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Research report

$GABA_A$ receptor stimulation blocks NMDA-induced bursting of dopaminergic neurons in vitro by decreasing input resistance

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Abstract

The effects of the $GABA_A$ agonist, isoguvacine, on NMDA-induced burst firing of substantia nigra dopaminergic neurons were studied with intracellular and whole cell recordings in vitro. NMDA application caused the neurons to fire in rhythmic bursts. Although the NMDA-induced bursty firing pattern was insensitive to hyperpolarization by current injection, it was reversibly abolished by the selective $GABA_A$ agonist, isoguvacine. The block of the rhythmic burst pattern by isoguvacine application occurred regardless of whether the chloride reversal potential was hyperpolarizing ($E_{Cl^-} = -70$ mV) or depolarizing ($E_{Cl^-} = -40$ mV). In either case, the input resistance of the dopaminergic neurons was dramatically decreased by application of isoguvacine. It is concluded that $GABA_A$ receptor activation by isoguvacine disrupts NMDA receptor-mediated burst firing by increasing the input conductance and thereby shunting the effects of NMDA acting at a distally located generator of rhythmic burst firing.

Keywords: GABA; NMDA; Dopamine; Burst; Isoguvacine; Basal ganglia

1. Introduction

Dopaminergic neurons of the ventral mesencephalon display three distinct firing patterns; pacemaker firing, random firing, and a bursty firing pattern in intact animals [8,11,21,30,31] whereas only the pacemaker pattern is normally encountered in vitro [11,13,16,17]. Burst firing appears to be important to the normal physiology of the basal ganglia since certain environmental stimuli and/or contingencies elicit bursting [25], which may increase dopamine overflow in the terminal fields [10,24]. In vivo and in vitro experiments have suggested the involvement of NMDA receptor activation in burst firing in dopaminergic neurons [4,5,16,19,28,32]. However, the majority (between 70% and 90%) of the afferents to dopaminergic neurons are GABAergic and inhibitory [1,14,18,22,23,29].

Recently, it has been demonstrated that GABAergic afferents influence dopaminergic neuron activity in vivo predominantly via $GABA_A$ receptors. Monosynaptic inhibition of dopaminergic neurons following electrical stimulation of striatum, globus pallidus or pars reticulata projection neurons is blocked by $GABA_A$ but not $GABA_B$ antagonists in vivo [12,20,30]. Furthermore, blockade of these receptors with selective $GABA_A$ antagonists causes dopaminergic neurons to switch to a bursty firing pattern regardless of their initial firing pattern. This effect is independent of any change in firing rate [20,21,30]. Therefore, both glutamatergic and GABAergic afferents appear to influence the firing pattern of dopaminergic neurons. The mechanism by which firing pattern is modulated by activation of $GABA_A$ receptors has yet to be demonstrated, and may involve an interaction between glutamatergic and GABAergic inputs. Activation of $GABA_A$ receptors, which open a chloride conductance [6], has two effects on dopaminergic neurons. The neuron is hyperpolarized and the input resistance is decreased, both due to opening of chloride channels. The purpose of the present experiments was to determine if stimulation of $GABA_A$ receptors could antagonize NMDA-induced burst firing, and if so, to determine if this is due to membrane hyperpolarization or to increased conductance.

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2. Materials and methods

Young Sprague–Dawley rats (13–18 days old for whole-cell recording, 30–45 days old for sharp-electrode recordings, Zivic-Miller) were anesthetized with ketamine (50 mg/kg) and xylazine (15 mg/kg) and transcardially perfused with artificial CSF. Coronal slices of the midbrain (400 μm thick) were prepared using a Vibroslice® (Campden Instruments). Slices were submerged in a continuously flowing solution (2 ml/min) containing (in mM): NaCl 125, KCl 2.5, $CaCl_2$ 2.5, MgCl 1.5, $NaHCO_3$ 26, NaH_2PO_4 1.25, and glucose 8.0 (pH 7.3–7) at 35°C.

Whole cell recordings were obtained using patch pipettes pulled from 1.5/1.1 mm o.d./i.d. borosilicate glass tubing (World Precision Instruments) on a Narishige PP-83 vertical pipette puller with an intracellular filling solution containing (in mM): 133.4 K-gluconate; 6.6 NaCl; 2 $MgCl_2$; 10 HEPES (free acid); 10 EGTA (free acid); 3 Na_2ATP; and 0.3 GTP (trisodium salt) (pH = 7.24 adjusted with KOH; $E_{Cl^-} = -70$ mV at 37°C). Some recordings were obtained using an intracellular filling solution containing a Cl^- concentration that would depolarize the neuron when Cl^- channels were activated and contained (in mM): 113 K-gluconate; 20.4 KCl; 6.6 NaCl; 2 $MgCl_{6\ 02}$; 10 HEPES (free acid); 10 EGTA (free acid); 3 Na_2ATP; 0.3 GTP (trisodium salt) (pH = 7.27 adjusted with KOH; $E_{Cl^-} = -40$ mV at 37°C). The in vitro patch pipette resistances were 4–10 MΩ. Current clamp whole cell recordings were visualized at 40× using infrared differential interference contrast microscopy with an Olympus BX50 fixed stage microscope.

Sharp electrode recordings were obtained using micropipettes pulled from 1.5/0.69 mm o.d./i.d. borosilicate tubing on a Sutter Instruments P-97 horizontal puller. Micropipettes were filled with 1–3 M potassium-acetate and possessed in vitro impedances of 80–125 MΩ. Membrane potentials were amplified by a Neurodata IR-183 or IR-283 amplifier, displayed on a Nicolet 4094C digital oscilloscope and recorded on an Apple Macintosh Quadra 950 computer using custom designed software. Neurons were identified as dopaminergic using well established electrophysiological criteria [13,15,17,32]. The input resistance was calculated by measuring the voltage deflection from −60 mV in response to hyperpolarizing current pulses of 0.05–0.2 nA for 300 ms. All input resistance measurements were taken at the same membrane potential for each neuron during each of the pharmacological treatments. All drugs were dissolved in artificial CSF and added to the perfusate at the following concentrations (in μM): NMDA 30 (Sigma, St. Louis, MO), apamin 0.05, isoguvacine 20–40, bicuculline methiodide 50, and picrotoxin 100 (RBI, Natick, MA).

3. Results

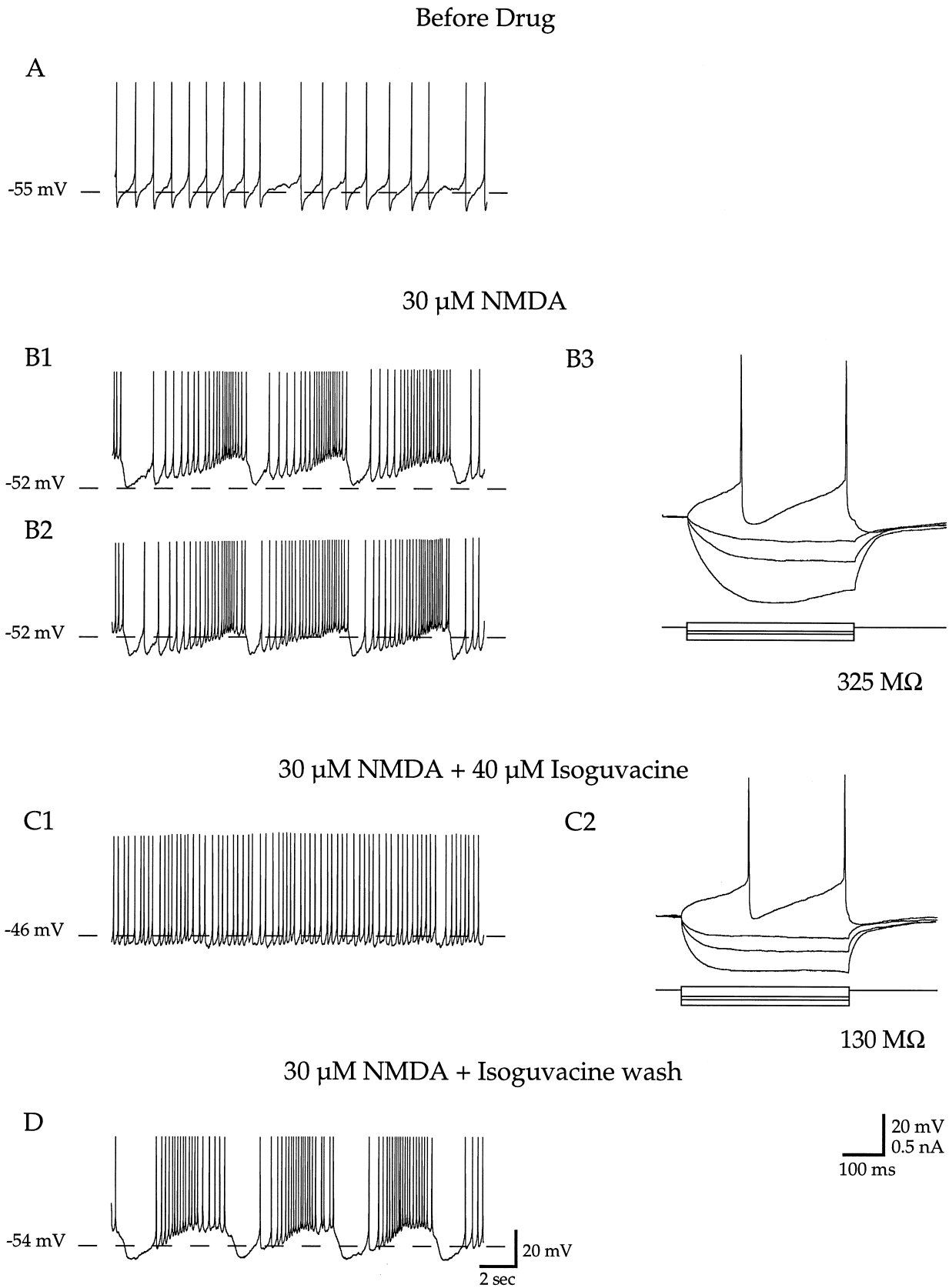
Dopaminergic neurons were electrophysiologically identified by a prominent spike afterhyperpolarization, a sag in the voltage deflection in response to a hyperpolarizing current injection due to activation of an H-current, and an action potential greater than 2 ms in duration [11,13,15,17,31]. All electrophysiologically identified dopaminergic neurons fired in a pacemaker mode either spontaneously or in response to depolarizing current injection. The firing rate increased linearly with membrane potential.

After application of 30 μM NMDA the spontaneous activity of all cells recorded in whole cell mode ($n = 26$) reversibly shifted to a rhythmic bursty pattern. Qualitatively similar effects were observed during sharp electrode recording although the incidence of neurons that shifted to the rhythmic bursty pattern of firing was less. Therefore, in all sharp electrode recording experiments ($n = 14$) 30 μM NMDA was administered along with 50 nM apamin, which caused all neurons to exhibit rhythmic burst firing [26].

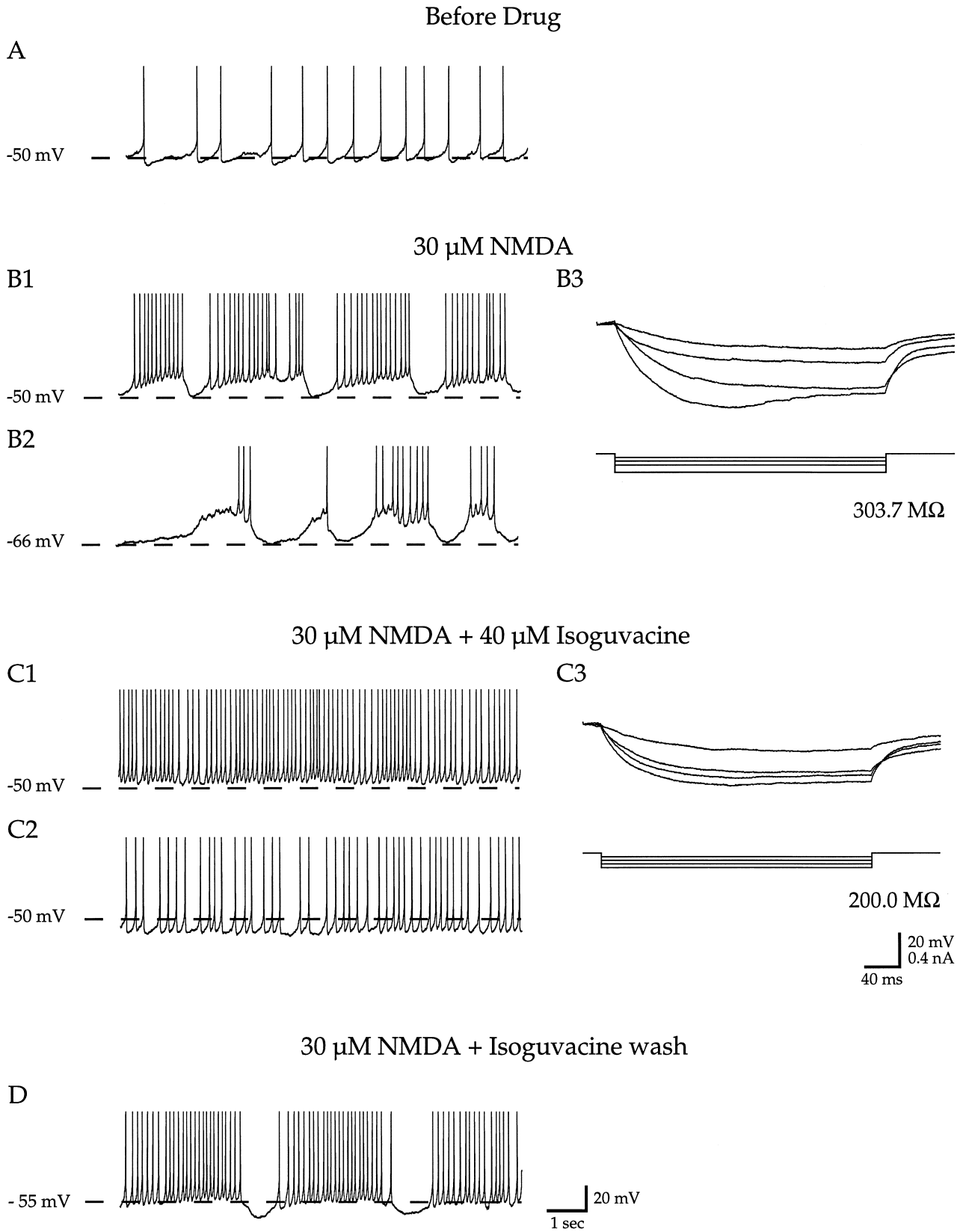
Regardless of recording mode or the presence of apamin, the NMDA-induced rhythmic bursty pattern was insensitive to alterations in membrane potential ranging from −40 to −80 mV ($n = 23$, Figs. 1–3). Application of the $GABA_A$ agonist isoguvacine (20–40 μM) in the presence of NMDA reversibly abolished the NMDA-induced bursty pattern during whole cell recordings ($n = 8$, Fig. 1). This was accompanied by a decrease in the input resistance of all neurons tested from 384.0 ± 76.0 MΩ to 234.3 ± 43.2 MΩ ($df = 6$, $t = 3.98$, $p < 0.05$, see Fig. 2). When the chloride reversal potential was adjusted to be depolarizing ($E_{Cl^-} = -40$ mV) with respect to the resting membrane potential by increasing the internal Cl^- concentration, isoguvacine still obliterated the rhythmic burst firing induced by NMDA ($n = 6$, Fig. 2). Under these conditions, isoguvacine still produced a decrease in input resistance from 297.1 ± 24.1 MΩ to 192.2 ± 8.1 MΩ ($df = 4$, $t = 3.67$, $p < 0.05$). Similar results were observed when isoguvacine was applied during NMDA-induced bursting with sharp electrode recordings ($n = 14$, Fig. 3). In some cases,

Fig. 1. Effects of NMDA and isoguvacine on a representative dopaminergic neuron in substantia nigra pars compacta recorded in whole cell mode. (A) Under control conditions the cell fires spontaneously in a pacemaker pattern. (B1) After bath application of 30 μM NMDA the neuron shifts to a rhythmic bursty firing pattern that is insensitive to hyperpolarization due to current injection (B2). (B3) Note the slow firing, large spike afterhyperpolarization, and sag in response to hyperpolarizing current injection characteristic of dopaminergic neurons. The input resistance is 325 MΩ. (C1) The rhythmic bursty pattern is completely abolished by application of the $GABA_A$ agonist, isoguvacine (40 μM), and the input resistance is decreased to 130 MΩ (C2). (D) The rhythmic bursty pattern returns after isoguvacine is washed out. Action potentials are truncated due to aliasing.

particularly during sharp electrode recording, addition of isoguvacine resulted in the reappearance of a pacemaker-like pattern nearly identical to that exhibited by the neuron prior to application of NMDA (Fig. 3). The input resistance during sharp electrode recordings decreased from 165.9 to 122.0 MΩ after addition of isoguvacine ($n = 2$).



Even when a depolarizing current was injected no cells fired in a rhythmic bursting pattern in the presence of isoguvacine plus NMDA ($n = 10$, Fig. 1). The effect of isoguvacine on input resistance and firing pattern was blocked by the $GABA_A$ antagonists, bicuculline ($n = 9$) and picrotoxin ($n = 2$, see Fig. 3), indicating that isogu-



vacine exerted its effects on $GABA_A$ receptors acting on chloride channels (Fig. 3).

4. Discussion

NMDA receptor activation has been shown to induce a rhythmic bursty firing pattern in dopaminergic neurons in vitro and in vivo [4,5,16,19,27,28,33]. Previous in vitro studies have demonstrated that $GABA_B$ receptor activation by baclofen can block or attenuate NMDA-induced rhythmic burst firing of dopaminergic neurons [27]. However, in vivo studies using local application of specific GABAergic antagonists have demonstrated that the GABAergic afferents to substantia nigra pars compacta inhibit the dopaminergic neurons predominantly via $GABA_A$ receptors [20,21]. $GABA_B$ receptors appear not to provide much of the inhibitory tone in vivo since $GABA_B$ antagonists applied locally or systemically have only modest effects on dopaminergic neuron firing pattern or rate [7,21]. Blockade of $GABA_A$ receptors on dopaminergic neurons leads to burst firing that is similar or identical to that seen spontaneously in vivo [21,30], an effect that appears to be due principally to blockade of the GABAergic input arising from substantia nigra pars reticulata [3,30]. It is unclear if NMDA receptor activation-induced bursting is the same type of bursting that occurs spontaneously or after $GABA_A$ receptor blockade in vivo. The relative influence of NMDA and $GABA_A$ receptor stimulation or blockade on firing pattern in vivo has never been investigated directly. It is possible that simultaneous NMDA receptor activation and cessation of $GABA_A$ receptor activation are necessary in order for bursting to occur in vivo. Phase-plane analysis and compartmental modeling of dopaminergic neuron rhythmic bursting induced by NMDA has shown that a linear conductance increase, simulating the effect of an increased $GABA_A$ receptor activation, can occlude the rhythmic bursting [2].

In this study, activation of $GABA_A$ receptors by the selective $GABA_A$ agonist, isoguvacine, abolished NMDA-induced rhythmic burst firing. This might have resulted because of a hyperpolarization of the membrane potential, a decrease in the input resistance of the cell, or both factors. The change in firing pattern is apparently caused solely by the decrease in the input resistance of the neurons as shifts in membrane potential elicited over a wide range in the presence or absence of isoguvacine did not affect firing pattern. The effect of isoguvacine on firing pattern was also insensitive to whether the chloride reversal potential was hyperpolarizing ($E_{Cl^-} = -70$ mV) or depolarizing ($E_{Cl^-} = -40$ mV), supporting the view that the change in membrane potential caused by $GABA_A$ receptor activation does not affect the firing pattern. Thus, it appears that it is the decrease in input resistance, which occurs regardless of whether the cell is depolarized or hyperpolarized by the increased chloride conductance, that has the most pronounced effect on NMDA-induced bursting. This predicts that other agonists that decrease input resistance should regularize the firing pattern of dopaminergic neurons induced to fire in rhythmic bursts by NMDA application. In fact, this is the case as demonstrated by Seutin et al. [27] where baclofen or dopamine acting at $GABA_B$ and D_2 receptors, respectively, decreased the membrane input resistance of dopamine neurons by increasing a potassium conductance and had the same antagonistic effect on NMDA-induced rhythmic bursting as isoguvacine.

NMDA-induced burst generation in dopaminergic neurons appears to be located in a distal dendritic compartment since voltage-clamp experiments are unable to clamp the rhythmic currents induced by NMDA receptor activation [27]. The high GABAergic tone encountered in vivo may, therefore, act to prevent burst firing in response to glutamatergic inputs by increasing the input conductance of the neuron and preventing the distal NMDA receptors from affecting the firing pattern. The effects of the distally located NMDA receptor-mediated burst generation would be shunted by the increased input conductance that expands the electrotonic length of the dendritic arbor. Dendritically released dopamine [9] could also affect the firing pattern of dopaminergic neurons by suppressing bursts in the vicinity of the site of release via a similar mechanism. These data suggest that in vivo, GABAergic afferents and perhaps dendritically released dopamine may interact with glutamatergic inputs to gate burst firing in dopaminergic neurons.

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Fig. 2. $GABA_A$ receptor-mediated abolition of NMDA-induced rhythmic burst firing is dependent on the decrease in input resistance. Whole cell recording obtained from a dopaminergic neuron using a pipette containing a high concentration of chloride ($E_{Cl^-} = -40$ mV). (A) The neuron fires in a pacemaker pattern under control conditions. (B1) After bath application of NMDA the cell fires in a rhythmic bursty pattern. (B2) Spiking is attenuated by hyperpolarization but the slow membrane potential oscillation underlying the burst is still present. (B3) The input resistance 303.7 MΩ. (C1) After bath application of the $GABA_A$ agonist, isoguvacine (40 μM), the bursty firing pattern is abolished even though the high chloride concentration causes a depolarization of the neuron after activation of $GABA_A$ receptors. (C2) Injection of hyperpolarizing current returns the neuron to a slower firing rate in a single-spike mode. (C3) The input resistance is decreased to 200 MΩ due to bath application of 40 μM isoguvacine. (D) Rhythmic burst firing resumes after isoguvacine is washed out. Action potentials are truncated due to aliasing.

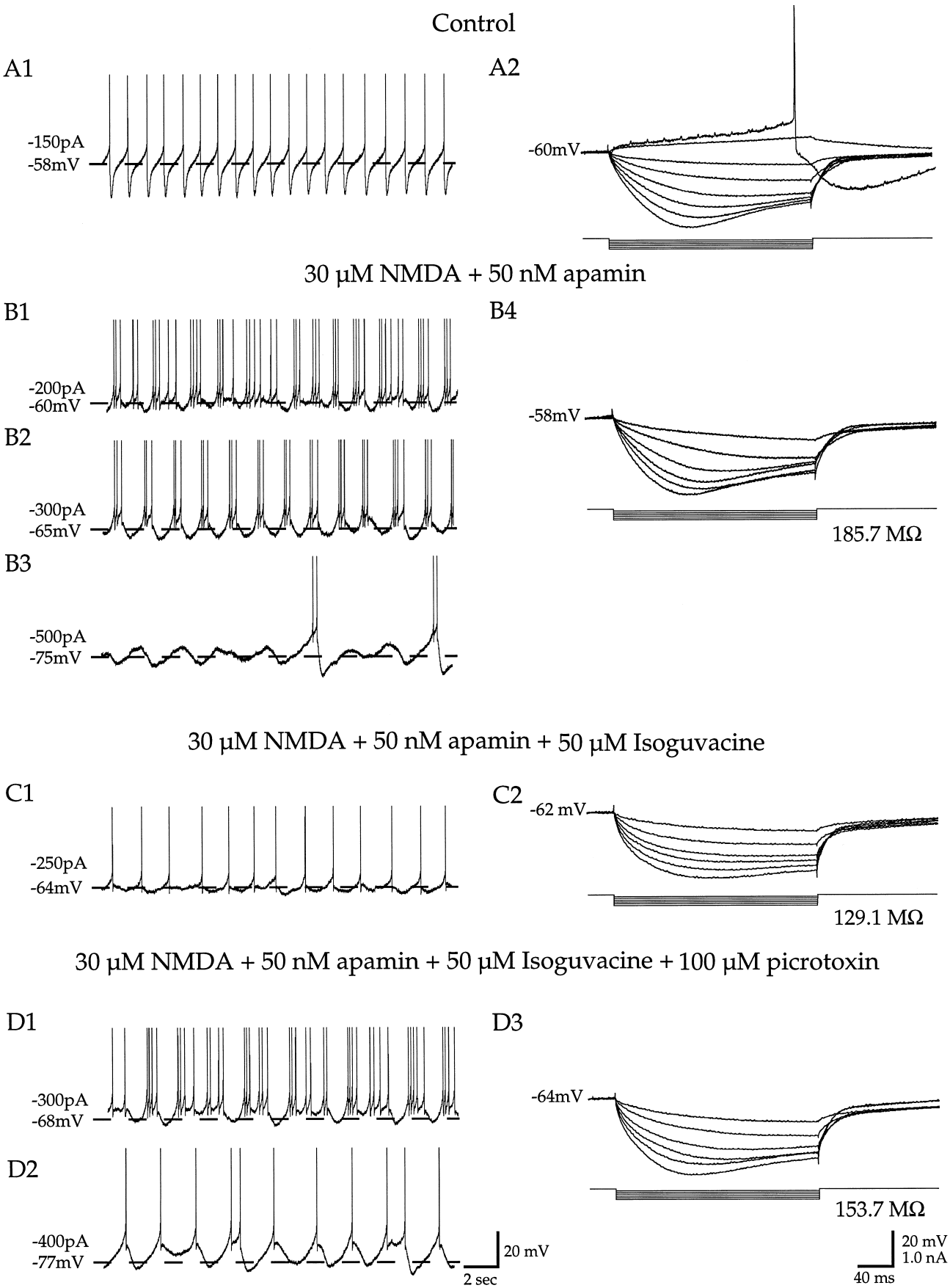
Control
A1
-150pA
-58mV
A2
-60mV
30 μM NMDA + 50 nM apamin
B1
-200pA
-60mV
B2
-300pA
-65mV
B3
-500pA
-75mV
B4
-58mV
185.7 MΩ
30 μM NMDA + 50 nM apamin + 50 μM Isoguvacine
C1
-250pA
-64mV
C2
-62 mV
129.1 MΩ
30 μM NMDA + 50 nM apamin + 50 μM Isoguvacine + 100 μM picrotoxin
D1
-300pA
-68mV
D2
-400pA
-77mV
20 mV
2 sec
D3
-64mV
153.7 MΩ
20 mV
1.0 nA
40 ms

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Fig. 3. Sharp electrode intracellular recordings from a dopaminergic neuron. (A1, A2) Under control conditions the neuron fires in a pacemaker pattern. (B1) Bath application of 30 μM NMDA and 50 nM apamin shifts the firing to a bursty pattern that cannot be abolished by hyperpolarization (B2, B3). (B4) The neuron has an input resistance of 185.7 MΩ. (C1) After application of the $GABA_A$ agonist, isoguvacine (50 μM), the firing returns to a slower, pacemaker pattern and the input resistance decreases to 129.1 MΩ (C2). (D1) The effect of isoguvacine on the NMDA-induced bursty firing pattern is blocked by the chloride channel blocker, picrotoxin. (D2) The rhythmic bursty pattern is still insensitive to hyperpolarization and the input resistance of the cell increases (153.7 MΩ) to near the input resistance measured before bath application of isoguvacine (D3). Action potentials are truncated due to aliasing.