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BASAL LOCAL CEREBRAL GLUCOSE UTILIZATION IS NOT ALTERED AFTER BEHAVIORAL SENSITIZATION TO QUINPIROLE

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Abstract

Sensitization to psychostimulants results in a behavioral response of a greater magnitude than that produced by a given single dose. Previously, we have shown that sensitization to the D₂/D₃ dopamine receptor agonist quinpirole produces alterations in quinpirole-stimulated local cerebral glucose utilization (LCGU) in ventral striatal and limbic cortical regions. To determine whether basal neuronal activity is altered in the sensitized animal, this study examined the effects of a sensitizing course of quinpirole on basal neuronal activity using the [¹⁴C]-2-deoxyglucose (2-DG) method in rats with verified sensitization. Adult, male Long-Evans rats (n = 7 or 10/group) were subjected to 10 injections of quinpirole (0.5 mg/kg, s.c.) or saline administered every 3rd day. Sensitization was verified on the basis of locomotor activity. The 2-DG procedure was performed in freely moving rats three days after the last quinpirole injection. LCGU was determined by quantitative autoradiography. No alterations in basal LCGU were detected in quinpirole-sensitized rats compared to those treated with saline. The present finding suggests that either the basal activity of very discrete populations of neurons is affected by sensitization to quinpirole or that the neurobiological changes that result in the sensitized behavioral response affect only stimulated, but not basal, neuronal activity.

Keywords

Sensitization; quinpirole; local cerebral glucose utilization; psychostimulant; [¹⁴C]2-deoxyglucose

Introduction

With repeated, intermittent administration, dopaminergic psychostimulants, such as the indirect agonists amphetamine and cocaine, or the direct agonists apomorphine, bromocriptine, and quinpirole, produce behavioral responses of greater magnitude than an acute dose; a phenomenon referred to as sensitization [24]. A long-lasting phenomenon, sensitization is hypothesized to contribute to the development of addiction and is believed to underlie the development of drug craving [5,27]. By an analogous process, sensitization to stimuli such as stressors, electrical stimuli, or chemicals is proposed to contribute to the etiology of neuropsychiatric disorders such as mania, multiple chemical sensitivity, obsessive-compulsive disorder (OCD), panic disorder, post-traumatic stress disorder, and psychosis [8,20,25,27,28, 31]. The specific mechanisms underlying sensitization remain to be fully determined; however,

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functional changes in the “motive circuit”, as well as changes in glutamatergic transmission, appear to be involved (for review: [19,30,35–37]).

Quinpirole is a highly specific direct dopamine agonist at D₂ and D₃ dopamine receptors [14, 33] that produces a sensitized locomotor response in rats. In addition to providing a model in which to study the specific contribution of D₂-like dopamine receptors to behavioral sensitization, the quinpirole-sensitized rat is also of interest because these animals exhibit “checking” behaviors similar to those observed in humans with OCD, and thus, represent a putative animal model of the disorder [31]. Previously, we have reported quinpirole-stimulated decreases in local cerebral glucose utilization (LCGU) in quinpirole-sensitized rats in brain regions associated with the “motive circuit”, such as the nucleus accumbens and limbic cortical regions [2,21]. However, one key feature of psychostimulant sensitization is that it is manifested behaviorally after a period of drug abstinence, suggesting that one or more aspects of basal neurobiology are altered by the previous drug exposure that facilitates the exaggerated behavioral response upon drug administration. Accordingly, this study examined basal neuronal activity in rats with verified behavioral sensitization at a time point when administration of quinpirole would be anticipated to elicit a sensitized behavioral response.

Materials and Methods

Research was performed in compliance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

Adult, male, Long-Evans rats (180–200 g; n=7 or 10 per group; Harlan, Indianapolis, IN) were treated with a sensitizing course of quinpirole, evaluated for the presence of sensitization, and LCGU determined as previously described in detail [2]. Briefly, rats were treated with 10 injections of quinpirole (0.5 mg/kg, s.c.; Sigma, St. Louis, MO) or saline vehicle, administered every 3rd day, based on the procedures of Culver and Szechtman [4]. Immediately after each injection, rats were placed in plastic boxes (30 × 56 × 30 cm) marked with a grid (3 × 4 cells). To verify sensitization, locomotor activity was quantified by direct observation on the basis of line crossings and rearings counted by a blinded observer for the 45-min period beginning 60 min after drug administration, which corresponds to the time period when quinpirole produces the greatest locomotor activation [6,34]. Two days after the 10th quinpirole injection, the femoral artery and vein were cannulated under pentobarbital anesthesia and rats were allowed to recover overnight. The 2-deoxyglucose (2-DG) procedure of Sokoloff and colleagues [29] was performed the next day, when administration of quinpirole would be anticipated to elicit a sensitized behavioral response. To ensure that all conditioned and environmental cues associated with the sensitized behavior were present during the assessment of basal LCGU, rats were injected with saline and placed in the locomotor apparatus. The [¹⁴C]2-DG was administered 60 min later to the freely moving rats. Rats were decapitated 45 min later. LCGU in specific brain areas was determined by quantitative autoradiography in 30 brain regions representing a survey of fore-, mid-, and hind brain regions, with emphasis on limbic structures, and expressed as $\mu\text{mol glucose}/100 \text{ g tissue}/\text{min}$ using NIH Image v. 1.6. Overall LCGU was calculated as the mean LCGU for all regions examined. Results are presented as the mean $\pm$ S.E.M.

Data were analyzed for statistically significant effects by 1- or 2-way ANOVA (Systat, 10.2) as appropriate, followed by post-hoc analysis with the Student-Newman-Keuls multiple comparisons test (GraphPad, Instat 3). Effects on overall LCGU were tested using Student's t-test. Significance was set at $P < 0.05$.

Results

Acute treatment with quinpirole produced a significant increase in locomotor activity over saline control. After 10 injections, quinpirole produced a 3.5-fold increase ($P < 0.05$) in locomotor activity compared to the initial dose, thus confirming sensitization (Figure 1).

In control rats, LCGU in the 30 brain regions examined was similar to previous reports [29]. Two-way ANOVA with factors of treatment and brain regions indicated significant main effects of treatment ($F(1,432) = 14.5$, $P < 0.0001$) and brain region ($F(29,432) = 46.4$, $P < 0.0001$); however, overall LCGU was not different between groups (Table 1). There was no interaction of treatment and brain region ($F(1,29) = 0.734$, $P = 0.834$). Post-hoc analysis using the Student-Newman Keuls multiple comparisons test indicated no significant differences in basal LCGU between quinpirole-sensitized rats and saline-treated controls in any brain region (Figure 2).

Discussion

The 2-DG procedure of Sokoloff et al. [29] measures net glucose uptake, which correlates with neuronal activity, in a sampled neuronal population. Using the 2-DG method, we have shown differences in quinpirole-stimulated LCGU between quinpirole-sensitized and drug naïve rats in the nucleus accumbens, limbic cortical areas, and other limbic regions, supporting the involvement of these regions in the sensitized response [2,21,22]. Because neurobiology must be altered at the time of drug administration in order for a sensitized response to be produced, this study examined the effects of a sensitizing course of quinpirole on basal neuronal activity as assessed on the basis of LCGU.

In contrast to the quinpirole-stimulated effects on LCGU in quinpirole-sensitized rats described in our previous studies [2,21,22], no alterations in basal LCGU were detected in rats with demonstrated sensitization at a time point when quinpirole would have produced a robust sensitized locomotor response. This lack of effect was observed despite the presence of conditioned and environmental cues associated with the sensitization treatment and a confirmed sensitized behavioral response after the 10th quinpirole injection. In agreement with the present findings, 14 consecutive days of amphetamine treatment (5 mg/kg) plus a 5–7 hour abstinence period did not produce any significant decreases in LCGU [18]. However, alterations in basal neuronal activity were reported in other studies of amphetamine- or cocaine-induced sensitization, though the brain regions affected were highly variable depending on drug (amphetamine or cocaine), dose (1, 5, or 10 mg/kg or 94.6 mg/kg/pellet), treatment paradigm (4 or 14 consecutive days), and/or abstinence period (20 hours, 3 days, or 7 days) [1,3,32]. The discrepancies between the present study and those that found alterations in basal LCGU are probably due to differences in the mechanisms of action between the direct and selective D₂/D₃ agonist quinpirole and the indirect agonists, amphetamine and cocaine, which affect serotonergic and noradrenergic systems in addition to the dopamine system, where amphetamine and cocaine affect not only the D₂ family of receptors but also the D₁ family of receptors [10]. The more specific actions of quinpirole might result in effects that are more subtle, and consequently less likely to be detected by a technique that measures the net changes in neuronal activity in the regions examined. Differences in the dosing schedule, route of administration, rat strain, post-anesthesia recovery time prior to the start of the 2-DG procedure, and use of freely-moving or restrained rats during the 2-DG procedure may also contribute to differences between these studies.

A variety of neurobiological alterations in the dopaminergic, glutamatergic, and other systems have been found after sensitization to amphetamine [35–37]. However, a lack of effect of amphetamine sensitization on basal dopamine content, neurotransmission, or receptor density

has also been reported in a number of studies similar to that observed in this study, even though sensitized behavioral effects were observed when a challenge dose was given [9,11–13,15–17,23,25,26]. Likewise, although some behavioral effects of quinpirole-sensitization, such as more repetitive travel in the open field and more perseveration during extinction in the water maze, persist for as much as 3 months after the last administration of quinpirole, other behavioral effects, such as increased overall activity level in the open field, do not [7]. These observations suggest that the long-term effects of a sensitizing course of quinpirole on undrugged behavior and basal neurobiology are subtle rather than robust, which may make any underlying differences in neuronal activity difficult to detect.

In conclusion, the present findings suggest that a quinpirole challenge is necessary to produce detectable alterations in neuronal activity using the 2-DG method after quinpirole-induced sensitization. Clearly, changes in basal neurobiology must underlie the sensitized behavioral response; however, changes in LCGU are not detectable in rats with verified sensitization to quinpirole suggesting that either the basal activity of very discrete populations of neurons is affected by sensitization or that the neurochemical, neurophysiological, or other changes that result in the sensitized behavioral response affect only stimulated, but not basal, neuronal activity.

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Fig. 1. Effects of sensitizing treatment with quinpirole on locomotor activity

Rats were given 10 injections of quinpirole (0.5 mg/kg, s.c.; n = 10) or saline (n = 7) administered every 3rd day. Data represent the mean $\pm$ SEM. *Different from saline, same injection, $P < 0.05$. †Different from injection 1, $P < 0.05$.

Fig. 2. Effects of quinpirole-sensitization on basal LCGU in specific brain regions

Rats were given 10 injections of quinpirole (0.5 mg/kg, s.c.; $n = 10$) or saline ($n = 7$) administered every 3rd day. The 2-DG procedure was initiated 60 minutes after an injection of saline administered 3 days after the last quinpirole injection. Brain regions are arranged in rostro-caudal order. Data represent the mean $\pm$ SEM. No statistically significant differences were detected.

Table 1

Effects of sensitization to quinpirole on overall basal local cerebral glucose utilization.

LCGU ($\mu\text{mol}/100\text{ g tissue}/\text{min}$)	
Saline	111 $\pm$ 4.5
Quinpirole	105 $\pm$ 4.5

Rats were treated with 10 injections of quinpirole (0.5 mg/kg, s.c.; n = 10) or saline (n = 7) administered every 3rd day. Data represent the mean $\pm$ SEM. Overall LCGU was calculated as the mean LCGU for all brain regions examined. Treatment groups were not different by Student's t-test.