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BEHAVIORAL RESPONSES TO INJECTIONS OF MUSCIMOL INTO THE SUBTHALAMIC NUCLEUS: TEMPORAL CHANGES AFTER NIGROSTRIATAL LESIONS

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Abstract—Changes in cellular activity in the subthalamic nucleus are a cardinal feature of Parkinson's disease and occur in rodents after lesions of the nigrostriatal pathway, a model of Parkinson's disease. GABA-ergic neurons from the globus pallidus provide a major input to the subthalamic nucleus. Previous electrophysiological studies revealed temporal changes in the activity of pallidal neurons after nigrostriatal lesions in rats. However, little is known about the impact of these changes on GABAergic transmission in the subthalamic nucleus. We have examined the behavioral responses to a local administration of the GABA A agonist muscimol into the subthalamic nucleus. Muscimol (0.01 and 0.1 μg) induced orofacial dyskinesia in normal rats; this response was blunted 2 weeks but enhanced 2 months after a unilateral lesion of the nigrostriatal pathway. The early decrease in the behavioral response occurred at a time when increased expression of mRNA for glutamic acid decarboxylase, the enzyme of GABA synthesis, and burst firing have been reported in the globus pallidus, suggesting an adaptive post-synaptic response to increased GABAergic transmission in the subthalamic nucleus. In contrast, we now show that glutamic acid decarboxylase mRNA is unchanged in the globus pallidus at the later time point, when electrophysiological changes also subside in this region. The increased behavioral response at this later time point may reflect a decreased activity in GABAergic inputs to the subthalamic nucleus. The results show time-dependent changes in behavioral responses to GABA A receptor stimulation in the subthalamic nucleus which may reflect adaptive changes in postsynaptic inhibitory responses after dopaminergic lesions. © 2005 Published by Elsevier Ltd on behalf of IBRO.

Key words: globus pallidus, GABA, GABAA receptor, Parkinson's disease, 6-hydroxydopamine, basal ganglia.

Parkinson's disease is characterized by the progressive development of motor symptoms resulting from the degeneration of dopaminergic inputs from the substantia nigra pars compacta to the striatum. The cardinal symptoms include tremor, rigidity and akinesia, and develop over the course of several years (Narabayashi, 1995; Poewe and Wenning, 1998). A major consequence of dopaminergic lesions is an increase in firing in the subthalamic nucleus (Albin et al., 1989). This effect has been observed in parkinsonian patients (Hutchison et al., 1998), monkeys treated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Bergman et al., 1994), and rats with a lesion of the nigrostriatal pathway induced by 6-hydroxydopamine (6-OHDA; Ni et al., 2001). In patients and animal models of Parkinson's disease, ablation, inhibition or high frequency stimulation of the subthalamic nucleus markedly improve akinesia and rigidity, suggesting that alterations in this brain region are crucial for parkinsonian symptoms (Bergman et al., 1990; Benazzouz et al., 1993; Kumar et al., 1998; Baron et al., 2002; Dostrovsky et al., 2002).

Models of basal ganglia circuitry predict that the increased activity of subthalamic neurons after nigrostriatal degeneration is due at least in part to a decreased activity of the globus pallidus (external pallidum in primates), which provides a dense input of GABA-containing nerve terminals to the subthalamic nucleus (Albin et al., 1989; Smith et al., 1990). Data from both rats and monkeys show that nigrostriatal lesions cause a decrease in spontaneous activity in neurons of the external pallidum; the same studies, however, show a simultaneous increase in burst firing (Pan and Walters, 1988; Filion and Tremblay, 1991). Therefore, the net effect of nigrostriatal degeneration on pallido-subthalamic transmission remains unclear. Similarly, studies of changes in glutamic acid decarboxylase (Mr 67,000; GAD67) GAD mRNA expression in the globus pallidus, an index of GABAergic activity, have provided ambiguous results (Chesselet and Delfs, 1996).

Previous studies from our laboratory and others have shown increased levels of mRNA encoding GAD67, one of the rate-limiting enzymes of GABA synthesis, in neurons of the globus pallidus 2–3 weeks after lesions of the nigrostriatal pathway in rats (Soghomonian and Chesselet, 1992; Kincaid et al., 1992; Delfs et al., 1995). According to a recent study (Billings and Marshall, 2004), this increase occurs both in parvalbumin-positive and parvalbumin-negative neurons of the globus pallidus, but to a larger extent in the latter. An increase in GAD67 mRNA in the external pallidum was also observed in MPTP-treated cats and monkeys (Soghomonian et al., 1994; Schroeder and Schneider, 2001). Another study, however, found no change in GAD67 mRNA in the external pallidum of MPTP-treated monkeys and in postmortem human tissue

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Abbreviations: ANOVA, analysis of variance; BTCP, ^{3}H-*N*-[1-92-benzo(*b*)thiophenylcyclohexyl]-piperidine; GAD67, glutamic acid decarboxylase (Mr 67,000); MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; 6-OHDA, 6-hydroxydopamine.

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(Herrero et al., 1996). These differences may be due to time-dependent changes in the pallido-subthalamic pathway following nigrostriatal degeneration. Indeed, Pan and Walters (1988) showed that the burst firing activity measured in the globus pallidus of rats peaked 1 week after lesions of the nigrostriatal pathway and decreased with time, returning to control levels by 6–9 weeks post-lesion. These data suggest that neuronal activity in the pallido-subthalamic pathway may be temporally regulated after acute nigrostriatal lesion.

Changes in GABAergic inputs can induce compensatory alterations in post-synaptic receptors that influence the response of neurons to GABAergic agonists (Penney and Young 1981; Brussaard and Herbison, 2000). The present study was designed to gain insight into the temporal changes of GABAergic transmission in the subthalamic nucleus after acute dopaminergic lesions in rodents. Dyskinesia induced by the local administration of the GABA-A receptor agonist muscimol into the subthalamic nucleus were examined 2–3 weeks and 8–9 weeks after unilateral lesions of the nigrostriatal dopaminergic neurons in adult rats. In addition, levels of expression of GAD67 mRNA in the globus pallidus were examined with quantitative *in situ* hybridization histochemistry and emulsion autoradiography at the later time point.

EXPERIMENTAL PROCEDURES

Animals

A total of 55 male Sprague–Dawley rats (270–310 g; Charles River, Wilmington, MA, USA) separated in four cohorts were used in this study. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and all efforts were made to reduce the number of animals. All experimental protocols were approved by the University of California Los Angeles animal care committee.

Surgery

Prior to surgery, rats were maintained on a 12-h light/dark schedule with food pellets and water *ad libitum*. Rats were anesthetized with equithesin (prepared as per instruction of Janssen-Salbutry Laboratories, Kansas City, MO, USA; 3.0 ml/kg, i.p.) and received an unilateral infusion of either 6-OHDA (8 μg/4 μl) or vehicle (4 μl 0.1% ascorbic acid in 0.9% saline) into the left substantia nigra pars compacta (AP=+3.4 mm, ML=+2.0 mm, both relative to interaural zero, and DV=7.4 mm below the surface of the cortex; Paxinos and Watson, 1998). Thirty minutes prior to surgery, all rats were pretreated with desipramine (25 mg/kg, i.p.) to prevent the uptake of 6-OHDA into noradrenergic neurons.

For delivery of muscimol into the subthalamic nucleus, rats were implanted with a guide cannula for unilateral microinfusion of drug into the left subthalamic nucleus (AP=+4.9 mm, ML=+2.5 mm, both relative to interaural zero, and DV=7.4 mm below the surface of the cortex; Paxinos and Watson, 1998). Details of the surgical procedure and injection equipment have been described previously (Parry et al., 1994). Briefly, the guide shafts were made from 22-gauge cannulae cut to a length of 17 mm and the injector was made of a piece of fused silica (o.d.=150 μm; i.d.=75 μm) threaded through a 28-gauge internal cannula such that the tip of the fused silica extended 1 mm from the end of the internal cannula shaft. In rats with prior lesion of the nigrostriatal pathway and their sham-operated controls, this procedure was done 12 days after the first surgery. In all cases, behavioral testing began 1 week after implanting the cannula.

Drug administration and behavioral testing

No behavioral testing was conducted to assess the level of nigrostriatal lesion to avoid any confounding effect on the behavioral analysis of muscimol effects. Loss of dopaminergic nerve terminals was determined histologically at the end of the experiment as described below.

In a first cohort of rats (cohort I; n=6), vehicle or muscimol at the concentrations of 0.001, 0.01, 0.1, 1.0 μg were injected into the subthalamic nucleus following a Latin-square design so that each rat received all the doses and vehicle, in a random order. Testing was done every 4 days. For cohort II (n=16) and cohort III (n=16), each rat was treated only once, either with muscimol or vehicle. Cohort II and cohort III were examined 2–3 and 8–9 weeks post-lesion, respectively, to avoid any confounding effect of prior treatment.

On the day of behavioral testing, each rat was adapted to a quiet room in a clear plastic cylindrical chamber (30 cm diameter×46 cm high) for 60 min prior to muscimol (or vehicle) infusion into the subthalamic nucleus. Immediately prior to the onset of behavioral observations, the awake rat was gently hand-held and muscimol (or vehicle) was infused directly into the subthalamic nucleus via an injector placed within the surgically implanted guide cannula. A volume of 0.1 μl was infused over 56 s. The injector was left in place for an additional 60 s to permit diffusion of the drug away from the injector tip.

Following the local administration of drugs into the subthalamic nucleus, the rats were returned to the testing chamber and observed continuously for 60 min for bouts of orofacial movements by an observer blind to the experimental conditions. Two rats were observed during each observation period and the observer sat approximately four feet from the observation chambers. An oral bout was defined as any combination of continuous, large amplitude non-directed orofacial movements including vacuous chewing, gaping, jaw tremor, and tongue protrusion. To improve the reliability of quantification, only the number of oral bouts, not the type of oral movement, was recorded as in our previous studies (Parry et al., 1994; Eberle-Wang et al., 1996; Mehta et al., 2000). Those oral movements that were directed toward an object or purpose, such as grooming or ingestion, were not counted. The number of oral bouts was measured over the 60 min observation period. The duration of each bout could not be accurately measured by visual observation and was not included in the analysis.

Drugs

Muscimol (MW=114.1 g/mol) was dissolved in saline. Desipramine (MW=302.8 g/mol) was dissolved in d_2H_2O. 6-OHDA (MW=250.1 g/mol) was dissolved in 0.1% ascorbic acid in 0.9% saline. All drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA). The pH of all solutions was in the neutral range.

Histology

All rats were anesthetized with equithesin (prepared as per instruction of Janssen-Salbutry Laboratories) and killed by decapitation. Brains were removed, rapidly frozen in powdered dry ice, and stored at −80 °C. Coronal sections (20 μm thick) were cut through the substantia nigra pars compacta and subthalamic nucleus with a cryostat (Leica CM1800; McBain Instruments, Chatsworth, CA, USA). Tissue sections from each brain were fixed for 30 min in 4% formaldehyde and stained with Cresyl Violet for verification of lesion of the substantia nigra pars compacta and cannula placement in the subthalamic nucleus. Only data from rats in which accurate cannula placement was verified were included in the statistical analysis (Fig. 1).