Excitatory GABAergic Effects in Striatal Projection Neurons

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Bracci, Enrico and Stefano Panzeri. Excitatory GABAergic effects in striatal projection neurons. J Neurophysiol 95: 1285–1290, 2006. First published October 26, 2005; doi:10.1152/jn.00598.2005. The ability of synaptically released GABA to facilitate action potential generation in striatal projection neurons was studied in brain slices using current-clamp, gramicidin-perforated whole cell recordings. Evoked GABAergic postsynaptic potentials (PSPs) were pharmacologically isolated from ionotropic glutamate receptor antagonists. Subthreshold depolarizing current injections were paired with GABAergic PSPs at different intervals. GABAergic PSPs were able to convert current injection-induced depolarizations from subthreshold to suprathreshold, but only when they preceded the current injection by an appropriate interval; accordingly, action potentials were observed 4–140 ms after the onset of the GABAergic PSP, and their likelihood was maximal after 50–60 ms. The GABAergic excitatory effects were fully blocked by the GABA_A receptor antagonist bicuculline. Appropriately timed GABA PSPs decreased the time taken by current injections to depolarize projection neurons, causing an apparent reduction in the spike threshold. In control solution, the ability of evoked PSPs (comprising both glutamatergic and GABAergic components) to reach spike threshold was often impaired by bicuculline. We conclude that GABAergic PSPs can exert excitatory effects on projection neurons and that this ability crucially depends on the timing between the GABAergic event and a concomitant depolarizing input.

INTRODUCTION

The basal ganglia are involved in motor control and cognitive processes (Brown et al. 1997; Graybiel 1995; Schultz et al. 2003). The striatum is the main input nucleus of the basal ganglia, receiving glutamatergic inputs from all cortical areas and from the thalamus (Bolam et al. 2000). While the function of the striatal networks is far from being satisfactorily understood, it has been proposed that a major task consists in detecting cortical representations of sensory events to trigger appropriate motor responses (Gillies and Arbuthnott 2000; Redgrave et al. 1999; Wilson 2000). Striatal projection neurons are medium-sized, spiny, GABAergic cells (MSs) and control the activity of the basal ganglia output nuclei (Parent et al. 2000). In turn, MSs receive GABAergic inputs from several neuronal sources, including other MSs (through local axon collaterals), and two classes of striatal interneurons (Tepper and Bolam 2004; Tepper et al. 2004). GABAergic connections among MSs have attracted strong computational interest, because they could create competitive dynamics that may provide an efficient code for classification of cortical inputs (Plenz and Kitai 2000; Wickens and Oorschot 2000). Interneuronal GABAergic inputs to MSs are also important, as the interneurons receive strong excitatory inputs from the cortex (Plenz 2003; Tepper and Bolam 2004), and can effectively delay MS firing (Koos and Tepper 1999).

GABAergic inputs to MSs have been traditionally considered inhibitory. Nevertheless, GABA_A receptor activation can exert excitatory effects on central neurons (Bracci et al. 1999, 2001; Cherubini et al. 1991; Gulledge and Stuart 2003). If GABAergic inputs excited, rather than inhibited, MSs under certain conditions, this would have important implications for our understanding of the dynamics of the striatal networks. Therefore we tested whether synaptically released GABA can exert excitatory effects on MSs.

Postsynaptic GABA effects critically depend on transmembrane chloride gradient (Cherubini et al. 1991). To prevent artifacts, we used gramicidin-perforated whole cell recordings, which do not perturb intracellular chloride (Kyrozis and Reichling 1995).

METHODS

Electrophysiological procedures

Male Wistar rats (18–28 days postnatal) were killed by cervical dislocation (in accordance with the UK Animals Act 1986), and coronal brain slices (300 μm thick) were maintained at 25°C in an oxygenated solution (composition in mM: 126 NaCl, 2.5 KCl, 1.3 MgCl_2, 1.2 NaH_2PO_4, 2.4 CaCl_2, 10 glucose, and 18 NaHCO_3). For infrared-visualized recordings (Bracci et al. 2003), slices were transferred to a submerged chamber and continuously superfused (2–3 ml/min) at 25°C.

Gramicidin-perforated, whole cell recordings were obtained with patch pipettes (2–5 MΩ) filled with a solution containing (in mM) 125 KCl, 10 NaCl, 1 CaCl_2, 2 MgCl_2, 1 BAPTA, 19 HEPES, 0.3 guanosine triphosphate, and 2 Mg-adenosine triphosphate and adjusted to pH 7.3 with KOH. Gramicidin was dissolved in dimethylsulfoxide (10 mg/ml) and diluted in the intrappetite solution to a final concentration of 10–15 μg/ml. The pipette tip was filled with gramicidin-free solution. The perfusion process lasted 20–40 min and was monitored with current injections. The perfusion was considered complete when 1) the amplitude of the action potentials was steady and >90 mV and 2) whole cell access resistance (measured with bridge balance) was steady and <50 MΩ. Accidental rupture of the membrane was detected because of a sudden decrease in the access resistance (which was regularly monitored and compensated with bridge balance) and a small but detectable increase (5–10 mV) in the amplitude of the action potentials. When this happened, the experiment was immediately terminated. Furthermore, at the end of each experiments, the membrane was ruptured by suction; in all cases, we were able to observe a sudden decrease in access resistance and a sudden increase in spike amplitude. As a further control, we did experiments with high chloride intrapipette solution (in which K^+-glucuronate was substituted with equimolar KCl) in the presence of
RESULTS

Gramicidin-perforated whole cell current-clamp recordings were obtained from 31 MSs identified based on their distinctive electrophysiological properties (Nisenbaum et al. 1994). Resting membrane potential (RMP) was $-81 \pm 4$ mV, whereas input resistance (measured with small negative current steps) was $161 \pm 39$ MΩ. Evoked GABAergic potentials were pharmacologically isolated by bath application of the ionotropic glutamate receptor antagonists 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) (10 μM) and D-(-)-2-amino-5-phosphono-pentanoic acid (d-AP5) (10 μM). Under these conditions, a single electrical stimulus delivered within the striatum evoked a depolarizing PSP that peaked after $16 \pm 4$ ms and was completely abolished by the GABA<sub>A</sub> receptor antagonist bicuculline (10 μM). The reversal potential for this GABAergic PSP (measured by polarizing the neuron at different levels) was $-64 \pm 4$ mV. Evoked GABAergic potentials per se did not elicit action potentials at any stimulation intensity. Increasing the stimulation intensity often triggered very short latency (<1 ms) action potentials, which were not abolished by bicuculline and seemed to be caused by direct activation of the recorded neuron.

We used trains of stimuli (2 Hz for $\leq 10$ min). The first five stimuli of a train often evoked PSPs significantly larger than the following ones and were therefore excluded from the analysis. After the first five stimuli, however, the amplitude of the GABAergic responses became steady. This was tested systematically in five MSs: in each cell, five to seven trains of stimuli at 2 Hz (2–10 min, separated by intervals >3 min) were delivered in the absence of current injections. The PSPs amplitudes after the fifth stimulus did not display a statistically significant trend of variation as a function of time (linear least squares fittings always had a slope <0.005 mV/s, never significantly different from 0; $P > 0.3$; bootstrap test). An example of this phenomenon is shown in Fig. 1A, where the amplitudes of the first 160 responses of a train are plotted versus time. The intrinsic variability of the evoked PSPs in a train (after the 5th stimulus) was measured by the CV of the PSP amplitude, which was $24 \pm 8\%$. The average PSP amplitude was $5.1 \pm 1.4$ mV.

To reveal possible facilitatory effects of GABAergic PSPs, we paired electrical stimuli with injections of positive current (100-ms duration), which elicited a subthreshold depolarization when delivered in isolation. The amplitude of the current injected was tuned in the preliminary part of the experiment to obtain subthreshold depolarizations that were close to threshold but did not elicit any action potential when applied at 2 Hz (in the absence of synaptic stimuli) for 1–3 min. After this procedure, the current injections remained fixed for each experiment. On average, the amplitude of these current-induced subthreshold depolarizations was $39 \pm 7$ mV. Because it was essential that the RMP of the MSs did not fluctuate during the experiments, we discarded experiments in which it varied by...
>1 mV during the protocol. All the experiments were performed at RMP (no steady current injected).

To test how the timing of the two stimuli affected the evoked responses, we varied the delay between the electrical stimuli and the onset of the current injections. As shown in the representative example of Fig. 1B, this protocol revealed that the GABAergic PSPs were able to convert the current-induced depolarization from subthreshold to suprathreshold in all neurons tested (n = 23), but only when the stimulus was delivered during an appropriate time window preceding the current injection. Facilitatory effects were never observed when the current injection preceded the GABAergic PSP. There were cases in which an electric stimulus delivered after the start of the current injection elicited an action potential at very short latency (<1 ms), but these events were caused by direct stimulation of the recorded neuron rather than GABAergic PSPs, because they persisted in the presence of the GABA_A receptor antagonist bicuculline (10 μM, n = 4). Therefore all cases where electrical stimulation elicited a spike at latencies <1 ms were excluded from the analysis.

The expansion of Fig. 1C shows how facilitatory GABAergic effects depended on the timing between electrical stimulation and current injection: when the stimulation was applied 58 ms after the onset of the current injection or 122 ms before the current onset, no action potential was elicited; however, if the current was applied 32 ms after the stimulus, a spike was observed (61 ms after the onset of the GABAergic PSP).

To more closely mimic the time-course of glutamatergic excitatory PSPs (EPSPs), we also used shorter duration (10 ms) current injections. This duration was chosen because it produced a depolarizing waveform similar in time-course to a glutamatergic EPSPs. This is shown in Fig. 2A, where an evoked EPSP (recorded in the presence of 10 μM bicuculline) and the depolarization induced by a current injection (220 pA, 10 ms) are compared. As in the case of longer current injections, we found that, in the presence of ionotropic glutamate receptor blockers, a preceding GABAergic PSP could convert a short current-induced depolarization from sub- to suprathreshold if appropriately timed (as quantified below). An example of this phenomenon is shown in Fig. 2B. The excitatory effects of evoked PSP preceding either long or short current injections were clearly caused by the activation of GABA_A receptors, because they were completely abolished by bath application of bicuculline (n = 8). This is shown in the example in Fig. 2B. In three of eight cases, it was possible to observe a full recovery of the facilitatory GABA effects after 10- to 20-min bicuculline washout.

We also tested whether spikes could be facilitated by GABAergic PSPs when evoked during long-lasting depolarizations. In four MSs, long (3–10 s) subthreshold depolarizations were induced by current injection. In all cases, PSPs evoked during these depolarizations failed to evoke spikes. In all cells tested, some electrical stimuli evoked spikes at very short latency (<1 ms), but these were not blocked by bicuculline (n = 2) and seemed to be caused by direct MS activation rather than synaptic effects.

To quantify the facilitatory effects, we measured the interval between the start of the evoked GABAergic PSP and the onset of the spike evoked by the subsequent current injection. The way this interval (named Δt) was defined is shown in Fig. 3A (shadowed area), for a case when an otherwise subthreshold current injection (gray trace) elicited a spike when preceded by a GABAergic PSP (black trace). The histogram in Fig. 3B shows the distribution of the interval Δt in a sample of 11 MSs in which the delay between the electrical stimulus and the current injection was systematically varied between −250 and 250 ms, in steps of 5 ms (see METHODS). This distribution provides a direct measure of the probability of observing excitatory effects as a function of time from the onset of the PSP. Excitatory GABAergic effects were observed in the range between 4 and 150 ms from PSP onset, and their likelihood was maximal between 50 and 60 ms. In four cells, we estimated the effectiveness of GABAergic facilitation by keeping the interval between the stimulus and the onset of the current fixed at 55 ms and applying trains of 40–100 just-subthreshold current injections at 2 Hz. With this protocol, in each cell, it was possible to obtain spikes in >97% of attempts when the stimulus was delivered, whereas no spike was observed with current injections alone.

We found no significant differences in the distribution of Δt when 10- or 100-ms current injections were used (Kolmogorov-Smirnov test; P < 0.01). This reflects the fact that the excitatory effects were only observed when the GABAergic PSP preceded the current injection and were limited to one.
excitable, and no spike was generated. In all neurons tested, to the current onset). In this case, the cell was apparently less injection alone, but this happened
Interestingly, this voltage level was also exceeded with current as a consequence, reached the level where a spike was trig-
The superimposed traces of Fig. 3 cast light on the dynamics underlying the excitatory GABA effects. The depolarizing trajectory induced by the current started from a more depolar-
gic PSP. These evoked responses can elicit action potentials (Kawaguchi et al. 1989). In three of seven MSs tested, bicuculline converted suprathreshold-evoked responses into subthreshold ones, as shown in the example in Fig. 4. This shows that a GABAergic PSP can actually facilitate a nearly simultaneous glutamatergic EPSP. The fact that these phenomena was only observed in a fraction of cells probably related to the variable proportion of GABAergic and glutamatergic fibers activated by the stimuli in different experiments.

We also attempted to study whether spikes evoked by suprathreshold current injections were abolished by appropriately timed GABA PSPs in resting MSs. We found that in all cases in which a GABAergic PSPs preceded a suprathreshold current step (10 ms long), it was not able to abolish the action potential, irrespective of the interval between the two. This was tested with similar results in eight cells from eight animals belonging to the three age groups defined above (3 to group I; 2 to group II; 3 to group III). An example of this phenomenon is shown in Fig. 5 (MS from a p19 rat). We attempted to assess the case when the current step preceded the evoked GABAergic PSP. These experiments were, however, hindered by the fact that when a stimulus was delivered during the depolarizing

FIG. 3. Temporal distribution of excitatory GABAergic effects. A: 2 superimposed traces recorded from a MS show effects of a preceding GABAergic PSP on the current-induced depolarization (180 pA, 100 ms). When such a PSP was present (black trace), the injection-induced depolarization was faster, and an action potential was triggered. In the absence of a GABAergic PSP (gray trace), the current-induced depolarization also exceeded the membrane potential level at which an action potential was triggered in the presence of a GABAergic PSP (dashed line), but this happened 5.5 ms later, and no spike was observed. B: distribution of the intervals \( \Delta t \) between the start of the GABAergic PSP and the spike elicited by a subsequent current injection (if present). \( \Delta t \) is positive if the PSP precedes the spike. Interval between the stimulus and onset of current injection varied between \(-250 \) and 250 ms in steps of 5 ms in the neurons used for this analysis.

spike, thus making the residual duration of the current injection irrelevant. Therefore results obtained with long and short injections were grouped together.

Furthermore, we did not find significant age-dependent differences in the range tested (18–28 days postnatal). This was tested by comparing the results obtained with rats belonging to three age groups (group I: p18–p20; n = 4; group II: p21–p23 n = 3; group III: p24–p28; n = 4). No significant differences in the distribution of \( \Delta t \) were found between the different age groups (Kolmogorov-Smirnov test; \( P < 0.01 \)).

The superimposed traces of Fig. 3A cast light on the dynamics underlying the excitatory GABA effects. The depolarizing trajectory induced by the current started from a more depolarized level in the presence of a preceding GABAergic PSP, and as a consequence, reached the level where a spike was triggered much earlier (in reference to the current injection onset). Interestingly, this voltage level was also exceeded with current injection alone, but this happened \(-5.5 \) ms later (with respect to the current onset). In this case, the cell was apparently less excitable, and no spike was generated. In all neurons tested, when traces were aligned to the onset of the current steps, the depolarization was more rapid in the presence of a preceding PSP than with a current injection alone. This was quantified by measuring the time needed to reach a depolarization of 30 mV (measured from resting membrane potential) in a sample of eight cells. This time, measured from current injection onset, was \( 3.7 \pm 1.7 \) ms shorter in the presence of a preceding PSP (evoked 30–70 ms before the current onset) than for current injections alone.

To test that the present excitatory effects of GABA did not depend on functional changes in GABAergic transmission caused by prolonged 2-Hz stimulation, in four cells in which repetitive stimuli had not been applied, we applied five or more stimuli at intervals \( >30 \) s. Each stimulus was followed (after 55 ms) by a current injection that was just subthreshold when delivered in isolation. In all cases, this led to the generation of a spike, similar to the case described above for 2-Hz trains.

It was of obvious interest to test whether GABAergic PSPs could also facilitate glutamatergic EPSPs. While we could not manipulate the timing between EPSPs and GABAergic PSPs, we studied the effects of bicuculline on potentials evoked in control solution, which is comprised of a mixture of glutamatergic and GABAergic PSPs. These evoked responses can elicit action potentials (Kawaguchi et al. 1989). In three of seven MSs tested, bicuculline converted suprathreshold-evoked responses into subthreshold ones, as shown in the example in Fig. 4. This shows that a GABAergic PSP can actually facilitate a nearly simultaneous glutamatergic EPSP. The fact that these phenomena was only observed in a fraction of cells probably related to the variable proportion of GABAergic and glutamatergic fibers activated by the stimuli in different experiments.

We also attempted to study whether spikes evoked by suprathreshold current injections were abolished by appropriately timed GABA PSPs in resting MSs. We found that in all cases in which a GABAergic PSPs preceded a suprathreshold current step (10 ms long), it was not able to abolish the action potential, irrespective of the interval between the two. This was tested with similar results in eight cells from eight animals belonging to the three age groups defined above (3 to group I; 2 to group II; 3 to group III). An example of this phenomenon is shown in Fig. 5 (MS from a p19 rat). We attempted to assess the case when the current step preceded the evoked GABAergic PSP. These experiments were, however, hindered by the fact that when a stimulus was delivered during the depolarizing

FIG. 4. GABAergic facilitation of a glutamatergic EPSPs. In control solution, an electrical stimulus evoked a suprathreshold synaptic response (left). Subsequent application of bicuculline reduced the amplitude of the evoked response and abolished the action potential (right).
and 60 ms. This action is mediated by GABAA receptors and from the onset of the PSP, with maximal effects between 50 current step.

Therefore we were not in the position to assess the inhibitory subcellular chloride dynamics. Consistent with this, when a mixture of GABAergic and glutamatergic PSPs was evoked in a resting MS, they seemed to cooperate to reach spike threshold, as spikes were caused by direct activation of the depolarized MS by the electric stimulus, rather than synaptic delivery. These spikes were caused by direct activation of the depolarized MS by the electric stimulus, rather than synaptic action, because they were not blocked by bicuculline \((n = 4)\). Therefore we were not in the position to assess the inhibitory actions of a GABAergic PSP elicited after a depolarizing current step.

**DISCUSSION**

The main result of this study is the novel demonstration that synaptically released GABA can exert excitatory effects on resting MSs, facilitating spikes in a time window of \(-140\) ms from the onset of the PSP, with maximal effects between 50 and 60 ms. This action is mediated by GABA\(_A\) receptors and was observed with a technique that does not perturb intracellular chloride dynamics. Consistent with this, when a mixture of GABAergic and glutamatergic PSPs was evoked in a resting MS, they seemed to cooperate to reach spike threshold, as subsequent blockade of GABA\(_A\) receptors decreased the probability that a spike was generated.

We could not assess if GABAergic PSPs were able to inhibit firing if triggered when the MS was close to spike threshold, because under the conditions of this study, electrical stimulation tended to evoke directly a spike. However, Koos and Tepper (1999) have shown with paired recordings that GABAergic potentials (originating from fast spiking interneurons) do inhibit MSs when they are triggered close to spike threshold during a depolarizing current injection. Therefore it seems likely that the excitatory action of GABA are confined to conditions when the MS membrane potential is close to resting level (approximately \(-80\) mV). This is a consequence of the large \((10–20\) mV\) negative driving force \((E_m - E_{GABA})\) existing under these conditions for GABA\(_A\) receptors, which are permeable to chloride and bicarbonate (Bracci et al. 2001).

GABA acts as an excitatory transmitter in early postnatal life, because of larger levels of intracellular chloride during the first 2 postnatal wk (Cherubini et al. 1991). We performed experiments on young adults (p18–p28), and in this range, did not find significant age-related differences. It seems therefore unlikely that these excitatory effects of GABA were caused by an immature transmembrane ion distribution.

An appropriately timed GABAergic potential provided an initial depolarization and decreased by a few milliseconds the time taken by a subsequent depolarizing current to drive the MSs to depolarized levels close to threshold. Under these conditions, a spike was often observed. The level at which a spike was triggered in the presence of a preceding PSP was often also increased when the cell was depolarized by a current injection alone, but no spike was observed in the latter case. This presumably reflects a different degree of sodium channels inactivation induced by the membrane potential trajectories in the two cases (Fricker et al. 1999).

Explaining these GABAergic excitatory effects is not straightforward; the membrane shunting associated with the GABAergic PSPs is expected to decrease the amplitude of the current-induced depolarization; the fact that the reversal potential for GABA (-64 mV on average) is markedly more negative than spike threshold is also expected to limit the ability of GABA to facilitate spikes. While realistic numerical simulations are likely to cast light on these observations, it should be noted that, because of the temporal filtering properties of the membrane (quantified by its time constant), the GABAergic depolarization outlasted the associated increase in membrane conductance. Spike facilitation was most likely between 20 and 90 ms from PSP onset. Voltage-clamp experiments in MSs suggest that in this interval the GABAergic conductance is considerably reduced from its peak (Koos et al. 2004). During this period, therefore, the influence of shunting effects and negative reversal potential is limited, and the concomitant depolarizing input is temporally isolated from the increase in GABAergic conductance but not from the residual depolarization (Gulledge and Stuart 2003).

In vivo, MSs receive strong bursts of cortical glutamatergic inputs that quickly shift their membrane potential from rest to a depolarized “Up” level, which is usually just below threshold (Wickens and Wilson 1998). While the Up-states are simultaneous in MSs, the associated spikes are not (Stern et al. 1998), and their timing may be highly informative, as that of their cortical inputs (Panzeri et al. 2001). Our results suggest that if a burst of cortical inputs is shortly preceded by a GABAergic PSP, this will increase the probability of the MS reaching firing threshold at the beginning of the Up-state. On the other hand, even if we were not able to address this issue directly in this study, other results suggest that GABAergic PSPs occurring during the plateau phase of an Up-state would be much less effective in facilitating cell firing.

MSs receive GABAergic contacts mainly from other MSs and two types of interneurons (Tepper and Bolam 2004; Tepper et al. 2004; Tunstall et al. 2002). The facilitatory effects

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**FIG. 5.** GABAergic PSPs do not inhibit action potentials when they precede current injections. A: in the presence of ionotropic glutamate receptors blockers, current injections (230 pA for 10 ms) elicited action potentials in a MS. Black arrow indicates the start of the injection. B: under the same conditions, electrical stimuli (gray arrow) elicited depolarizing GABAergic PSPs. C and D: when the GABAergic PSPs (gray arrow) preceded current injection onset (black arrow) by 9 (C) or 2 ms (D), they failed to abolish the spike.
reported here may be caused by the activation of some subtypes of GABAergic connections but not others. Interneurons tend to synapese on MS somata or proximal dendrites, whereas MSs tend to synapese on MS distal dendrites (Tepper et al. 2004). In the cortex, the ability of distal GABAergic inputs to produce excitatory effects is larger than that of proximal ones (Gulledge and Stuart 2003). If this conclusion can be extended to the striatum, the connections between MSs may be particularly effective at producing excitatory effects. Thus the view that the striatum is governed by competitive dynamics will have to be updated to include the fact that groups of synaptically connected MSs and interneurons could actually cooperate, rather than compete, when they receive appropriately timed glutamatergic inputs.

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References


