Dopaminergic Substantia Nigra Neurons Express Functional NMDA Receptors in Postnatal Rats

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Lin, John Yu-luen and Janusz Lipski. Dopaminergic Substantia Nigra neurons express functional NMDA receptors in postnatal rats. J Neurophysiol 85: 1336–1339, 2001. Activation of N-methyl-d-aspartate (NMDA) receptors in the Substantia Nigra zona compacta (SNc) may determine the degree of physiological apoptosis during the early postnatal period. However, the expression of these receptors during this stage of development is uncertain, as a recent study failed to detect responses to NMDA in unidentified SNc neurons isolated from 2-wk-old rats. Using conventional or perforated-patch whole cell recordings, we examined the presence of NMDA-evoked responses in SNc neurons acutely dissociated from P4 to P16 rats, applying strict criteria for identification of these neurons as nigrostriatal and dopaminergic. The SNc neurons were identified by retrograde labeling after striatal injection of Fluoro-Gold; the presence of TH, current; and the inhibition of firing by dopamine (50 μM). NMDA (100 μM, V_hold = −60 mV) evoked inward, APV-sensitive currents (56.4 ± 8.6 pA) in all tested neurons (n = 29). Strong depolarizing responses were observed under current-clamp. These results indicate that NMDA receptors play a functional role in SNc neurons during the first two postnatal weeks.

INTRODUCTION

Dopaminergic neurons of the Substantia Nigra zona compacta (SNc) project to striatum where they control neurons involved in the execution of motor programs (e.g., Pucak and Grace 1994). The SNc neurons receive glutamatergic inputs from the prefrontal cortex, the subthalamic nucleus, and the pedunculopontine tegmental nucleus (Kitai et al. 1999), which normally regulate the firing of these neurons through both N-methyl-d-aspartate (NMDA) and non-NMDA receptors (e.g., Chergui et al. 1993; Christoffersen and Meltzer 1995). There is also evidence that increased glutamatergic input may contribute, through excitotoxicity, to degeneration of SNc neurons in Parkinson’s disease in humans or related animal models (Blundin et al. 1996) and that the hyperactive input to neurons that survive neurodegeneration may lead to some symptoms of the disease (e.g., Rodriguez et al. 1998).

Recent studies indicate that CNS glutamatergic synapses not only control neural activity and mediate excitotoxic injury but also determine neuron survival during the pre- and postnatal periods through activation of the NMDA receptors (see DISCUSSION for references). However, the expression of these receptors in the postsynaptic membrane of SNc neurons in the early stages of development remains uncertain. In a recent physiological study, Wu and Partridge (1998) were unable to demonstrate any NMDA-induced currents in neurons isolated from 2-wk-old rats, a postnatal period associated with a transient peak in naturally occurring SNc cell death (Janecek and Burke 1993; Oo and Burke 1997). Since downregulation of NMDA receptor expression may have important consequences for neuronal survival during development, we felt it was important to re-examine the presence of functional NMDA receptors in SNc of young rats.

METHODS

Experiments were conducted on SNc neurons acutely dissociated from P4 to P16 Wistar rats. Rat pups (<8 days old) were anesthetized by hypothermia, and 0.6 μl injections of 0.5% Fluoro-Gold (Fluorochrome) were made bilaterally into striatum (cf. Silva et al. 1990). Neuron dissociation was conducted using a protocol similar to that applied by us previously to isolate cells from the medulla oblongata (Lipski et al. 1998). In brief, 2–9 days after injection of the retrograde label, rats were anesthetized with CO2 and decapitated. The brains were removed and the midbrain region cut transversely with a vibratome (200 μm). The sections containing SNc were mildly digested with papain (20 U/ml 10–15 min, 32°C; Worthington), the SNc region dissected out using a scalpel blade, and the tissue gently triturated with fire polished Pasteur pipettes. Cells were plated on poly-l-lysine-coated coverslips that were placed in a recording chamber (volume, 0.4 ml) mounted on an inverted microscope equipped with fluorescence attachment (filter block: excitation, 355–425; dichroic mirror, 455; barrier, 460 nm). The chamber was perfused (~0.4 ml/min) at room temperature (22–24°C) with a solution containing (in mM) 150 NaCl, 3 KCl, 2.4 CaCl2, 10 HEPES, 0.01 glycine, and 15 glucose (pH 7.4). Magnesium ions were omitted unless stated otherwise. Fluoro-Gold labeled cells were typically multipolar or oval in shape (long axis, 25–40 μm) and had several (3–6) truncated dendrites. In one experiment, dissociated cells were fixed in 4% formaldehyde and examined for tyrosine hydroxylase (TH) immunoreactivity using a monoclonal TH antibody (Boehringer Mannheim) and Texas Red-labeled secondary antibody (cf. Lipski et al. 1998). Over 90% of Fluoro-Gold labeled neurons were also TH immunoreactive (Fig. 1, C and D).

Whole cell recordings were made using a conventional tight-seal, or perforated-patch, configuration. Conventional recordings (seal, >10 GΩ) were made with pipettes filled with a solution containing (in mM) 130 KF, 5 NaCl, 11 EGTA, 10 HEPES, 1 CaCl2, 10 glucose, and 3 Na ATP or 140 CsF, 10 tetraethylammonium chloride, 10 HEPES, 5 EGTA, 10 glucose, and 3 Na ATP (pH = 7.25). For perforated-patch recording (access resistance, <20 MΩ), the pipette solution...
RESULTS

The following criteria were used to select neurons for testing with NMDA: healthy appearance (smooth plasma membrane, bright under phase-contrast, lack of signs or swelling or shrinkage, dendrites without “beading”); the presence of retrograde labeling after striatal injection of Fluoro-Gold (Fig. 1C); hyperpolarization-induced time-dependent inward ($I_h$) current under voltage clamp or a depolarizing “sag” in current clamp (both are characteristic of these neurons) (e.g., Silva et al. 1990; Washio et al. 1999) (Fig. 2, B and C); the ability to fire repetitive action potentials in the current-clamp mode in response to depolarizing current (Fig. 2C) (Silva et al. 1990: Yung et al. 1991); and inhibition of the firing (in animals older than P9) by application of 50 μM dopamine (Fig. 2D) (Silva et al. 1990; Washio et al. 1999). Most cells showed periods of spontaneous regular firing, with action potentials (duration, 4.9 ± 0.3 ms; $n = 25$) preceded by slowly rising depolarizations (indicative of pacemaker potentials; see Fig. 2C) and were followed by long-lasting afterhyperpolarizations (Fig. 2C) (Grace and Om 1989; Yung et al. 1991).

A total of 29 cells (14 from P4 to P9 and 15 from P10 to P16 animals) fulfilled the preceding criteria and were examined for responses to NMDA application (100 μM; 1 s). In the voltage-clamp mode ($V_{hold} = −60$ mV), all cells responded with an inward current that reached a peak amplitude near the end of injection (Fig. 3A). When depolarizing voltage ramps were used (together with Cs-based internal solution), the current reversed in polarity at around 0 mV ($n = 3$, Fig. 3C2). In current-clamp, NMDA induced membrane depolarization and high-frequency firing (or depolarizing block of firing; not illustrated) in all 20 tested cells from both age groups (Fig. 3B). The peak amplitudes of the responses, together with the values of the membrane capacitance (indicative of cell size) and a measure of the $I_h$ current, are given in Table 1. No statistically significant difference in the peak amplitude of the NMDA-induced inward current, or the membrane depolarization, was found in neurons from the two examined age groups. The inward current could be blocked by co-application of 200 μM APV ($n = 5$, Fig. 3A). It was also strongly reduced or abolished in the presence of 1 mM Mg$^{2+}$. The effect was voltage dependent ($n = 3$; Fig. 3C, 1 and 2). Responses to NMDA application were observed in all cells tested with the conventional ($n = 24$) or perforated-patch ($n = 5$) recording technique.

DISCUSSION

The expression of NMDA receptors in adult rat dopaminergic SNc neurons has been documented with receptor binding studies (Albin et al. 1992) and molecular identification of receptor subunits (particularly the NMDAR1 and R2C) (Albers et al. 1999). These receptors are believed to regulate the firing rate and the bursting pattern of activity of SNc neurons (e.g., Chergui et al. 1993; Christoffersen and Meltzer 1995; Johnson et al. 1992), and to modulate release of dopamine both from the somato-dendritic region and presynaptic terminals in the striatum (Araneda and Bustos 1989; Cheramy et al. 1996). To our knowledge, there are no data on NMDA receptor expression (using ligand binding, RNA or protein analysis) in rats under the age of P14. Our electrophysiological results demonstrate the presence of functional NMDA receptors in SNc neurons isolated during this early postnatal period. The NMDA-induced responses were postsynaptic (i.e., evoked in the soma and/or proximal dendrites) as, due to acute cell isolation, any presynaptic effects were eliminated. It is uncertain why our results differ from those published by Wu and Partridge (1998), who concluded that glutamate depolarizes SNc neurons in 2-wk-old rats only by non-NMDA receptors. It is unlikely...
that the difference is due to various enzymes used for cell isolation (papain versus pronase), as Wu and Partridge (1998) did observe NMDA-induced currents in cells isolated from an adjacent region. The SNc region is not entirely homogenous with respect to axonal projections and also contains nondopaminergic neurons (Yung et al. 1991). Therefore the different findings could be explained by the fact that we used more strict criteria for identification of SN neurons as dopaminergic and projecting to the striatum, systematically identifying the cells by retrograde labeling, the presence of the characteristic $I_h$ current, and the response to dopamine.

Unless “stem cells” are present in SNc in mature animals (see Janson et al. 2000), the final number of neurons present in this nucleus in mature animals is determined by the proportion of cells that are not “trimmed out” by physiological apoptosis during the early stages of development (Jackson-Lewis et al. 2000). Studies conducted in rodents revealed that a major age-dependent peak in cell death is unclear. One possibility is that the peak is transient downregulation of NMDA receptors on SNc neurons and the lack of the survival-promoting effect of NMDA receptor activation. Previous studies have demonstrated that synaptic activation of NMDA receptors by glutamate promotes neuronal survival in widespread brain regions during development and that blockade of these receptors triggers apoptotic neurodegeneration (Gould et al. 1994; Ikonomidou et al. 1999). Our results, in contrast to the findings of Wu and Partridge (1998), show consistent expression of functional NMDA receptors in the SNc during the second postnatal week and therefore argue against such a mechanism. In fact, in the first weeks of postnatal life, NMDA receptors often undergo a period of hypersensitivity rather than downregulation (Ikonomidou et al. 1989).

**REFERENCES**


**TABLE 1. Properties of SNc neurons isolated from two examined age groups**

<table>
<thead>
<tr>
<th></th>
<th>P4–P9</th>
<th>P10–P16</th>
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<tr>
<td>Membrane capacitance, pF</td>
<td>28.6 ± 1.8</td>
<td>30.7 ± 2.5</td>
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<tr>
<td>$I_h$ current, pA</td>
<td>153 ± 25</td>
<td>180 ± 37</td>
</tr>
<tr>
<td>NMFA response (voltage-clamp, $V_{hold} = -60$ mV), pA</td>
<td>58.3 ± 14.3</td>
<td>54.6 ± 10.3</td>
</tr>
<tr>
<td>NMFA response (current-clamp), mV</td>
<td>18 ± 4</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>12</td>
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Data represent means ± SE. Differences between the neurons from two age groups in input capacitance, $I_h$ current and N-methyl-D-aspartate (NMDA)-induced responses were not significant ($P > 0.05$; Student’s $t$-test). The amplitude of $I_h$ current was measured at 1.7 s after the onset of hyperpolarizing step from −60 to −130 mV.


Janson AM, Delfani K, and Zhao M. Demonstration of tyrosine-hydroxylase-positive apoptotic neurons in adult substantia nigra although total nigral neuronal numbers remain constant during the life span of mice. Euro J Neurosci 12, Supp 11: S7, 2000.


