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Chronic dopamine depletion augments the functional expression of K-ATP channels in the rat subthalamic nucleus

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Abstract

Symptoms of Parkinson's disease caused by dopamine depletion are associated with burst firing in the subthalamic nucleus (STN). Moreover, regularization or suppression of STN neuronal activity is thought to improve symptoms of Parkinson's disease. We reported recently that *N*-methyl-D-aspartate (NMDA) receptor stimulation of rat STN neurons evokes ATP-sensitive K⁺ (K-ATP) current via a Ca²⁺- and nitric oxide-dependent mechanism. The present studies were done to determine whether or not K-ATP channel function in STN neurons is altered in a model of chronic dopamine depletion. Brain slices were prepared from rats with unilateral dopamine depletion caused by intracerebral 6-hydroxydopamine (6-OHDA) injections. Whole-cell patch-clamp recordings showed that NMDA evoked more outward current at −70 mV and greater positive slope conductance in STN neurons located ipsilateral to 6-OHDA treatment compared to neurons located contralateral. Moreover, extracellular, loose-patch recordings showed that NMDA increased spontaneous firing rate in STN neurons in slices from normal rats, whereas NMDA produced a tolbutamide-sensitive inhibition of firing rate in STN neurons located ipsilateral to 6-OHDA treatment. These results show that K-ATP channel function in STN neurons is up-regulated by chronic dopamine deficiency. We suggest that K-ATP channel activation in the STN might benefit symptoms of Parkinson's disease.

Keywords

ATP-sensitive K⁺ channel; subthalamic nucleus; *N*-methyl-D-aspartate; sulfonylurea; tolbutamide; brain slice

1. Introduction

The tremor, rigidity and bradykinesia of Parkinson's disease are caused by progressive loss of substantia nigra dopamine neurons and chronic dopamine denervation of the basal ganglia. One consequence of chronic dopamine depletion is increased burst firing of neurons in the subthalamic nucleus (STN) [23]. Because the STN is an excitatory, glutamate-containing nucleus that innervates the major output nuclei of the basal ganglia, the STN exerts a powerful excitatory influence on basal ganglia output [15]. Moreover, abnormal firing in STN neurons correlates with symptoms of Parkinson's disease [4,22].

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Experimentally induced parkinsonism in animals is improved by lesioning the STN [5]. Deep brain stimulation of the STN also improves many symptoms of Parkinson's disease, most likely by interrupting the abnormal firing pattern in basal ganglia output neurons [10]. These studies support the hypothesis that either silencing or regularizing STN neuronal output improves symptoms of Parkinson's disease.

There is increasing interest in the ATP-sensitive K⁺ (K-ATP) channel as an inhibitory regulator of activity in central neurons. K-ATP channels are inwardly rectifying K⁺ channels that are coupled with a sulfonylurea receptor [1]. Although best characterized in the pancreas as regulators of insulin secretion, K-ATP channels are also widely distributed throughout the brain including the STN [6,8]. Using the 6-hydroxydopamine (6-OHDA) model of Parkinson's disease in rats, Wang et al showed that chronic dopamine depletion up-regulates the expression of K-ATP channel subunits in striatum and other brain regions [20]. These investigators also showed that systemic administration of the K-ATP channel opener iptakalim reversed the catalepsy and hypolocomotion induced by haloperidol treatment. Moreover, local injection of the K-ATP channel opener diazoxide into the basal ganglia has been shown to reduce akinesia and increase locomotion in the reserpine rat model of Parkinson's disease [11]. These reports support the hypothesis that K-ATP channel openers might be helpful in the treatment of Parkinson's disease.

We reported recently that K-ATP currents can be evoked in rat STN neurons by activation of *N*-methyl-D-aspartate (NMDA) receptors via a calcium- and nitric oxide-dependent mechanism [18]. Moreover, we showed that concurrent K-ATP channel activation attenuated the excitatory influence of NMDA on action potential generation in STN neurons recorded *in vitro*. In the present study, we used rats pretreated with unilateral, intracerebral injections of 6-OHDA to study the effects of chronic dopamine depletion on currents evoked by NMDA in STN neurons. Using patch pipettes to record currents and potentials in slices of rat brain, we found that dopamine depletion up-regulates the functional expression of K-ATP channels in the STN. We suggest that pharmacological modulation of K-ATP channels in the STN could potentially be beneficial in the treatment of symptoms of Parkinson's disease.

2. Materials and methods

All procedures were carried out in accordance with a protocol approved by the Institutional Animal Care and Use Committee of the Portland Veterans Affairs Medical Center. Sprague-Dawley rats (275–325 g, Harlan, Indianapolis, IN) were euthanized by severing thoracic vessels under isoflurane anesthesia. Horizontal slices containing midbrain and diencephalon (300 μ m thick) were cut with a vibratome in an ice-cold solution of the following composition (in mM): sucrose, 196; KCl, 2.5; MgCl₂, 3.5; CaCl₂, 0.5; NaH₂PO₄, 1.2; glucose, 20 and NaHCO₃, 25, equilibrated with 95% O₂ and 5% CO₂. A slice containing the STN was then placed on a supporting net and submerged in a continuously flowing solution (2 ml/min) of the following composition (in mM): NaCl, 126; KCl, 2.5; CaCl₂, 2.4; MgCl₂, 1.2; NaH₂PO₄, 1.2; NaHCO₃, 19; glucose, 11, gassed with 95% O₂ and 5% CO₂ (pH 7.4) at 36°C. Using a dissection microscope for visual guidance, the STN was located as grey matter approximately 2.7 mm lateral to the midline and 2 mm rostral to the center of the substantia nigra pars reticulata.

Some rats received unilateral intracerebral injections of 6-OHDA 7–8 weeks prior to preparation of brain slices. Under isoflurane anesthesia, rats received stereotaxic injections of 6-OHDA (2.75 mg/ml) into the right substantia nigra pars compacta (anteroposterior = +3.7; mediolateral = +1.2; dorsoventral = –8.0) and the right median forebrain bundle (anteroposterior = +4.2; mediolateral = +1.2; dorsoventral = –8.0) [16]. 6-OHDA infusions

were performed at a rate of 1 $\mu\text{l}/\text{min}$ using a 30-gauge infusion needle and a 10- μl Hamilton syringe that was connected to a constant infusion pump. To protect noradrenergic pathways, desipramine (25 mg/kg i.p.) was given 30 min before 6-OHDA infusions. Unilateral dopamine depletion was assessed by measuring rate of contralateral rotations in response to apomorphine (0.25 mg/kg, i.p.); only rats that turned more than five times per min were used for brain slice recordings.

Whole-cell recordings were made with pipettes (3–8 M Ω) that contained (in mM): potassium gluconate, 138; MgCl₂, 2; CaCl₂, 1; EGTA, 0.2; HEPES, 10; ATP, 0.5; GTP, 0.3 (pH 7.3). Membrane currents were recorded under voltage clamp and amplified with an Axopatch-1D amplifier (Molecular Devices, Foster City, CA, USA). Current-voltage (I–V) relationships were constructed by measuring currents during a series of hyperpolarizing voltage steps (400 ms duration) with 10 mV increments from a holding potential of –70 mV. Currents were measured immediately after capacitive transients to minimize the influence of hyperpolarization-activated cation currents. Slope conductance was calculated for each cell as the slope of a straight line in the I–V plot at voltages between –70 and –100 mV. Mean slope conductance and S.E.M. were obtained by averaging slope conductance from all cells. I–V plots are displayed as “subtracted” currents in which currents recorded during the experimental treatment were subtracted from control currents. Therefore, these subtracted currents represent “net” currents that were produced by the experimental treatment. Membrane potentials were corrected for the liquid junction potential (10 mV). In some experiments, we used the “loose-patch” method to record extracellular potentials [14]. Pipettes for loose-patch recordings were filled with standard extracellular solution; loose-patch recordings were made with a seal resistance typically 5–10 times the starting value of electrode resistance (3–8 M Ω). Data were acquired using a personal computer with a Digidata analog/digital interface and analyzed using pCLAMP software (Molecular Devices, Axon Instruments, Foster City, CA, USA). Currents and potentials were recorded continuously using a MacLab analog/digital interface, Chart software (AD Instruments, Castle Hill, Australia) and a Macintosh computer.

All drugs were dissolved in aqueous or dimethyl sulfoxide stock solutions and added to the slice superfusate at a dilution of 1:1000. Approximately 30 sec were required for the drug solution to enter the recording chamber; this delay was due to passage of the superfusate through a heat exchanger. NMDA, tolbutamide, desipramine, apomorphine, and 6-OHDA were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). Drug effects were analyzed using two-way analysis of variance (ANOVA) with repeated measures (RM) followed by the Holm-Sidak pairwise comparison test (SigmaStat, Jandel Scientific, San Rafael, CA, USA) unless stated otherwise. Numerical data in the text and error bars in figures are expressed as mean $\pm$ S.E.M.

3. Results

We recorded whole-cell currents evoked by NMDA in STN neurons that were located either ipsilateral or contralateral to previous 6-OHDA injections. Brain slices were superfused with NMDA (20 μM) for 3 min and recovery was recorded for at least 10 min after washout. As shown in the current traces in Fig. 1A, NMDA evoked a small inward current followed by a much larger outward current when recorded at –70 mV. However, outward currents evoked in STN neurons located ipsilateral to 6-OHDA treatment were significantly larger than those evoked in neurons located contralateral to 6-OHDA. On average, outward current in neurons located ipsilateral to 6-OHDA measured 147 ± 39 pA ($n = 10$), which was larger than the 8 ± 10 pA of outward current ($n = 10$) that was evoked at –70 mV in neurons contralateral to 6-OHDA treatment ($P < 0.01$, t test). In order to investigate the voltage-dependence of NMDA-evoked currents, I–V plots were constructed for “net” NMDA currents. As shown in

Fig. 1B, NMDA currents in neurons ipsilateral to 6-OHDA treatment exhibited a voltage dependence that was significantly different from those recorded in contralateral neurons ($F_{1,7} = 9.041$, $P < 0.001$, two-way ANOVA with RM). We also compared average slope conductances for NMDA-gated currents as measured between -70 and -100 mV. The average NMDA-dependent slope conductance in neurons ipsilateral to 6-OHDA (7.6 ± 1.6 nS) was significantly larger than that in contralateral neurons (1.7 ± 0.3 nS; $P < 0.01$, t test). Reversal potentials for NMDA-evoked currents in ipsilateral neurons (-88 ± 2 mV) were also significantly more hyperpolarized compared to those in contralateral neurons (-65 ± 8 mV; $P < 0.05$, t test). Both these average reversal potentials are much more hyperpolarized than the 0 mV that would be typical for NMDA ligand-gated ion channel currents in central neurons [3]. In our previous study, we reported an average reversal potential of -60 mV for NMDA-evoked current, which we showed to have a prominent contribution from K-ATP channels [18]. Due to a more hyperpolarized reversal potential and steeper conductance increase in ipsilateral versus contralateral neurons, these results are consistent with the hypothesis that NMDA evokes more K-ATP current in STN neurons in dopamine-depleted tissue.

In order to assess the effect of altered K-ATP function on neuronal excitability, we performed loose-patch recordings to measure the effects of NMDA ($20 \mu\text{M}$) on spontaneous firing rates in the presence and absence of tolbutamide ($100 \mu\text{M}$). STN neurons located ipsilateral to 6-OHDA treatment were compared to STN neurons in slices prepared from normal, untreated rats. When recording from STN neurons ipsilateral to 6-OHDA treatment, NMDA caused a reversible suppression of neuronal discharge, as illustrated in Fig. 2A. Figure 2A also shows that superfusing the slice with the K-ATP blocker tolbutamide converted the inhibitory effect of NMDA on firing rate to that of excitation. Summarized data in Fig. 2B show effects of NMDA on neurons ipsilateral to 6-OHDA treatment. Tolbutamide caused a significant change in the effect of NMDA on firing rate in dopamine-depleted slices ($F_{1,35} = 10.88$; $P < 0.001$, two-way ANOVA with RM). In the absence of tolbutamide, a five-minute application of NMDA reduced the frequency of spontaneous spikes from a control value of 8.3 ± 1.9 Hz to 2.5 ± 1.7 Hz ($n = 6$). In the presence of tolbutamide, however, the same concentration of NMDA greatly increased the firing rate from 8.4 ± 2.8 Hz to 16.7 ± 3.5 Hz in those same neurons. As a percentage change in firing rate, tolbutamide increased the NMDA-induced firing rate by 568%, from 2.5 Hz to 16.7 Hz. Pairwise comparison tests showed that tolbutamide produced significant changes in the response to NMDA beginning 110 seconds after starting NMDA superfusion ($P < 0.01$, Holm-Sidak test). These effects contrast with those obtained on STN neurons recorded in brain slices from normal, untreated rats, as shown in Fig. 2C. In control slices without tolbutamide, a five-minute application of NMDA increased the frequency of spontaneous spikes from a value of 9.9 ± 1.5 Hz to 18.7 ± 4.3 Hz after NMDA ($n = 8$). In the presence of tolbutamide, however, the same concentration of NMDA increased the firing rate from 12.1 ± 2.6 Hz to 33.1 ± 5.7 Hz in those same neurons. Two-way ANOVA with RM showed that the effect of tolbutamide significantly altered the effect of NMDA on spontaneous firing ($F_{1,35} = 5.392$, $P < 0.001$). As a percentage change in firing rate, tolbutamide increased the NMDA-induced firing rate by 77%, from 18.7 Hz to 33.1 Hz. Pairwise comparison tests showed that tolbutamide produced significant changes in the response to NMDA beginning 90 seconds after starting NMDA superfusion ($P < 0.01$, Holm-Sidak test). Although tolbutamide significantly increased the excitatory effect of NMDA in both experimental groups, the percentage increase in firing rate in the dopamine depleted slices was much greater than the increase seen in normal slices. Moreover, tolbutamide converted an inhibitory effect of NMDA to that of excitation in dopamine depleted slices. These results suggest that 6-OHDA treatment up-regulates the influence of K-ATP channels in the STN.

When recording in the presence of tolbutamide, Figures 2B & 2C also show that NMDA produced a smaller increase in firing rate in 6-OHDA-treated slices compared to slices from normal rats. Thus, there was a significant difference in NMDA-evoked firing rate (in tolbutamide) depending upon whether neurons were recorded in slices from normal, untreated rats or from neurons ipsilateral to 6-OHDA treatment ($F_{1,35} = 3.321$, $P < 0.001$; two-way ANOVA with RM). Five minutes after starting NMDA superfusion in the presence of tolbutamide, firing rate in neurons ipsilateral to 6-OHDA treatment had increased to 16.7 ± 3.5 Hz ($n = 6$), which was significantly slower than the 33.1 ± 5.7 Hz firing rate that was evoked by NMDA in normal slices ($n = 8$; $P < 0.05$, Holm-Sidak test). Nevertheless, the superfusion of tolbutamide-alone did not significantly alter the spontaneous firing rate of STN neurons in either normal slices ($F_{1,5} = 0.187$) or in slices ipsilateral to 6-OHDA treatment ($F_{1,5} = 0.549$). Furthermore, the average basal firing rate of STN neurons before NMDA or tolbutamide treatment in dopamine-depleted slices (8.3 ± 1.9 Hz, $n = 6$) was not significantly different from that in normal STN (9.9 ± 1.5 Hz, $n = 8$; $F_{1,5} = 0.822$). These results suggest that NMDA has a weaker excitatory effect in dopamine-depleted tissue compared to control STN. The fact that tolbutamide produced no significant change in firing rate before NMDA superfusion suggests that K-ATP channels are not tonically active at rest in either experimental group.

4. Discussion

In agreement with previous studies, the present study shows that NMDA evokes K-ATP currents in STN neurons in slices of rat brain [18]. However, the present study also shows that chronic dopamine depletion augments the inhibitory influence of K-ATP currents in the STN. These results agree with those of Wang et al who showed that unilateral 6-OHDA lesions increased the expression of K-ATP subunits in striatum and other brain regions in rats [20]. Although human studies failed to find altered sulfonylurea binding in striatum of Parkinson's disease subjects, the STN was not examined in that study [7]. Because the STN plays such an important role in the expression of symptoms of Parkinson's disease, we suggest that pharmacological manipulation of K-ATP channels in the STN could be explored as a possible way to manage these symptoms.

Because previous studies have shown that dopamine exerts an excitatory effect on STN neuronal activity [12,13,19,24,25], it might be surprising that the present study did not demonstrate a significant reduction in spontaneous firing rate in STN neurons from 6-OHDA-treated rats. It is possible that this lack of statistical significance may be due to the small number of observations in the current study. It is also interesting to note that when K-ATP channels were blocked with tolbutamide, we found that the excitatory effect of NMDA was significantly weaker in STN neurons ipsilateral to 6-OHDA treatment compared to neurons in slices from normal, untreated rats. This is consistent with results from an earlier study from our laboratory that showed that unilateral dopamine depletion caused by 6-OHDA treatment reduced NMDA-evoked currents while augmenting AMPA-mediated currents in STN neurons [17]. Thus, our finding that the excitatory effect of NMDA is reduced after chronic dopamine depletion is more likely to be due to a change in NMDA receptor function rather than a loss of a direct, excitatory effect of dopamine.

It is interesting to speculate as to how NMDA-evoked K-ATP current could be up-regulated by chronic dopamine depletion even while NMDA receptor function appears to be down-regulated. Certainly, increased surface expression of K-ATP channels by dopamine depletion might account for a large part of this effect [20]. Even though dopamine depletion appears to down-regulate NMDA receptor function, it is possible that dopamine depletion could up-regulate the calcium- and nitric oxide-dependent second messenger systems that are needed for NMDA receptor stimulation to activate K-ATP channels [18]. Further work

will be needed to ascertain how dopamine depletion alters interactions between NMDA receptors and K-ATP channels.

Increased burst firing of STN neurons is well-established as a pathological correlate of the symptoms of Parkinson's disease [2,23]. If K-ATP channel function in the STN is up-regulated in Parkinson's disease, then one might expect that K-ATP channel openers might improve symptoms of Parkinson's disease [21]. Indeed, studies in the reserpine-treated rat model of parkinsonism showed that hypokinesia and rigidity were improved by systemic administration of the K-ATP channel opener diazoxide [11]. However, K-ATP channels are also expressed in the substantia nigra zona compacta [9], and the inhibitory effect of K-ATP channel openers on residual dopamine neurons would be expected to worsen symptoms of Parkinson's disease. Thus, systemic administration of K-ATP channel openers would likely have unintended side effects. Further work will be needed to find ways to limit the actions of K-ATP channel openers to effective sites of action such as the STN.

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Abbreviations

STN	subthalamic nucleus
NMDA	N-methyl-D-aspartate
K-ATP	ATP-sensitive K ⁺
6-OHDA	6-hydroxydopamine
ANOVA with RM	analysis of variance with repeated measures

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Highlights

- NMDA evokes K-ATP currents in rat subthalamic nucleus (STN) neurons
- STN neurons were recorded in slices from rats treated with 6-OHDA
- NMDA evoked more outward current in STN neurons from dopamine-depleted rats
- NMDA inhibited firing rate in slices from 6-OHDA-treated but not normal rats
- We conclude that dopamine deficiency up-regulates functional K-ATP expression

Fig. 1. NMDA evokes a larger conductance increase in STN neurons located ipsilateral to 6-OHDA treatment

(A) Whole-cell current traces from STN neurons located ipsilateral and contralateral to 6-OHDA treatment. NMDA (20 μ M) evoked larger outward current (at -70 mV) in the neuron located ipsilateral compared to that recorded contralateral to 6-OHDA treatment. Vertical deflections are caused by voltage steps that were used to measure series resistance or membrane currents. (B) Summarized I–V plots showing net (subtracted) currents evoked by NMDA (20 μ M) in STN neurons located ipsilateral (filled circles) and contralateral (open circles) to 6-OHDA treatment. Lines indicate average conductances measured between -70 and -100 mV. Note that NMDA evoked a larger positive-slope conductance in STN neurons

located ipsilateral ($n = 10$) compared to those located contralateral ($n = 10$) to 6-OHDA injections ($P < 0.001$, two-way ANOVA with RM). Asterisks indicate significance differences at specific potentials (*, $P < 0.05$; **, $P < 0.01$).

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Fig. 2. K-ATP channels exert a greater influence on STN neuronal excitability in dopamine-depleted tissue compared to normal tissue

Loose-patch recordings were made from STN neurons in brain slices prepared from normal, untreated rats and from STN in slices ipsilateral to 6-OHDA treatment. (A) Representative voltage traces obtained from an STN neuron located ipsilateral to 6-OHDA treatment. Record shows firing rates before, during, and after washout of NMDA (20 μ M) in the absence (upper traces) and presence (lower traces) of tolbutamide (100 μ M). Note that tolbutamide converted an inhibitory effect of NMDA to one of excitation. All recordings are from the same neuron. (B) Effects of NMDA (20 μ M) on firing rates of STN neurons in slices ipsilateral to 6-OHDA treatment. Note that NMDA depressed the spontaneous firing

rate in the absence of tolbutamide, whereas NMDA significantly accelerated firing rate in the presence of tolbutamide. Data were obtained from the same 6 neurons. (C) Effects of NMDA on firing rates of STN neurons from slices prepared from normal rats. NMDA increased firing rates before and during superfusion with tolbutamide. Data were obtained from the same 8 neurons.

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