Equilibrium Potential of $GABA_A$ Current and Implications for Rebound Burst Firing in Rat Subthalamic Neurons In Vitro

MARK D. BEVAN,[1,2] CHARLES J. WILSON,[2,3] J. PAUL BOLAM,[1] AND PETER J. MAGILL[1]
[1]*Medical Research Council Anatomical Neuropharmacology Unit, University Department of Pharmacology, Oxford OX1 3TH, United Kingdom;* [2]*Department of Anatomy and Neurobiology, University of Tennessee, Memphis, Tennessee 38163; and* [3]*Division of Life Science, University of Texas, San Antonio, Texas 78294*

Bevan, Mark D., Charles J. Wilson, J. Paul Bolam, and Peter J. Magill. Equilibrium potential of $GABA_A$ current and implications for rebound burst firing in rat subthalamic neurons in vitro. *J. Neurophysiol.* 83: 3169–3172, 2000. Reciprocally connected glutamatergic subthalamic and GABAergic globus pallidus neurons have recently been proposed to act as a generator of low-frequency oscillatory activity in Parkinson's disease. To determine whether $GABA_A$ receptor-mediated synaptic potentials could theoretically generate rebound burst firing in subthalamic neurons, a feature that is central to the proposed oscillatory mechanism, we determined the equilibrium potential of $GABA_A$ current (E_{GABA_A}) and the degree of hyperpolarization required for rebound firing using perforated-patch recording. In the majority of neurons that fired rebounds, E_{GABA_A} was equal to or more hyperpolarized than the hyperpolarization required for rebound burst firing. These data suggest that synchronous activity of pallidal inputs could underlie rhythmic bursting activity of subthalamic neurons in Parkinson's disease.

INTRODUCTION

Subthalamic neurons possess an intrinsic pacemaker mechanism which underlies their rhythmic discharge in vitro and their function as a driving force of neuronal activity in the basal ganglia in vivo (Bevan and Wilson 1999). Removal of hyperpolarizing current can produce a rebound depolarization and a burst of firing in subthalamic neurons (Nakanishi et al. 1987). Rebound excitations of this type do not play a role in the spontaneous rhythmic firing of subthalamic neurons because the necessary degree of hyperpolarization is not attained during the afterhyperpolarization from a single action potential (Bevan and Wilson 1999). Rhythmic bursting activity of subthalamic neurons is phase-related to resting tremor in idiopathic and animal models of Parkinson's disease (Bergman et al. 1994; Rodriguez et al. 1998) and has been suggested to arise from interactions with reciprocally connected GABAergic neurons of the globus pallidus through a mechanism that is similar to that reported for thalamic nuclei (McCormick and Bal 1997; Plenz and Kitai 1999). The aim of this study was to test whether $GABA_A$ current could generate sufficient hyperpolarization in subthalamic neurons to produce rebound burst firing. Thus we determined E_{GABA_A} and the hyperpolarization required for rebound burst firing in subthalamic neurons using perforated-patch recording. We used the cation selective pore-forming substance gramicidin to maintain a natural intracellular concentration of chloride, the major permeant ion of the $GABA_A$ receptor (Ulrich and Huguenard 1997).

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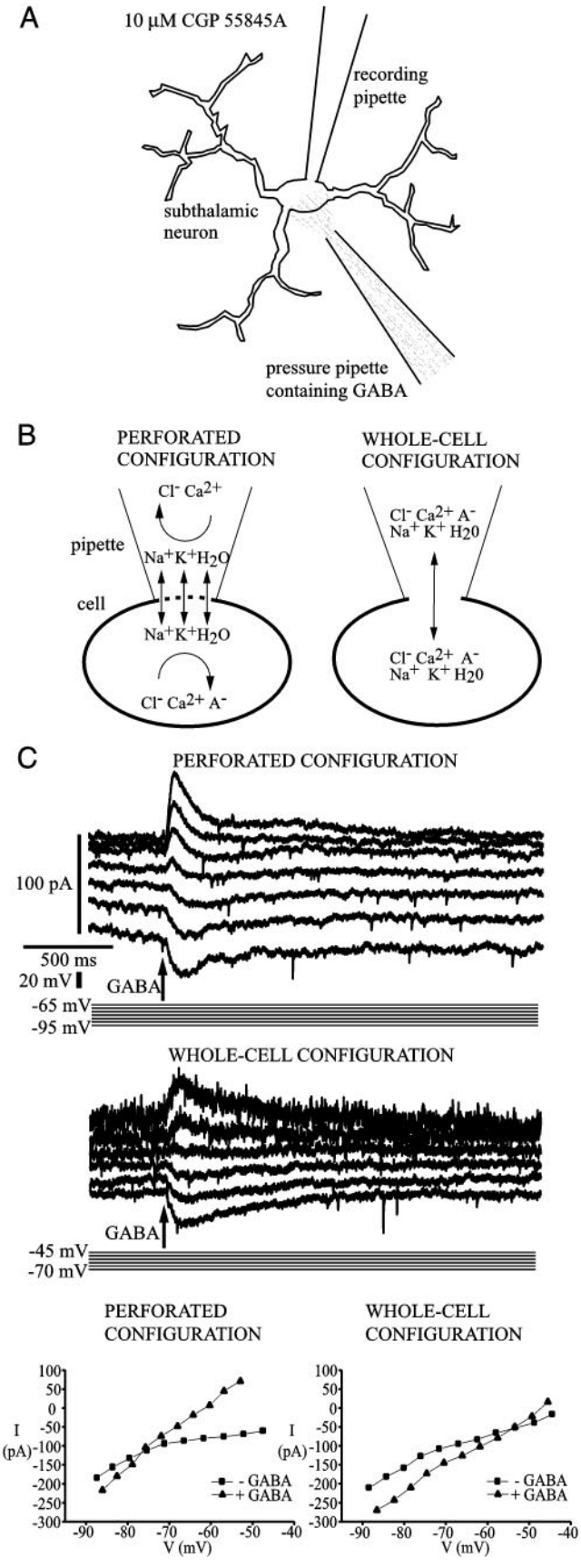
METHODS

Slice preparation and visualized recording

Coronal slices (300-μm thickness) of the subthalamus were prepared from male Sprague-Dawley rats (16- to 23-day old) as described previously (Bevan and Wilson 1999). Individual slices were transferred to a recording chamber, perfused with ACSF at 30–32 or 35–37°C and were examined using infrared differential interference contrast video microscopy (Infrapatch Workstation, Luigs and Neumann, Ratingen, Germany). Somatic recordings were made using patch pipettes prepared from thick-wall borosilicate glass and filled with a solution containing (in mM) 106 K-$MeSO_4$, 25 KCl, 1 $MgCl_2.6H_2O$, 0.1 $CaCl_2.2H_20$, 10 HEPES, and 1 EGTA, pH, 7.3; osmolarity, 290–300 mosmol. Gramicidin was added to the intracellular solution at a concentration of 5 μg/ml. Resistance of the filled pipettes ranged from 3 to 6 MΩ. Fast capacitative transients of the pipette were nulled on-line but voltage errors due to series resistance were compensated off-line. Recordings were made in the perforated and whole-cell configurations using an EPC 9/2. C amplifier (HEKA, Lambrecht, Germany) and Pulse 8.3 (HEKA). Signals were low-pass filtered at a frequency (1.7–33.3 kHz) that was three times less the frequency of digitization (5–100 kHz).

Measurement of E_{GABA_A}

Pressure pulses of GABA (100 μM in the pipette) were directed at the soma of recorded neurons (Fig. 1*A*). The selective $GABA_B$ antagonist CGP 55845A (10 μM; supplied by Novartis) was bath applied at a concentration that saturated $GABA_B$ receptors. Responses were recorded at various holding potentials in current- and voltage-clamp modes. Changes in holding potential were made between 800 and 1,000 ms before the GABA spritz to allow the membrane potential to reach its steady-state value. In current clamp, E_{GABA_A} was measured as the potential at which GABA evoked no response or as the mean of the two voltages at which the smallest depolarizing and hyperpolarizing responses were evoked. In voltage clamp, E_{GABA_A} was taken as the intersection of peak GABA current and baseline current plotted against voltage. Baseline current was measured as the current flowing at the same time as the peak GABA response by repeating the protocol in the absence of GABA and/or by extrapolation from monoexponential fits of currents flowing before and after the GABA response. Voltages errors were corrected according to the equation $V_{corrected} = V_{command} - (I \times R_{series})$.



Measurement of hyperpolarization required for rebound burst firing

Injections of varying amounts of hyperpolarizing current were made for 500 ms and the maximum degree of hyperpolarization during a pulse was measured. A "rebound burst" after removal of negative current was defined as a burst that contained one or more intervals that were at least three times shorter than those associated with spontaneous activity. The threshold for rebound burst firing was defined as the minimum value of peak hyperpolarization that preceded a rebound burst.

Statistical comparisons were made using the Mann-Whitney *U* test. Probability values of <0.05 were considered significant. Data are expressed as means ± SD.

RESULTS

Stable series resistances of between 25 and 75 MΩ were obtained 40–60 min after sealing. E_{GABA_A} was determined using current clamp (-78 ± 5 mV, $n = 20$) and in most cases also using voltage clamp (-78 ± 4 mV, $n = 15$). Similar values were obtained with the two techniques ($P = 0.85$), and the difference in E_{GABA_A} in individual neurons was small (2 ± 1 mV, $n = 15$). The whole-cell configuration was established after perforated recording in six cells. In these cases, E_{GABA_A} shifted significantly toward more positive values (Fig. 1, *B* and *C*: $P = 0.004$; -77 ± 6 mV, perforated; -52 mV ± 6 mV, whole-cell) predicted by the Nernst equation (-42 mV). This observation confirmed that E_{GABA_A} was measured using the perforated configuration. E_{GABA_A} was not altered by the application of the carbonic anhydrase inhibitor ethoxyzolamide ($P = 0.58$; -77 ± 6 mV, $n = 6$, control; -79 ± 5 mV, $n = 6$, ethoxyzolamide); this suggests that neurons were not chloride-loaded by the protocol and E_{GABA_A} was dominated by chloride gradient (Staley et al. 1995). The response of subthalamic neurons to GABA were due solely to actions at $GABA_A$ receptors because the $GABA_A$ antagonist bicuculline (30 μM) abolished responses ($n = 4$).

Rebound burst firing was observed in 17 of 20 neurons (Figs. 2 and 3). The threshold for rebound bursts was -78 ± 3 mV ($n = 17$). Neurons fired either short (Figs. 2, *A* and *C*, and 3*D*, $n = 12$) or long duration bursts (Fig. 2*B*, $n = 5$). E_{GABA_A} was equal to, or more negative than, the threshold for rebound burst firing in 14 of the 17 neurons that fired rebound bursts (Fig. 3). E_{GABA_A} and burst thresholds were not significantly different at the two recording temperatures (E_{GABA_A}: $P = 0.25$; -77 ± 5 mV, $n = 11$, 30–32°C; -80 ± 4 mV, $n = 9$, 35–37°C. Burst threshold: $P = 0.34$; -78 ± 3 mV, $n = 11$, 30–32°C; -77 ± 3 mV, $n = 6$, 35–37°C).

DISCUSSION

These data suggest that E_{GABA_A} in subthalamic neurons is sufficiently hyperpolarized for $GABA_A$ receptor-mediated syn-

FIG. 1. Measurement of E_{GABA_A} in subthalamic neurons. *A*: GABA was directed toward the soma of recorded neurons. *B*: the perforated configuration allows the regulation of intracellular anions (A^-) and divalent cations by the recorded neuron. In contrast, the intracellular concentration of all ions and neutral molecules is dominated by the pipette solution when the whole-cell configuration is established. *C*: perforated recording of a subthalamic neuron followed by a whole-cell recording. Because the pipette solution contained a high [Cl^-], this produced a depolarizing shift in E_{GABA_A} that can be observed from the size and direction of the peak current evoked by GABA at various holding potentials in the two configurations (perforated $E_{GABA_A} = -77$ mV; whole-cell $E_{GABA_A} = -54$ mV).

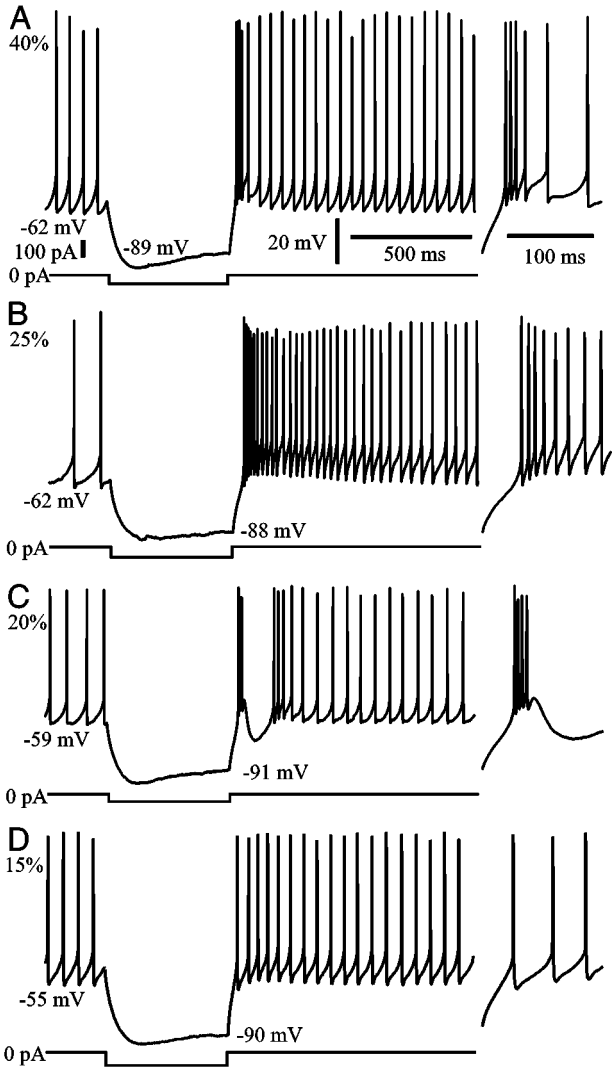


FIG. 2. Heterogeneity of rebound burst firing in subthalamic neurons after the removal of hyperpolarizing current. *A–D*: rebound bursts were of 2 types. The most common was of short duration (<100 ms; *A* and *C*) and was followed immediately by spontaneous firing (*A*) or by a deep afterhyperpolarization (*C*), which was sometimes followed by a second, weaker rebound (*C*). The less common type had long duration rebound bursts (several hundred ms; *B*). Some neurons did not display rebound bursts (*D*). The rebound is shown at a larger time scale to the right of each figure. Voltages to the *left* and the *right* refer to the first point and the peak hyperpolarization, respectively. Scale bars in *A* apply to *A–D*.

aptic potentials to produce rebound burst firing. The value of E_{GABA_A} and the magnitude and duration of hyperpolarization required for burst firing suggest that sufficient hyperpolarization could only be generated by synchronous barrages of GABAergic synaptic potentials. During normal movement, sufficient hyperpolarization is unlikely to occur as subthalamic and pallidal neurons discharge asynchronously in an irregular single spike or burst mode (Nini et al. 1995; Wichmann et al. 1994). Under these conditions, it is likely that burst firing of subthalamic neurons is generated by excitatory drive from the cortex or thalamus and asynchronous feedback inhibition from the globus pallidus acts to limit or time action potential generation. In contrast, in idiopathic and models of Parkinson's disease, the activity of subthalamic and pallidal neurons becomes highly correlated and rhythmic bursting activity

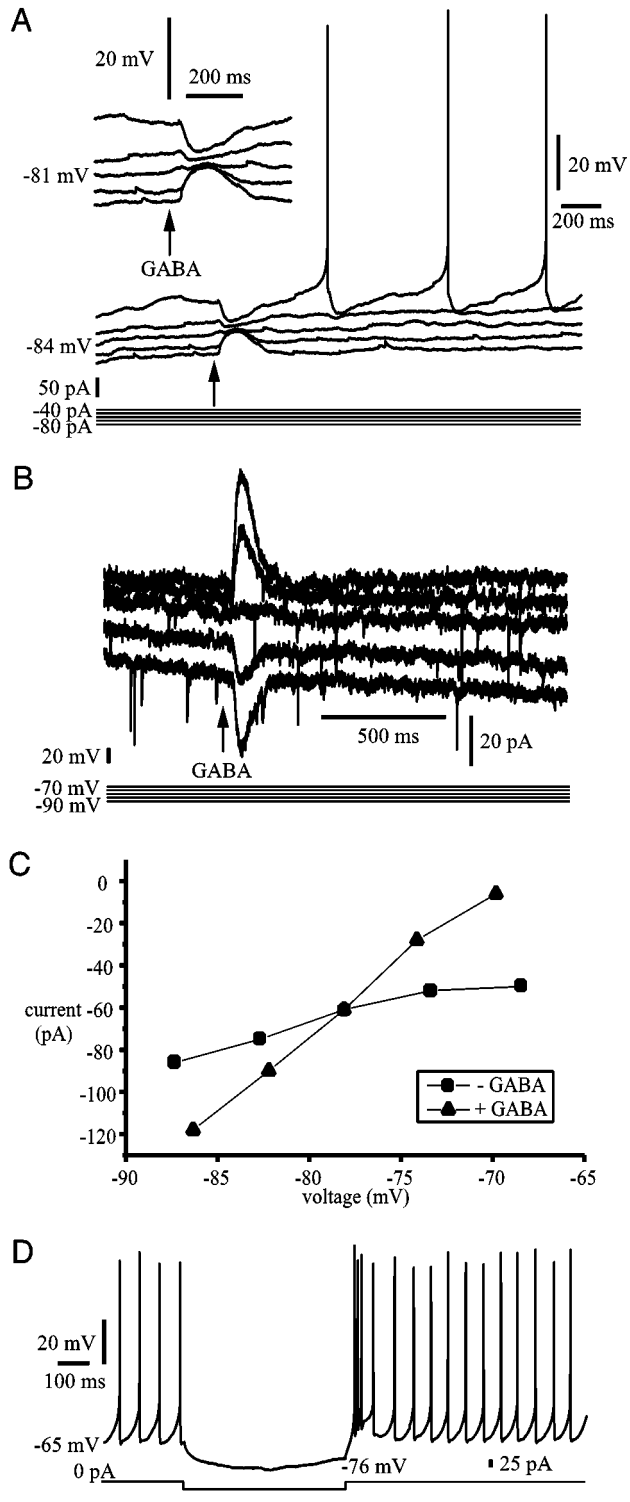


FIG. 3. E_{GABA_A} was more hyperpolarized than burst thresholds in subthalamic neurons. *A*: current-clamp recordings demonstrating the depolarizing and hyperpolarizing responses of subthalamic neurons to the application of GABA (↑). E_{GABA_A} was measured from these recordings as −79 mV (*A*). *B*: voltage-clamp recordings demonstrating the reversal of GABA$_A$ current. Note the slowly increasing inward baseline current, which is due to the activation of hyperpolarization activated cationic current at potentials below −70 mV (Bevan and Wilson 1999). *C*: plots of peak GABA$_A$ current and current that flowed in the absence of GABA application against voltage (point of intersection: −78 mV). *D*: removal of hyperpolarizing current while holding the membrane potential close to E_{GABA_A} elicited rebound burst firing.

emerges within the network (Bergman et al. 1994; Nini et al. 1995; Rodriguez et al. 1998). Under these conditions, synchronous GABAergic inputs from the globus pallidus may generate rebound firing in subthalamic neurons and oscillatory network behavior underlying tremor may emerge in a manner similar to that described in the thalamus (McCormick and Bal 1997; Plenz and Kitai 1999).

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Address for reprint requests: M. D. Bevan, MRC Anatomical Neuropharmacology Unit, Mansfield Road, University Department of Pharmacology, Oxford OX1 3TH, UK.

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