Conductance-Based Model of the Voltage-Dependent Generation of a Plateau Potential in Subthalamic Neurons

Takeshi Otsuka, Takaumi Abe, Takahisa Tsukagawa, and Wen-Jie Song
Department of Electronic Engineering, Graduate School of Engineering, Osaka University, Suita 565-0871, Japan

Submitted 27 May 2003; accepted in final form 23 February 2004

Otsuka, Takeshi, Takaumi Abe, Takahisa Tsukagawa, and Wen-Jie Song. Conductance-based model of the voltage-dependent generation of a plateau potential in subthalamic neurons. J Neurophysiol 92: 255–264, 2004; 10.1152/jn.00508.2003. Because the subthalamic nucleus (STN) acts as a driving force of the basal ganglia, it is important to know how the activities of STN neurons are regulated. Previously, we have reported that a subset of STN neurons generates a plateau potential in a voltage-dependent manner. These plateau potentials can be evoked only when the cell is hyperpolarized. Here, to examine the mechanism of the voltage-dependent generation of the plateau potential in STN neurons, we constructed a conductance-based model of the plateau-generating STN neuron based on experimental observations and compared simulation results with recordings in slices. The model consists of a single compartment containing a Na\(^+\) current, a delayed-rectifier K\(^+\) current, an A-type K\(^+\) current, Ca\(^{2+}\) current, a T-type Ca\(^{2+}\) current, a Ca\(^{2+}\)-dependent K\(^+\) current, and a leak current. Our simulation results showed that a plateau potential in the model could be induced in a voltage-dependent manner that depended on the inactivation properties of L-like long-lasting Ca\(^{2+}\) current. The model could also reproduce the generation of a plateau potential as a rebound potential after termination of hyperpolarizing current injection. In addition, we tested the stability of simulated plateau potentials against inhibitory perturbation and found that the model showed similar properties observed for the plateau potentials of STN neurons in slices. The effects of K\(^+\) channel blockade by TEA and intracellular Ca\(^{2+}\) ion chelation by BAPTA on the plateau duration were also tested in the model and were found to match experimental observations. Thus our STN neuron model could qualitatively reproduce a number of experimental observations on plateau potentials. Our results suggest that the inactivation of L-type Ca\(^{2+}\) channels plays an important role in the voltage-dependent generation of the plateau potential.

INTRODUCTION

The subthalamic nucleus (STN) is an oval-shaped small nucleus, consisting only of glutamatergic projection neurons, in the basal ganglia. It projects to the output structures of the basal ganglia, the globus pallidus (GP), and the substantia nigra (Kita and Kitai 1987; Kita et al. 1983b; Kitai and Kita 1987; Van der Kooy and Hattori 1980). Despite its small size, the STN plays important roles in motor control. Pathological changes in the nucleus cause hemiballism (Whittier 1947), suggesting that the STN plays a pivotal role in voluntary movement control (Mink and Thach 1993; Wichmann and DeLong 1996). Furthermore, manipulation of the activities of STN neurons strongly affects motor behavior (Hamada and Hasegawa 1996; Wichmann et al. 1994b). These observations indicate the importance of controlling outputs of the basal ganglia by STN neurons. Therefore it is crucial to know how activities of STN neurons are regulated.

In slice preparations, STN neurons show rhythmic single-spike activities at resting membrane potentials (Beurrier et al. 1999; Bevan and Wilson 1999; Overton and Greenfield 1995). This may contribute to the tonic discharge of STN neurons observed in resting animals (DeLong et al. 1985; Matsumura et al. 1992; Wichmann et al. 1994a). In response to depolarizing current pulses, STN neurons increase their firing frequencies linearly with the magnitude of injected current (Bevan and Wilson 1999; Nakaniishi et al. 1987), suggesting STN neurons work as linear transformers relaying the strength of inputs from, for example, the cortex (Magill et al. 2000). STN neurons, however, have intrinsic membrane properties that can produce more complex firing patterns. In a subset of STN neurons, the generation of a plateau potential, a long-lasting depolarizing potential, have been reported in several studies (Beurrier et al. 1999; Nakaniishi et al. 1987; Otsuka et al. 2001a; Overton and Greenfield 1995). The plateau potential can induce long-lasting high-frequency discharge in the absence of synaptic inputs. One feature of plateau potentials in STN neurons is the voltage dependence in the generation. STN neurons can generate a plateau potential only when the cells are hyperpolarized (Beurrier et al. 1999; Nakaniishi et al. 1987; Otsuka et al. 2001a; Overton and Greenfield 1995). By enabling this feature in the generation of a plateau potential, STN neurons can form short-lasting synaptic excitation into long-lasting burst spikes in a voltage-dependent manner (Otsuka et al. 2001a) and change their spontaneous activities from single-spike to bursting firing pattern (Beurrier et al. 1999).

In addition, voltage-dependence of a plateau potential may play important roles in the generation of oscillatory bursting activity of the STN neurons, characterized by bursts of long duration and repeating at a low frequency, which correlated with parkinsonian resting tremor (Bergman et al. 1994; Rodriguez et al. 1998). A recent organotypic culture study has suggested that the network of the STN and the GP, which are reciprocally connected, acts as a generator of oscillatory burst activity of STN neurons (Plenz and Kitai 1999). Because the STN receives inhibitory inputs from the GP (Groenewegen and Berendse 1990; Kita et al. 1983b; Morizumi and Hattori 1992), long-duration bursts in STN neurons may be produced by long-lasting rebound depolarizations. The plateau potential in STN neurons, which can be evoked as a rebound potential (Beurrier et al. 1999; Otsuka et al. 2001a; Overton and Green-
field 1995), is one candidate for such potentials. It has also been shown that GABA_A receptor activation can hyperpolarize STN neurons sufficiently for them to produce rebound burst firing (Bevan et al. 2000).

Although voltage-dependent generation of a plateau potential in STN neurons has been described in several studies (Baufreton et al. 2003; Beurrier et al. 1999; Nakaniishi et al. 1987; Otsuka et al. 2001a; Overton and Greenfield 1995), the mechanism of the voltage dependency in the generation of a plateau potential remains unknown. To explore the mechanism, we have constructed a model for the plateau-generating STN neurons, which contains a set of ionic currents identified previously in physiological experiments. We also obtained whole cell recordings from STN neurons in slices to compare side-by-side with simulation results. Simulation results showed that our model could reproduce a number of experimental observations on plateau potentials and suggest that the voltage-dependent generation of a plateau potential can be attributed to the inactivation of the L-type Ca^{2+} channel. Some of the results have been published in abstract form (Otsuka et al. 2000).

**METHODS**

**Whole cell recordings in slice**

All experiments were conducted in compliance with the Guidelines for Use of Laboratory Animals of Osaka University. Slice preparation including the STN from Sprague-Dawley rats aged P14–P27 was obtained with the use of procedures similar to those we have previously described (Otsuka et al. 2001a). Briefly, rats were anesthetized with ether and decapitated; brains were removed, iced, and blocked for slicing. The blocked tissue was cut into 350- or 400-μm-thick slices with a Microslicer (Dosaka EM) while being bathed in a high-sucrose solution composed of (in mM) 200 sucrose, 2.5 KCl, 0.5 CaCl_2, 10 MgSO_4, 1.25 KH_2PO_4, 26 NaHCO_3, and 10 glucose (300 ± 5 mM osmol/l, pH 7.4). The slices were incubated at room temperature for ≥1 h in oxygenated Krebs’s solution composed of (in mM) 126 NaCl, 2.5 KCl, 1.25 KH_2PO_4, 1 MgSO_4, 2 CaCl_2, 26 NaHCO_3, and 10 glucose (300 ± 5 mM osmol/l, pH 7.4, bubbled with 95% O_2-5% CO_2). The slice was transferred to a recording chamber mounted on an upright microscope (Olympus) and continuously perfused with Krebs’s solution.

Whole cell recordings from slices employed standard techniques (Edwards et al. 1989; Stuart et al. 1993). Temperature of the bath solution in the recording chamber was adjusted to 30 °C with a thermostable plate (Tokai Hit). Identification of recorded STN neurons with biocytin was previously described (Otsuka et al. 2001a), and all recorded neurons were found within the STN in this study. The recording pipettes were filled with a solution containing (in mM) 70 K_2SO_4, 30 N-methyl-D-glucamine, 2.5 MgCl_2, 35 HEPES, 1.0 EGTA, 0.1 CaCl_2, 2 Na_3ATP, 0.2 Li_GTP, and 0.1 leupeptin (pH 7.2, 270 ± 5 mM osmol/l). Recordings were obtained with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA) controlled with a Pentium PC running pCLAMP (Axon Instruments).

**Computer simulation**

Previously, we examined the subcellular origin of a plateau potential by taking advantage of a space-clamp problem (Otsuka et al. 2001a). When electrotonically distant synaptic inputs that triggered a plateau potential in current-clamp mode were activated, generation of plateau potentials (currents) was prevented by somatic voltage clamp, suggesting that the subcellular origin of a plateau potential is the soma and/or proximal dendrites. This notion is supported by the observation that acutely dissociated STN neurons, consisting only of the soma and proximal dendrites, can generate a plateau potential (Do and Bean 2003). Therefore we constructed the STN model neuron as a single compartment. The model contains several sets of currents. First, to produce action potentials, the model includes a sodium current (I_Na), potassium currents, and a leak current (I_l). Because STN neurons express Kv3-type and A-type K^+ currents (I_K) as voltage-dependent K^+ channels (Wigmore and Lacey 2000), the model contains two types of K^+ currents: a delayed rectifier K^+ current (I_K), which has relatively high activation threshold and fast activation time constant as features of Kv3-type K^+ currents (Rudy and McBain 2001), and an I_L, which has low activation threshold and fast activation and inactivation time constants. Second, the model includes L-type long-lasting Ca^{2+} currents (I_{Ca_L}) and low threshold T-type Ca^{2+} currents (I_{Ca_T}) as Ca^{2+} currents. L-type Ca^{2+} channels have been shown to be involved in the generation of plateau potentials in our previous study (Baufreton et al. 2003; Beurrier et al. 1999; Otsuka et al. 2001a), and the involvement of I_T in the induction of a plateau potential was suggested by Beurrier et al. (1999). STN neurons also express other types of high-threshold Ca^{2+} channels such as N- and P/Q-types (Song et al. 2000), but there is no indication that these channels are involved in plateau potentials, so these were omitted for simplicity. Last, the model includes Ca^{2+}-activated K^+ current (I_{Ca-K}), which has been observed as a repolarization component of plateau potentials (Otsuka et al. 2001a). The membrane potential of the plateau-generating STN model neuron is described by

\[
\frac{dV}{dt} = -I_Na - I_K - I_L - I_{Ca-K} - I_I
\]

where \(C_m\) is membrane capacitance and takes 1 μF/cm^2, and \(V\) is membrane potential. The ionic currents are given by the following Hodgkin-Huxley type equations

\[
I_{Na} = g_{Na} m^3 h (V - \theta_{Na})
\]

\[
I_K = g_K h a (V - \theta_K)
\]

\[
I_L = g_L c d d (V - \theta_L)
\]

\[
I_I = g_L d (V - \theta_L)
\]

where \(a, b, c, d, h, m, n, p, q, r\) and \(r\) are activation or inactivation variables; \(V_{Na}, V_K, V_Ca,\) and \(V_I\) are the reversal potentials of the sodium, potassium, calcium, and leak current, respectively (potentials in mV); and \(g_{Na}, g_K, g_{Ca}, g_T, g_{Ca-K}\) and \(g_I\) are maximal conductances (in mS/cm^2). Because inactivation of L-type Ca^{2+} channel depends on both voltage and intracellular Ca^{2+} concentration (Imredy and Yue 1994; Shi and Soldatov 2002; Singer et al. 1991), L-like long-lasting Ca^{2+} current in our model has two inactivation variables: voltage- and Ca^{2+}-dependent inactivation (\(d_1\) and \(d_2\), respectively). \(V_Ca\) is simulated to change with intracellular concentration of Ca^{2+} and is given by the Nerst equation. The extracellular Ca^{2+} concentration of the model cell takes 2 mM to match with our physiological experiments. Reversal potentials of other ionic currents were assumed as constants.

The gating kinetics of the ionic conductances are governed by equations of the following form

\[
\frac{dw}{dt} = w_c (v) - w
\]

where \(w\) stands for one of \(a, b, c, d, h, m, n, p, q, r\); the steady-state activation and inactivation functions are given by
where \( \theta_a \) and \( k_a \) are the half-activation/half-inactivation voltage and slopes, respectively. In the case of \( d_2 \) (Ca\(^{2+}\)-dependent inactivation variable of \( I_a \)) and \( r \) (Ca\(^{2+}\)-dependent K\(^+\) conductance), steady-state inactivation and activation depend on intracellular Ca\(^{2+}\) concentration (in \( \mu \)M). In Xenopus oocytes, half-inactivation voltage for voltage-dependent inactivation of L-like long-lasting current ranged from -68 to -62 mV, depending on the expression of subunits (Singer et al. 1991). We assumed half-inactivation voltage of L-like long-lasting Ca\(^{2+}\) currents to be -60 mV. Half-inactivation Ca\(^{2+}\) concentration of Ca\(^{2+}\)-dependent inactivation was assumed to be 100 nM (Ohya et al. 1988). Half-activation voltage of \( I_k \) was set according to values obtained in STN neurons (Wigmore and Lacey 2000). Kinetics of \( I_x \) was modified from data obtained in striatal cholinergic interneurons (Song et al. 1998). Half-activation and inactivation voltages of \( I_x \) took values obtained in brain slice (Perez-Reyes 2003). The activation time constants \( (\tau_a \text{ in ms}) \) for \( I_{Na} \) and \( I_K \), which have fast onset kinetics, are given by the following sigmoidal function

\[
\tau_a = \tau_a^0 + \tau_a^d \cdot \left[ 1 + \exp\left( - \left( \theta_a - \theta_a^0 \right)/\theta_a^d \right) \right]
\]

The activation/inactivation time constants of other conductances are given by the following bell-shaped function

\[
\tau_i = \tau_i^0 + \tau_i^d \cdot \left[ 1 + \exp\left( - (\nu - \theta_i^0)/\theta_i^d \right) \right]
\]

In motoneurons that generate plateau potentials mediated by L-type Ca\(^{2+}\) channels (Kiehn 1991), activation time constant of the L-type Ca\(^{2+}\) current ranges from 30 to several hundreds of milliseconds, depending on the strength of activation (Schwindt and Crill 1984). We used an activation time constant ranging from 45 to 55 ms for \( I_L \). Ca\(^{2+}\)-dependent inactivation time constant of \( I_w \) was estimated as 130 ms from the observation in myocytes (Findlay 2002). The activation time constant of \( I_{Ca-K} \) was set at 2 ms. The activation time constants of \( I_a \) ranged from 2 to 5 ms, a feature of K\(_{V3}\)-type K\(^+\) channels (Rudy and McBain 2001). Other parameter values used in simulations are given in Table 1.

Intracellular Ca\(^{2+}\) concentration, [Ca], depends on the total Ca\(^{2+}\) current, \( I_{Ca} \), and is given by the following equation

\[
\frac{d[Ca]}{dt} = -\alpha I_{Ca} - K_{Ca}[Ca] \]

\[
\alpha = 1/2F
\]

where \( F \) is the Faraday constant, \( Z = 2 \) is the valence of calcium ion, and \( K_{Ca} = 2 \text{ ms}^{-1} \) is the Ca\(^{2+}\) removal rate. The first term on the right side has a minus sign, because \( I_{Ca} \) is minus. In our model, intracellular Ca\(^{2+}\) concentration was 5 nM at rest and reached 70 nM during a plateau potential.

All simulations reported here were performed using Visual C++ (Microsoft). Differential equations were solved by a fourth order Runge-Kutta algorithm (time step, 0.01 ms).

**RESULTS**

**Firing properties of the STN model neuron**

Firing properties of the STN model neuron were adjusted to experimental observations on slice preparations. In slice preparations, STN neurons showed rhythmic spontaneous spiking activities at 2-10 Hz (\( n = 21/56 \) ch. 1A, top; see also Beurrier et al. 1999; Bevan and Wilson 1999; Overton and Greenfield 1995). The model neuron fires spontaneously at -10 Hz (Fig. 1A, bottom). Action potentials in the model were repolarized quickly, and membrane potentials were depolarized to the threshold of spikes following a slow hyperpolarization, similar to those in the STN neurons (Fig. 1A, inset). Figure 1B displays the individual ionic currents (bottom) during spontaneous activity of the model. Rhythmic spontaneous activities of the model are driven largely by \( I_{Na} \). The fast upstroke of the action potential in the model is driven by \( I_{Na} \) (Fig. 1C). After activation of \( I_{Na} \), \( I_K \) activates immediately to repolarize the potential. Although Ca\(^{2+}\) and Ca\(^{2+}\)-dependent K\(^+\) currents contribute little during interspike interval and action potential, manipulation of maximal conductances of these currents affects the frequency of spontaneous activities (data not shown). In response to depolarizing current pulse injection, STN neurons fired repetitively \( \leq 100 \text{ Hz} \) (\( n = 5 \), Fig. 1D, left). Firing frequency increased in a near-linear manner with the increase of the magnitude of current pulse (Fig. 1E, top) (Bevan and Wilson 1999; Nakamichi et al. 1987). In the STN model neuron, injection of depolarizing current pulse induced repetitive action potentials during current injection. Increase of the current intensity induced high-frequency spikes during current injection (Fig. 1D, right). The relationship between firing frequency and current intensity of the model neuron is close to linear (Fig. 1E), similar to that observed in slice preparations. Thus our STN model neuron qualitatively reproduces the characteristics of spontaneous spiking activity and spiking responses to depolarizing current pulse injection at the resting membrane potential.

**Voltage-dependent generation of a plateau potential**

A plateau potential can be induced in STN neurons that are hyperpolarized (Beurrier et al. 1999; Nakamichi et al. 1987;
steady-state activation and inactivation of L-like long-lasting Ca\textsuperscript{2+} current have to hyperpolarized state; namely, the channels giving rise to this current with a negative slope only when the cell is held at hyperpolarized state. In the presence of inactivation of the long-lasting Ca\textsuperscript{2+} current, the steady-state conductance (Fig. 1F, insets), currents evoked by a slow voltage ramp had a negative slope region, but only when the model was held at hyperpolarized potentials before the ramp protocol (Fig. 1F). No voltage dependence of the negative slope was observed when the inactivation was removed. Thus inclusion of the inactivation variable to the L-like long-lasting Ca\textsuperscript{2+} current allows the model to change the shape of the steady-state I-V curve in a voltage-dependent manner.

We then examined whether our STN model could reproduce voltage-dependent generation of a plateau potential by comparing the responses of the model to injection of depolarizing current pulses at the resting and hyperpolarized membrane potentials with those of plateau-generating STN neurons. We recorded from 56 STN neurons. Twenty-seven STN neurons were plateau-generating neurons, and 15 of them generated repetitive action potentials during a plateau potential. Examples of recordings from a plateau-generating STN neuron are Otsuka et al. 2001a; Overton and Greenfield 1995). In principle, generation of a plateau potential requires the steady-state current-voltage (I-V) curve to cross zero current with a negative slope (Kiehn 1991). Therefore for a plateau potential to occur in a voltage-dependent manner as it does in STN neurons, the steady-state I-V curve of the cell must cross zero current with a negative slope only when the cell is held at hyperpolarized state; namely, the channels giving rise to this current have to (1) decay slowly to maintain a plateau potential, (2) be inactivated at the resting membrane potential, and (3) be deinactivated at hyperpolarized potentials. The L-type Ca\textsuperscript{2+} channel, which is involved in the generation of a plateau potential in STN neurons (Baufreton et al. 2003; Beurrier et al. 1999; Otsuka et al. 2001a), is one candidate for such a conductance, because of its slow inactivation kinetics. We therefore examined whether voltage-dependent generation of plateau potentials in the STN model neuron depends on inactivation properties of L-like long-lasting Ca\textsuperscript{2+} conductance. Because inactivation of L-type Ca\textsuperscript{2+} channels depends on both voltage and Ca\textsuperscript{2+} (Imredy and Yue 1994; Shi and Soldatov 2002; Singer et al. 1991), our L-like long-lasting Ca\textsuperscript{2+} current model includes both voltage- and Ca\textsuperscript{2+}-dependent inactivation variables. In the presence of inactivation of the long-lasting Ca\textsuperscript{2+} conductance (Fig. 1F, insets), currents evoked by a slow voltage ramp had a negative slope region, but only when the model was held at hyperpolarized potentials before the ramp protocol (Fig. 1F). No voltage dependence of the negative slope was observed when the inactivation was removed. Thus inclusion of the inactivation variable to the L-like long-lasting Ca\textsuperscript{2+} current allows the model to change the shape of the steady-state I-V curve in a voltage-dependent manner.

We then examined whether our STN model could reproduce voltage-dependent generation of a plateau potential by comparing the responses of the model to injection of depolarizing current pulses at the resting and hyperpolarized membrane potentials with those of plateau-generating STN neurons. We recorded from 56 STN neurons. Twenty-seven STN neurons were plateau-generating neurons, and 15 of them generated repetitive action potentials during a plateau potential. Examples of recordings from a plateau-generating STN neuron are
shown in Fig. 2A. At the resting membrane potential, injection of depolarizing current pulses evoked repetitive action potentials during current injection (Fig. 2A, top). Spontaneous rhythmic spiking activities were also observed before and after injection of current pulse. When the cell was hyperpolarized by injection of constant currents, spontaneous activities disappeared. At hyperpolarized potentials, injection of current pulse induced a plateau potential with burst firing, which outlasted current injection (Fig. 2A, bottom). In the model, injection of depolarizing current pulse similarly evokes repetitive action potentials during current injection at the resting membrane potentials, and spontaneous rhythmic activities remain before and after current injection (Fig. 2B, top). When the model is hyperpolarized by injection of constant current, spontaneous activities disappear as observed in slice preparations. At hyperpolarized states, a plateau potential with burst firing is elicited that outlasted current injection in the model neuron, similar to those in STN neurons (Fig. 2B, bottom). Maximal firing frequencies of STN neurons and the model after current injection were 61.5 ± 6.69 (n = 15) and ~150 Hz, respectively. Although firing frequencies during plateau potentials are somewhat different between our model and plateau-generating STN neurons, decrease of firing frequency during a plateau potential in the model is similar to those in STN neurons (Fig. 2C). When the inactivation variable of $I_L$ was removed from the model, no voltage dependency of the generation of plateau potentials was observed (data not shown). These results suggest that inactivation of L-type Ca$^{2+}$ channels plays an important role in the voltage-dependent generation of a plateau potential.

We next compared the voltage-dependence of the generation of plateau potentials in the model with that of plateau-generating STN neurons. For this purpose, membrane potentials of the STN neurons and the model were gradually hyperpolarized by changing the amounts of injected constant currents, while a depolarizing current pulse was injected to test whether a plateau potential could be induced. Examples of responses of a STN neuron to current pulse injection are shown in Fig. 3A. Plateau potentials in STN neurons were triggered when membrane potential was below ~70 mV (n = 5). In the model, plateau potentials developed within a similar range of membrane potentials (Fig. 3B). The relationship between plateau duration, defined as the duration from the pulse end to the time when membrane potential returned to the baseline level, and membrane potential is shown in Fig. 3C. Development of plateau potentials in STN neurons and the model showed similar behavior. Thus our model qualitatively reproduces the voltage-dependent generation of a plateau potential in STN neurons.

**Generation of a plateau potential as rebound potentials**

Another feature of the voltage-dependent generation of a plateau potential is that a plateau potential can be evoked as a rebound potential after termination of negative current injection (Beurrier et al. 1999; Nakanishi et al. 1987; Otsuka et al. 2001a; Overton and Greenfield 1995). Because the equilibrium potential of GABA$_A$ receptors in STN neurons is lower than the threshold potential of a plateau potential (Bevan and Wilson 1999), a volley of inhibitory inputs to STN neurons would be expected to induce a plateau potential as rebound potentials. Therefore generation of a plateau potential as rebound potentials might be an important physiological property of STN neurons, considering that these cells receive massive inhibitory inputs from the GP (Groenewegen and Berendse 1990; Kita et al. 1983b; Morizumi and Hattori 1992). We thus examined whether our model can reproduce the generation of a plateau potential as rebound potentials.

Examples of rebound responses recorded from a plateau-generating STN neuron are shown in Fig. 4A. When the amplitude of the hyperpolarizing current was kept constant, the generation of a plateau potential was directly related to the duration of the current pulse. Injection of a short-duration current pulse (100 ms, −50 pA) induced rebound potentials of small amplitude and short duration (Fig. 4A, top). An increase of the duration of the current pulse, however, induced long duration rebound plateau potentials with burst discharges (Fig. 4A, middle). The duration of the plateau potential became longer with further increase in the duration of current pulses (Fig. 4A, bottom). Sufficient duration of negative current pulse (intensity, −50 pA), which triggered a rebound plateau potential, ranged from 100 to 200 ms (n = 9). These behaviors were qualitatively reproduced in the model. Generation of a plateau...
potentials as a rebound potential depends on the duration of a hyperpolarizing current pulse (Fig. 4B). Here, spontaneous spiking activity was suppressed by injecting a small amount of hyperpolarizing constant current (−1.5 μA/cm²) to the model to obtain a clear rebound response. Rebound plateau potentials in the model, however, were triggered by negative current pulse of shorter duration than that in STN neurons. When hyperpolarizing current pulse was injected at resting membrane potentials in the absence of negative constant current, spiking frequencies of rebound responses gradually decreased following a long-lasting burst and returned to the rate of spontaneous firing in both the model and STN neurons (Fig. 4, C and D). The above results suggest that plateau potentials induced as a rebound potential in the model are qualitatively similar to those induced in STN neurons.

Stability of a plateau potential

Previously, we have shown that the early part of a plateau potential in STN neurons is resistant to inhibitory perturbations, but the late part is not (Otsuka et al. 2001a). Because STN receives inhibitory inputs from the GP (Groenewegen and Berendse 1990; Kita et al. 1983b; Morizumi and Hattori 1992), the stability of a plateau potential against inhibitory inputs would be important to shape the firing pattern of STN neurons in vivo. Therefore we examined whether the plateau potential in our model shows similar stability to that observed experimentally, as in our previous study (Otsuka et al. 2001a). To this end, we simulated an inhibitory synaptic current by experimentally, as in our previous study (Otsuka et al. 2001a). By the ratio of the potential after the negative current pulse to the potential before the current, defined as the stability index. The stability index of a stable potential equals to one. Shown in Fig. 5A, Plateau potentials were induced by injection of a depolarizing current pulse (20 ms, 5 μA/cm²) at a hyperpolarized state. Na⁺ conductance was set to 0 mS/cm² to suppress action potentials. The stability was estimated, as in our previous experiments (Otsuka et al. 2001a), by the ratio of the potential after the negative current pulse to the potential before the current.

FIG. 4. Generation of a plateau potential as a rebound potential. A: rebound responses of a plateau-generating STN neuron after hyperpolarizing current pulses of various durations (bottom: currents). Plateau potentials were evoked after termination of current injection and the duration depended on the duration of hyperpolarizing current pulse. B: responses of the STN model neuron to different duration current pulses. Generation and duration of a plateau potential depends on the duration of hyperpolarizing current pulse as in STN neurons. To block spontaneous activities, −1.5-μA/cm² constant current was injected to the model. Top: spikes were shown only for a part to better show subthreshold potentials. C and D: rebound responses of the STN neuron and the model to hyperpolarizing current pulse injection (0.5 s, −50 pA for STN neuron; −5 μA/cm² for the model). Firing frequencies of rebound responses were decreased gradually and returned to spontaneous firing rate.

FIG. 3. Voltage dependence of the induction of a plateau potential. A and B: responses of STN and model neuron to current pulse injections (50 ms, 50 pA, 5 μA/cm²). STN and model neuron were held at different membrane potentials by injecting constant currents. C: relations between holding membrane potentials and plateau duration in STN neuron (○) and the model (●). Plateau durations of the STN and the model neuron were normalized against maximal plateau durations, respectively.
the nature of a plateau potential on inhibitory perturbations. Reproduction not only behaviors of plateau generation but also part of plateau potentials (data not shown). Thus our model produces these effects.

STN model neuron, we examined whether the model reproduces behaviors resembling those observed in physiological experiments, it does not constitute a physiologically complete description of STN neurons. Ionic currents having less obvious relationships with plateau potentials were not behaviors of plateau generation but also the nature of a plateau potential on inhibitory perturbations.

Reproduction of experimental pharmacological observations

We have previously shown that chelation of intracellular Ca\textsuperscript{2+} by bis-(o-aminophenoxyl)-N,N,N',N'-tetraacetic acid (BAPTA), applied through the patch pipette, or bath-application of the K\textsuperscript{+} channel blocker, tetraethylammonium chloride (TEA), enhanced the duration of a plateau potential (Otsuka et al. 2001a). To confirm the validity of our plateau-generating STN model neuron, we examined whether the model reproduces these effects.

The effects of TEA were simulated by decreasing the value of maximal delayed-rectifier type K\textsuperscript{+} conductance (g\textsubscript{K}). Decreasing the value of g\textsubscript{K} increases the duration of plateau potentials (Fig. 6, A and B). When g\textsubscript{K} was set to 1 mS/cm\textsuperscript{2}, the plateau potential lasted about 1.8 times the initial duration. In addition, the peak of a plateau potential is also elevated with decreasing g\textsubscript{K}. These effects are consistent with those observed in our previous experiments (Otsuka et al. 2001a).

To simulate the effect of Ca\textsuperscript{2+} chelation by BAPTA, we divided the Ca\textsuperscript{2+} flux by a scalar, X, called the rate of Ca\textsuperscript{2+} chelation. Intracellular Ca\textsuperscript{2+} concentration was represented by the following equation

\[
\frac{d[Ca]}{dt} = -\frac{\alpha I_{ca}}{X} - K_{ca}[Ca]
\]

The duration of a simulated plateau potential was sensitive to

\[\text{Ca}^2+\text{ chelation. Increasing the value of } X \text{ leads to increases in the duration of a plateau potential (Fig. 6C). This effect, however, is saturated at a duration around 1.3 times the initial duration (Fig. 6D). Thus our model reproduces the effects of Ca}^2+\text{ chelation and } K^+ \text{ channel blocking on plateau duration, suggesting that ionic mechanisms of plateau potential repolarization in the model are the same as those in STN neurons.}

DISCUSSION

In this study, we investigated the mechanism of the voltage-dependent generation of a plateau potential in STN neurons with computer simulations. Voltage-dependent generation of a plateau potential in STN neurons could be reproduced with the inactivation variable of L-like long-lasting Cu\textsuperscript{2+} current. Simulation results showed that our STN model neuron could reproduce a number of experimental observations on plateau potentials. Inactivation of L-type Ca\textsuperscript{2+} channel may play important roles in the voltage-dependent generation of a plateau potential in STN neurons.

Conductance of STN neurons

We constructed a model of plateau-generating STN neurons with a single compartment containing Na\textsuperscript{+} current, Kv3-like high-threshold delayed rectifier K\textsuperscript{+} current, A current, L-like long-lasting Ca\textsuperscript{2+} current, T-type Ca\textsuperscript{2+} current, Ca\textsuperscript{2+}-dependent K\textsuperscript{+} current, and leak current. Although our model shows a variety of firing behaviors resembling those observed in physiological experiments, it does not constitute a physiologically complete description of STN neurons. Ionic currents having less obvious relationships with plateau potentials were
not included in our model. Nevertheless, our model qualitatively reproduced similar firing behavior and plateau potentials observed in our slice experiments (Otsuka et al. 2001a). Conductances that have been physiologically identified in STN neurons but not considered in our model include TTX-sensitive sustained Na⁺ current (I_{NaP}) (Beurrier et al. 2000; Bevan and Wilson 1999), a hyperpolarization-activated inward current (Ih) (Song et al. 2000), and several types of high-threshold Ca²⁺ channels (Song et al. 2000). These channels may contribute to the voltage-dependent generation of a plateau potential. I_{NaP} can impart a negative slope region to the steady-state I-V curve of STN neurons (Beurrier et al. 2000; Bevan and Wilson 1999). However, Ih appears not essential for plateau generation, because the plateau potential in STN neuron could be evoked in the presence of TTX (Baufreton et al. 2003; Beurrier et al. 1999; Otsuka et al. 2001a). TTX-sensitive subthreshold membrane oscillations were observed in STN neurons (Otsuka et al. 2001a). I_{NaP} may contribute to the rhythm of spontaneous spiking activities as in type I neurons of the tonemal pedunculopontine nucleus (Bevan and Wilson 1999; Do and Bean 2003; Takakusaki and Kitai 1997). Because Ih activates with hyperpolarization and deactivates slowly (McCormick and Pape 1990), it may be involved in the generation of a plateau potential in STN neurons in a voltage-dependent manner. However, bath-applied low concentration of Cs⁺ or ZD 7288, a specific Ih blocker (Harris and Constanti 1995), did not prevent the generation of a plateau potential (unpublished data). Furthermore, when the model included Ih instead of I_L, plateau potentials could not be induced at hyperpolarized state, although long-lasting rebound depolarizations occurred. These observations suggest that Ih does not play an important role in the voltage-dependent generation of a plateau potential in STN neurons. Plateau potentials in STN neurons decayed at a lower rate compared with simulated plateau potentials in our model (see Fig. 2). Repolarization kinetics of a plateau potential may be regulated not only by the inactivation of L-type Ca²⁺ channels and the activation of Ca²⁺-dependent K⁺ channels, but also by the deactivation of Ih. In addition, Ca²⁺-dependent cation channels may also slow the decay of a plateau potential (Beurrier et al. 1999).

Conductance of the STN model neuron

Previous studies of the STN model have focused on the network behavior of the STN and the GP (Gillies et al. 2001; Humphries and Gurney 2001; Terman et al. 2002). They presented STN model neurons as the integrate-and-fire neuron (Humphries and Gurney 2001) or the model where spikes were convolved with an α-type response functions (Gillies et al. 2001), for simplicity. Terman et al. (2002) have also constructed an STN model with Hodgkin-Huxley type equations and have elegantly simulated a number of activity patterns in a modeled STN-GP network. The firing behavior at resting membrane potentials, the firing response to current injection, as well as a long-lasting rebound potential in STN neurons were also simulated in Terman et al. (2002). In this study, we focused on simulating the voltage-dependent generation of plateau potentials and rebound potentials in STN neurons and provided a side-by-side comparison of experimental results and simulation results. Long-lasting rebound potentials were simulated with T-type Ca²⁺ currents in Terman et al. (2002), whereas in our model, L-type currents play an essential role in the voltage-dependent generation of plateau potentials and long-lasting rebounds. We modeled plateau potentials with L-type currents because of experimentally shown sensitivity of plateau potentials to dihydropyridines (Baufreton et al. 2003; Beurrier et al. 2000; Otsuka et al. 2001a). Although the STN consists only of projection neurons (Kitai and Kita 1987), STN neurons can be divided into two cell groups, namely, plateau-generating and non–plateau-generating neurons (Otsuka et al. 2001a). Non–plateau-generating neurons can also generate rebound potentials after termination of hyperpolarizing current injection (Otsuka et al. 2001a). These rebound potentials, however, were terminated within 100 ms and mediated by T-type currents (Beurrier et al. 1999; Song et al. 2000).

All ionic conductances included in the plateau-generating STN model described here are those obtained from physiological experiments. Because the kinetic parameters of conductances of STN neurons remain unknown, the values of these parameters were assigned. In our model, generation of a plateau potential is mediated by L-like long-lasting Ca²⁺, Kv₃-type K⁺, and Ca²⁺-dependent K⁺ currents, which were identified experimentally in our previous study (Otsuka et al. 2001a). Kv₃ channels in STN neurons, characterized in slice preparations, activate at around −40 mV, with half-activation voltage around −15 mV (Wigmore and Lacey 2000). Our Kv₃ current model also activates at around −40 mV, with a half-activation voltage of −17.5 mV. Expression of apamin-sensitive Ca²⁺-activated K⁺ current has been described in STN neurons (Bevan and Wilson 1999). The apamin-sensitive Ca²⁺-activated K⁺ channel is activated by submicromolar Ca²⁺ (Rudy 1988). Our Ca²⁺-activated K⁺ current model activates with a half-activation of 240 nM Ca²⁺. Thus our parameter values for K⁺ currents used here are biophysically plausible. Among high-threshold Ca²⁺ channels in STN neurons, L-type channels activate from lowest voltages, with a half-activation voltage of −20 mV (Song et al. 2000). Here we modeled L-like long-lasting Ca²⁺ channels with −25 mV for half-activation voltage. The observations in acutely dissociated preparations, however, were obtained with Ba²⁺ as the charge carrier. Because activation and inactivation properties of Ca²⁺ channels depend on the species and concentration of charge carrier (Bargas et al. 1994; Lorenzon and Foehring 1995), physiological values of Ca²⁺ channel parameters of STN neurons remain unknown. Among L-type channels, Cav 1.3 channels activate from lower voltages (Xu and Lipscombe 2001), with half-activation voltage ranging from −40 to −20 mV, depending on concentration of charge carriers. Currently it is not known which subtypes of L-type channels are expressed in STN neurons. In this study, we used parameter values for voltage- and Ca²⁺-dependent inactivation obtained from experiments in other cells (Ohya et al. 1988; Singer et al. 1991). It must be pointed out, however, that currently there is conflicting experimental evidence on voltage-dependent inactivation of L-type channels; whereas some report little inactivation (Xu and Lipscombe 2001), others find full inactivation (Singer et al. 1991). The inactivation behavior of L-type channels in STN neurons remains to be determined. In the model, dependency of plateau generation as a rebound potential on hyperpolarizing current pulse injection was somewhat different from that in the STN neuron (see Fig. 4). This difference may in part
be attributable to possible difference in recovery kinetics of L-type channels in STN neurons and in our model.

Even if L-type Ca\(^{2+}\) channels activate at low voltages, currents mediated by Ca\(^{2+}\) channels would be small during a plateau potential. STN neurons express a Kv3-type delayed rectifier and an A current (\(I_A\)) as depolarization-activated K\(^+\) channels (Weis et al. 1994; Wigmore and Lacey 2000). The high threshold of activation of Kv3 channels (Rudy and McBay 2001) and the fast inactivation of \(I_A\) (Rudy 1988) would make STN neurons have high-input resistances at potentials close to resting, enabling the generation of a plateau potential by small amounts of inward currents.

**STN-GP circuit**

Oscillatory long-duration burst activity in STN neurons has been the subject of an increasing number of studies, because this activity correlates with parkinsonian resting tremor (Bergman et al. 1994; Rodriguez et al. 1998). In the study of organotypic slice culture, it has been suggested that the network of the STN and the GP, which forms a recurrent excitatory–inhibitory interaction, acts as a generator of oscillatory bursting activities (Plenz and Kitai 1999). Oscillatory bursting activities in STN neurons are either in-phase or anti-phase with those in GP neurons (Plenz and Kitai 1999). Because STN receives inhibitory inputs from the GP, STN must generate long-duration depolarizing potentials as a rebound potential to induce long-duration burst. Voltage-dependent generation of a plateau potential in STN neurons, which can be evoked as a rebound potential, would be one candidate for such potentials. A rebound plateau potential has been suggested to occur in type I cerebellar nuclei neuron, in response to strong inhibition from Parkinje cells (Czubayko et al. 2001). Recent computer modeling studies have shown that oscillatory bursting activities could be reproduced in a modeled STN-GP circuit (Gillies et al. 2001; Humphries and Gurney 2001; Otsuka et al. 2001b; Terman et al. 2002). In a modeled STN-GP circuit, oscillatory activities are due to rebound responses mediated by Ca\(^{2+}\) current in STN neurons (Humphries and Gurney 2001; Otsuka et al. 2001b). In addition, both in-phase and anti-phase oscillatory bursts were reproduced in a modeled network of plateau-generating STN and GP neurons (Otsuka et al. 2001b). Thus plateau potentials in STN neurons may play important roles in generating oscillatory bursting activities.

Given that oscillations are due to long-duration rebound responses in STN neurons, what causes STN neurons to switch activity patterns to the oscillation? Why is oscillatory bursting activity only observed in parkinsonian, but not normal, subjects? Recent evidence suggests that membrane potentials of STN neurons are depolarized by dopamine application (Zhu et al. 2002). STN neurons in normal subjects would be depolarized by dopamine, and thus GP inhibitory inputs would not hyperpolarize STN neurons enough for plateau potentials to be induced. After dopamine depletion, because Parkinson’s disease is caused by dopamine depletion, the membrane potential of STN neurons in affected patients would be hyperpolarized; therefore a plateau potential would be more likely to be induced in STN neurons, resulting in oscillatory bursting activities.

**ACKNOWLEDGMENTS**

We thank J. Wesseling and A. Horton for helpful comments on the manuscript.

Present address of T. Otsuka: Department of Neurobiology, Duke University Medical Center, Durham, NC 27710.

**GRANTS**

This work was supported by the Uehara Foundation and Grants-in-Aid for Scientific Research on Priority Areas (15500213, 15029228, and 15016065) from the Ministry of Education, Science, and Culture, Japan to W.-J. Song.

**REFERENCES**


