCUSSION

The results of this study demonstrated that glucoprivic stress induced by 2DG administration is associated

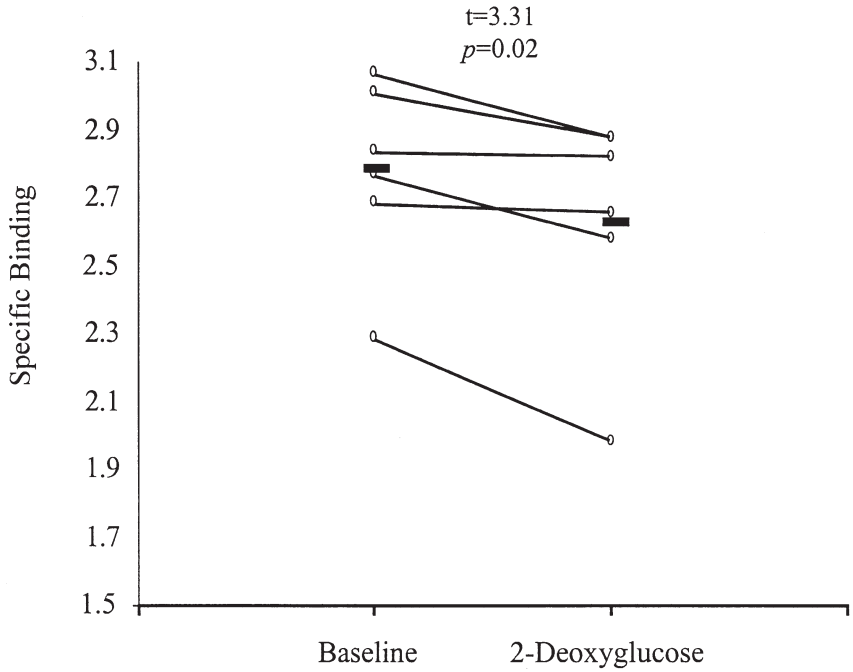

Figure 1. The effects of 2-deoxyglucose on [^{11}C]raclopride striatal specific binding (striatum/cerebellum-1) in healthy volunteers ($n = 6$).

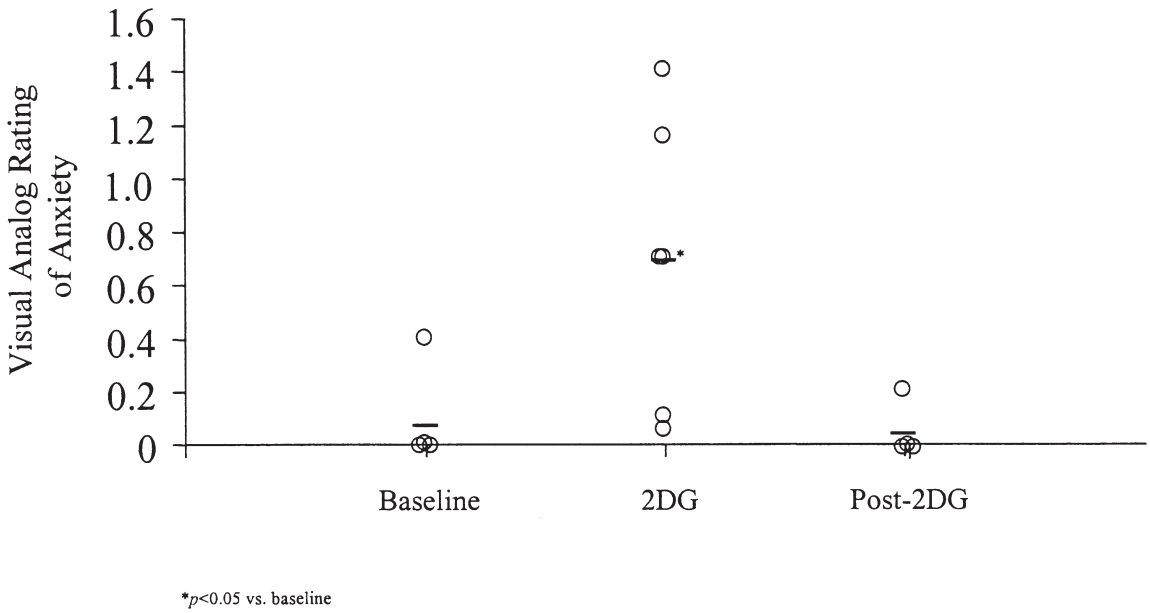

Figure 2. The effects of 2-deoxyglucose (2DG) on anxiety self-rating on a visual analog scale ($n = 6$).

with increased experience of subjective anxiety measured by a visual analog scale, as well as reductions in [^{11}C]raclopride specific binding in healthy volunteers, probably reflective of 2DG-induced striatal dopamine release. Our results are consistent with previous studies indicative of increased dopamine turnover, as measured by indirect peripheral indices, with glucoprivic stress in healthy volunteers (Cottet-Emard and Peyrin 1982; Woolf et al. 1983; Breier et al. 1993b). Our findings are also consistent with observations of striatal dopamine release in healthy volunteers using a very different, psychological, stress paradigm (Koepp et al. 1998).

The magnitude of decrements in specific binding associated with 2DG administration is somewhat lower than we have previously observed with either amphetamine (15.5%) (Breier et al. 1997) or the NMDA antagonist, ketamine (11.3%) (Breier et al. 1998), implying that while glucoprivic stress stimulates striatal dopamine release, it does not do so as robustly as either direct dopaminergic or indirect glutamatergic pharmacologic stimulation. Observations that stress activates brain catecholamine systems (Thierry et al. 1968; Dunn 1988; Roth et al. 1988; Kalén et al. 1989; Nisenbaum et al. 1991) and increases levels of excitatory amino acids (Moghaddam 1993), suggest several possible alternate mechanisms for 2DG-induced striatal dopamine release. Further studies will be necessary to identify specific pathways activated by neural glucoprivation. The prominent variability in baseline and post-2DG specific binding is consistent with previous studies utilizing this technique to study the effects of amphetamine and ketamine.

A few caveats need to be considered in interpreting our data. The degree to which 2DG-related glucoprivation is comparable to other types of stress is not yet entirely clear. While 2DG administration did induce anxiety measured with a self-rating scale, increased anxiety with 2DG did not correlate with degree of striatal dopamine release. While sensitive, the self-rating scale may be susceptible to influence by expectations. Moreover, the necessity of requiring subjects to recall their peak anxiety experience after the scan was complete may have furthered affected findings. Nonetheless, 2DG appears to influence many neurophysiological systems in ways that are similar to the effects of environmental stress. The lack of correlation may be related to the small sample. Further, the small sample size and single gender of the study population raise separate issues of generalizability to the population as a whole. While our findings should be considered preliminary, the power was sufficient to detect significant effects with 2DG administration. Another issue is the potential effect of a 2DG-induced increase in cerebral blood flow on determination of specific binding. While Elman et al. (1999) observed increased basal ganglia blood flow to be associated with 2DG administration, blood flow peaked at 20 minutes after administration. By 40 minutes after administration, cerebral blood flow was returning to normal and by 60 minutes was essentially at baseline. Time points used to measure post-2DG specific binding in this study were obtained from 35 to 60 minutes after 2DG administration when any putative increase in cerebral blood flow was returning to normal. Moreover, the raclopride bolus/infusion methodology employed here is relatively resistant to blood flow changes (Logan et al. 1994; Carson et al. 1997; Endres et al. 1997).

These data suggest that the [^{11}C]raclopride displacement paradigm may be a useful tool in broadening our understanding of physiological and behavioral responses to acute stress. Moreover, these data may provide a neurophysiological underpinning for observations that some psychiatric populations may be particularly sensitive to environmental stress (Gruen and Baron 1984; Hultman et al. 1997). Pathologic response to stress in schizophrenic patients might be related to hypothesized decrements in tonic striatal dopamine release in this population leading to stimulus-induced supranormal dopamine release (Grace 1991). Breier's (1993b) findings that 2DG administration is associated with greater increases in plasma HVA levels in schizophrenic patients than in healthy controls are consistent with this suggestion.

Our preliminary findings demonstrate that 2DG-induced glucoprivation is associated with a change in striatal dopamine synaptic concentration in healthy volunteers. Further studies comparing these findings with 2DG-induced dopamine changes in cohorts of psychiatric patients might help to clarify the importance of striatal dopamine pathways in the pathological response of some psychiatric patients to stress, particularly in illnesses such as schizophrenia for which dopamine dysregulation is thought to play an important role.

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