GABA<sub>A</sub> Mediated Afterdepolarization in Pyramidal Neurons From Rat Neocortex

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Cerne, R. and W. J. Spain. A GABA<sub>A</sub> mediated afterdepolarization in pyramidal neurons from rat neocortex. J. Neurophysiol. 77: 1039–1045, 1997. We report a novel slow afterdepolarization (sADP) in layer V pyramidal neurons when brain slices from somatosensory cortex are perfused with γ-aminobutyric acid (GABA). Whole cell recordings were made from visually identified neurons in slices from 3- to 5-wk-old rats. The firing of action potentials at 100 Hz for 1 s, evoked by a train of brief current pulses, typically is followed by a slow afterhyperpolarization (sAHP). When GABA (1 mM) was applied to the perfusate, the sAHP was replaced by a sADP of ~18 mV in amplitude, which on average lasted for 26 s. The sADP was not evoked or terminated as an all-or-none event: it grew in amplitude and duration as the number of evoked action potentials was increased; and when the sADP was interrupted with hyperpolarizing current steps, its amplitude and duration were graded in a time- and voltage-dependent manner. The sADP did not depend on Ca<sup>2+</sup> entry into the cell: it could be evoked when bath Ca<sup>2+</sup> was replaced by Mn<sup>2+</sup> or in neurons dialyzed with 20 mM bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid. We hypothesized that the sADP was generated predominantly in the dendrites because it was associated with the firing of small-amplitude action potentials that continued after the somatic membrane potential was repolarized to ~70 mV by steady current injection. We tested this hypothesis by evoking the sADP in neurons with surgically amputated apical dendrites. In those neurons, the average duration of the sADP was 78% shorter than in neurons with an intact apical dendrite and there were no associated small action potentials. The sADP also was evoked by muscimol, but not by baclofen, and was blocked by bicuculline or picrotoxin but not by CGP 35348, indicating that it is mediated through the activation of GABA<sub>A</sub> receptors. Our results suggest that intense activity in the presence of GABA results in a long-lasting enhancement of excitability in the apical dendrite that in turn could lead to amplification of distal excitatory synaptic potentials.

INTRODUCTION

The principal role of γ-aminobutyric acid (GABA) in neocortex is as an inhibitory neurotransmitter (Connors 1992). The inhibition results from fast and slow hyperpolarizing synaptic potentials (associated with a conductance increase), which are mediated through GABA<sub>A</sub> and GABA<sub>B</sub> receptors, respectively, (Avoli 1986; Benardo 1994; Connors et al. 1988; Howe et al. 1987; Kang et al. 1994; Kawaguchi 1992; Knezevic and Schwartz 1967; McCormick 1989; van Brederode and Spain 1995).

GABAergic activity in neocortex can be sustained and intense. The smooth interneuron (one source of GABA release in neocortex) (Kawaguchi and Kubota 1993; Meinecke and Peters 1987) can maintain discharge rates >200 Hz (McCormick et al. 1985). Upon release from presynaptic terminals, the concentration of GABA reaches >500 μM in the synaptic cleft (Jones and Westbrook 1995; Maconochie et al. 1994).

When high concentrations of GABA are applied near the soma of neocortical pyramidal neurons (Weiss and Hablitz 1984) or when GABA is applied focally to the distal dendrites (Connors et al. 1988; Scharfman and Sarvey 1985), a prolonged GABA<sub>A</sub>-mediated depolarizing response develops. A similar depolarization has been observed during high-frequency inhibitory synaptic activity in the hippocampus (Grover et al. 1993; Staley et al. 1995). Although the depolarization is thought to result in decreased excitability by acting as a current shunt, it recently was shown to increase the excitation caused by N-methyl-D-aspartate (NMDA) receptor activation (Staley et al. 1995).

Here we report that sustained application of GABA to slices of neocortex results in a prolonged afterdepolarization, which replaces the slow afterhyperpolarization that normally follows repetitive firing. This afterdepolarization is associated with increased excitability in the apical dendrite. We have reported some of these results previously in abstract form (Cerne and Spain 1995).

METHODS

Experiments were performed on layer V pyramidal neurons in slices from rat sensorimotor cortex. Three- to five-week-old Sprague-Dawley rats were anesthetized with intraperitoneal injection of ketamine and xylazine. Coronal slices (300 μm) were cut on a Vibratome and maintained at 35°C in a carbogenated (95% O<sub>2</sub>-5% CO<sub>2</sub>) Ringer solution containing (in mM) 130 NaCl, 3 KCl, 2 CaCl<sub>2</sub>, 2 MgCl<sub>2</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, and 10 dextrose (pH 7.4). Slices were transferred to a submerged-type of recording chamber (volume, 1 ml) and perfused at 3 ml/min with the carbogenated Ringer solution (33 ± 1°C). Recordings were made from somata of visually identified neurons 10–80 μm deep in the slice using infrared video-microscopy (Stuart et al. 1993). All experiments were done in the whole cell configuration, using current clamp in bridge mode with an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA). The electrodes were drawn from 75-μm hematocrit tubing (VWR, San Francisco, CA) using a two-stage puller, coated with Polystyrene Q-dope (GC Electronics, Rockford, IL) to reduce stray capacitance, and the tips heat polished to DC resistance of 1.5–3 MΩ. In most experiments, the internal solution contained (in mM) 130 KCH<sub>3</sub>SO<sub>4</sub>, 2 MgCl<sub>2</sub>, 5 KCl, 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid, 2 NaATP, 0.2 NaGTP and 0.1 ethylene glycol-bis(β-aminomethyl ether)-N,N,N′,N′-tetraacetic acid (pH 7.2). Chemicals were obtained from Sigma except KCH<sub>3</sub>SO<sub>4</sub> from ICN and MgCl<sub>2</sub> from Aldrich.
FIG. 1. \(\gamma\)-Aminobutyric acid (GABA) induces a slow afterdepolarization. A: voltage (top) and current (bottom) records before and during application of GABA. One hundred action potentials were evoked by a train of 3-ms depolarizing current steps (2.7 nA, 100 Hz). GABA was added to perfusate during time indicated by the horizontal line. GABA (1 mM) resulted in a depolarization from resting membrane potential of 24 \pm 1 mV (\(n = 66\)) and a 62 \pm 3% decrease in input resistance (\(n = 54\)). Membrane potential was repolarized to \(-71\) mV by means of constant current injection and a train of 100 action potentials was again evoked. Inset: superimposed voltage records of response to 1-s, \(-100\) pA hyperpolarizing current steps before (○) and during (●) GABA application that were used to monitor input resistance. CGP-35348 (200 μM) was present continuously in superfusing medium.

B: records from another cell of response after 100 evoked action potentials before (control), during 1 mM GABA application (GABA), and after removal of GABA (wash). Timing of 100-Hz stimulus is indicated by a bar above each voltage record. In this and subsequent figures, action potentials are clipped by digitization at scales shown.

All records were corrected for a measured liquid junction potential of 10 mV. Use of modified internal solutions is indicated in the results. In some experiments, 0.5–1% biocytin was included. Slices containing biocytin-filled neurons were processed in whole-mount sections (DAB reaction; Horikawa and Armstrong 1988).

D-2-amino-5-phosphonovaleric acid (APV; 50 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM) were present continuously in the bath solution throughout all experiments [except when excitatory postsynaptic potentials (EPSPs) were evoked]. All other drugs were added directly to the perfusate where indicated. Drug sources were as follows: GABA, bicuculline methiodide, baclofen, and tetrodotoxin (TTX), (Sigma); APV (Cambridge Research Biochemicals); CNQX (Tocris Neuramin); CGP 35348 (a gift from Ciba-Geigy); and picrotoxin (RBI).

Current and membrane potential were low-pass filtered (5 kHz), digitized at 44 kHz, and stored on video cassette tape. The recordings were analyzed off-line using a computer. Measured values are reported as the means \(\pm SE\), and statistical comparisons were performed using a two-tailed Student’s \(t\)-test with significance set at \(P < 0.05\).

RESULTS

Results are based on recordings from 122 layer V pyramidal neurons. Neurons were accepted for analysis if a greater than gigaohm seal was obtained between the electrode and the cell membrane before entering the whole cell configuration, if they had stable resting potentials more negative than \(-60\) mV, if they generated action potentials that overshot 0 mV, and if they fired repetitively to depolarizing current pulses. Average resting membrane potential was \(-74 \pm 0.4\) mV (\(n = 122\)). Average input resistance was 77 \pm 5 MΩ (\(n = 66\)) when measured at the end of small (5–10 mV) hyperpolarizing responses evoked by 1-s negative current pulses.
FIG. 2. Slow afterdepolarization (sADP) does not occur as an all-or-none event. A: in presence of GABA (1 mM), size of sADP increased as number of evoked action potentials was increased. Action potentials were evoked at 100 Hz (a, 25 action potentials; b, 80 action potentials; and c, 100 action potentials). B: in presence of GABA (1 mM), sADP was evoked as in Fig. 1A. Voltage records (a–c) show a graded decrease in amplitude and duration of sADP when it was interrupted by 1-s hyperpolarizing current steps of increasing magnitude. Current records are shown superimposed (bottom). sADP had a similar graded decrement in size when duration of a given hyperpolarizing current step was increased (not shown).

A GABA induced slow afterdepolarization

Repetitive firing evoked during continuous perfusion with GABA caused the slow-afterhyperpolarization that normally follows repetitive firing to be replaced by a long-lasting afterdepolarization associated with repetitive discharge of action potentials. Figure 1A illustrates the protocol that was used to characterize this GABA-induced slow-afterdepolarization (sADP). A control response was obtained by evoking a train of 100 action potentials in response to 3-ms depolarizing current steps delivered at 100 Hz. As previously shown, the action potentials are followed by a medium and slow afterhyperpolarization (mAHP and sAHP; Fig. 1B) (Schwindt et al. 1988b). Perfusion with GABA, resulted in a prolonged depolarization of membrane potential (Fig. 1A). The neuron was repolarized to the control resting potential by means of constant current injection, and the train of 100 action potentials was evoked again. The entire afterhyperpolarization was replaced by a sADP (Fig. 1, A and B). In the presence of GABA, the sADP also could be induced after a train of antidromic action potentials (n = 6, not shown), a train of suprathreshold EPSPs (n = 2, not shown), or a 1-s step of membrane potential to ∼0 mV that inactivated sodium dependent action potentials (n = 11; Fig. 3B). The sADP typically reverted to a sAHP within 3–7 min of washout of GABA from the perfusate (Fig. 1B).

To test whether the size of the sADP depends on GABA concentration, we evoked 100 action potentials at 100 Hz (as shown in Fig. 1A) in the presence of two different bath concentrations of GABA. In 100 μM GABA, the sADP duration was 3.1 ± 1.1 s (n = 14; peak voltage 8.0 ± 1.6 mV), whereas with 1 mM GABA, the duration was 26 ± 3 s (n = 58) and the peak amplitude reached the threshold for firing (∼18 mV). Thus the duration and amplitude of the sADP depended on the concentration of GABA (e.g., compare A with B in Fig. 5).

At a given concentration of GABA, the size of the sADP also depended on the number of action potentials used to evoke the sADP. It was seen after as few as five evoked action potentials (100 Hz), but grew in both amplitude and duration after more prolonged periods of evoked firing (Fig. 2A). This, however, was not due to intracellular calcium accumulation because the duration of the sADP was not significantly shorter during >30-min perfusion with medium containing nominally 0 calcium (Mn2+ substituted for equimolar Ca2+ in the perfusate, n = 9) or by inclusion of bis-(o-aminophenoxy)−N,N′,N′,N′-tetraacetic acid 10–20 mM in the patch pipette (n = 5). Consistent with these results, the sADP also demonstrated both voltage and time dependence when interrupted by hyperpolarizing current steps (Fig. 2B).
FIG. 3. Tetrodotoxin (TTX)-sensitive action potentials during sADP originate remote from soma. A: in presence of 1 mM GABA, sADP was evoked as in Fig. 1A. During sADP, membrane potential was hyperpolarized to approximately equal to −73 mV by constant current injection. Continuous firing of action potentials during sADP (a) and after membrane repolarization (b) are shown using a faster sweep rate in correspondingly labeled traces (bottom). During GABA but before evoking sADP, an action potential was evoked by a brief somatic current pulse (c). Current required to generate this action potential was 220 pA larger than current required to generate an action potential in same cell before GABA application (also, action potential threshold moved 12 mV positive in GABA). Note that threshold of evoked action potential is positive to potential from which sADP-associated action potentials arose.

B: another slice was perfused with nominally calcium-free Ringer solution (MnCl₂ substituted for CaCl₂) for 21 min. GABA (1 mM) then was added to the bath. Two superimposed sweeps are shown (before and during addition of 1 μM TTX to bath) of membrane response after a 1-s depolarizing current step. Decreased the duration of the sADP to 6.6 ± 2.5 s compared with 15.6 ± 4.0 s before TTX was added (n = 5) and there were no associated small action potentials (Fig. 3B).

Long time-course of the sADP depends on an intact apical dendrite

As shown in Fig. 3A, the sADP was associated with firing of action potentials with small and variable amplitude. When the sADP was repolarized to the resting potential by constant somatic current injection, the action potentials continued and arose directly from the baseline voltage, suggesting that they have a dendritic origin (Wong and Stuart 1992).

The small action potentials occurred during perfusion with nominally 0 calcium perfusate in nine of nine cells, indicating that they are not calcium spikes. However, when the typical sADP was evoked after a 1-s depolarizing step of membrane potential to 0 mV in perfusate containing GABA (1 mM) and 0 calcium, the subsequent addition of TTX decreased the duration of the sADP to 6.6 ± 2.5 s compared with 15.6 ± 4.0 s before TTX was added (n = 5) and there were no associated small action potentials (Fig. 3B).

To test if there was a dendritic origin for the sADP and the small action potentials, we amputated the apical dendrite with a scalpel ≈70 μm from its origin at the soma (Fig. 4A). In control perfusate, the amputated neurons had normal appearing repetitive firing and sAHPs in response to depolarizing steps of current (Fig. 4B). Average resting potential was −73 ± 1 mV, similar to neurons with intact dendrites, and average input resistance was 91 ± 16 MΩ. We then performed the same protocol shown in Fig. 1A (GABA 1 mM). The sADP was significantly shorter in duration (5.8 ± 1.6 s, n = 5; Fig. 4C) when compared with the sADP evoked in neurons with an intact apical dendrite (P < 0.01).
**sADP is mediated by activation of GABA A receptors**

To determine the type of GABA receptor involved in generation of the sADP, we tested the effect of the GABA A agonist, muscimol, the GABA A antagonists bicuculline and picrotoxin, and the GABA B agonist, baclofen, and antagonist CGP-35348. Muscimol (100 μM) caused a sADP after a train of 100 action potentials (100 Hz) that was not statistically different from the results shown above for 1 mM GABA (amplitude = 17.5 ± 4.9 mV; duration = 24.2 ± 13.6 s; n = 4). Bicuculline (20 μM) blocked the induction of the sADP by GABA (100 μM) in 4/4 neurons tested (Fig. 5A). Upon washout of bicuculline, the sADP could again be evoked. The duration of the sADPs was reduced markedly when picrotoxin (100–500 μM) was added to GABA (1 mM) (GABA alone: 21.2 ± 8.6 s; GABA plus picrotoxin: 3.7 ± 0.7 s; n = 12, Fig. 5B). The presence of CGP-35348 (0.2–1 mM, n = 24), however, did not prevent the induction of the sADP by either 100 μM or 1 mM GABA (note that 200 μM CGP-35348 was present during the experiment shown in Fig. 1A). Perfusion with baclofen (50 and 500 μM, n = 3) did not result in a sADP after 1 s of evoked 100 Hz firing (not shown). These results indicate that activation of GABA A receptors was necessary to evoke the sADP.

**DISCUSSION**

During sustained activation of GABA A receptors, an evoked train of action potentials was followed by a sADP associated with prolonged and continuous firing of small-amplitude action potentials. Long-lasting afterdepolarizations have been observed in layer V pyramidal neurons in response to muscarine (Andrade 1991; Schwindt et al. 1988a), serotonin (Spain 1994), and glutamate (Greene et al. 1994). The ionic mechanism of those ADPs is clearly distinct from the one reported here because the former all depend on calcium entry for their expression whereas our results indicate that the GABA-induced sADP is calcium independent.

The sADP could not maintain its characteristic prolonged time course in the presence of TTX or in neurons with amputated apical dendrites. These results suggest that the size of the sADP was enhanced by the activation of sodium channels located in the apical dendrite (Huguenard et al. 1989; Kim
and Connors 1993; Schwindt and Crill 1995; Stuart and Sakmann 1994). However, the ionic mechanism underlying the sADP is unknown and will require further study to be determined unequivocally.

**Implications for synaptic transmission**

Although the precise function of the GABA-induced sADP is unknown, we suggest the following. Recent studies show the presence of voltage-dependent calcium channels on the apical dendrite of neocortical pyramidal cells (Kim and Connors 1993; Markram and Sakmann 1995; Yuste et al. 1994) as well as voltage-dependent sodium channels (referenced above). The presence of prolonged dendritic depolarization during sustained GABAergic activity could increase the activation of those channels (or bring them closer to activation threshold), which in turn could increase the gain of distal excitatory synaptic inputs. Indeed, our data strongly suggest that the sADP activates dendritic sodium-dependent action potentials. Likewise, through the activation of dendritic calcium channels, the sADP could provide a pathway for sustained calcium entry long after glutamatergic excitatory inputs had ceased.

In the present study, the entire neuron was bathed in GABA; consequently, there was an increase in somatic chloride conductance (Thompson et al. 1988) and thus shunting of current flowing from the apical dendrite to the initial segment (i.e., spike generating region) (Stuart and Sakmann 1994). It remains to be tested if selective activation of GABAergic inputs to the apical dendrite could result in a sADP, without an increase in somatic chloride conductance. In such a case, we predict that the sADP would depolarize sufficiently the initial segment to cause action potentials that would be transmitted down the axon.

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