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STRIATAL, PALLIDAL, AND PARS RETICULATA EVOKED INHIBITION OF NIGROSTRIATAL DOPAMINERGIC NEURONS IS MEDIATED BY $GABA_A$ RECEPTORS *IN VIVO*

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Abstract—Dopaminergic neurons express both $GABA_A$ and $GABA_B$ receptors and GABAergic inputs play a significant role in the afferent modulation of these neurons. Electrical stimulation of GABAergic pathways originating in neostriatum, globus pallidus or substantia nigra pars reticulata produces inhibition of dopaminergic neurons *in vivo*. Despite a number of prior studies, the identity of the GABAergic receptor subtype(s) mediating the inhibition evoked by electrical stimulation of neostriatum, globus pallidus, or the axon collaterals of the projection neurons from substantia nigra pars reticulata *in vivo* remain uncertain. Single-unit extracellular recordings were obtained from substantia nigra dopaminergic neurons in urethane anesthetized rats. The effects of local pressure application of the selective $GABA_A$ antagonists, bicuculline and picrotoxin, and the $GABA_B$ antagonists, saclofen and CGP-55845A, on the inhibition of dopaminergic neurons elicited by single-pulse electrical stimulation of striatum, globus pallidus, and the thalamic axon terminals of the substantia nigra pars reticulata projection neurons were recorded *in vivo*. Striatal, pallidal, and thalamic induced inhibition of dopaminergic neurons was always attenuated or completely abolished by local application of the $GABA_A$ antagonists. In contrast, the $GABA_B$ antagonists, saclofen or CGP-55845A, did not block or attenuate the stimulus-induced inhibition and at times even increased the magnitude and/or duration of the evoked inhibition. Train stimulation of globus pallidus and striatum also produced an inhibition of firing in dopaminergic neurons of longer duration. However this inhibition was largely insensitive to either $GABA_A$ or $GABA_B$ antagonists although the $GABA_A$ antagonists consistently blocked the early portion of the inhibitory period indicating the presence of a $GABA_A$ component.

These data demonstrate that dopaminergic neurons of the substantia nigra pars compacta are inhibited by electrical stimulation of striatum, globus pallidus, and the projection neurons of substantia nigra pars reticulata *in vivo*. This inhibition appears to be mediated via the $GABA_A$ receptor subtype, and all three GABAergic afferents studied appear to possess inhibitory presynaptic $GABA_B$ autoreceptors that are active under physiological conditions *in vivo*. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: GABA autoreceptor, substantia nigra, bicuculline, saclofen, CGP-55845A, picrotoxin.

The important role of dopaminergic neurons in various neurological and psychiatric phenomena and in the action of stimulant drugs of abuse has made them the focus of intensive study over the past 25 years. For example, pathophysiology of dopaminergic neurons of the ventral mesencephalon (ventral tegmental area, retrorubral field, and substantia nigra pars compacta) is critically involved in movement disorders such as Parkinson's disease,[32] and psychoses such as schizophrenia.[1,7] Much progress has been made investigating the intrinsic properties of dopaminergic neurons,[18,19,35,36,40,58] but our understanding of their afferent control is more limited. Afferent modulation can affect different aspects of dopamine neuron physiology including firing rate and pattern.[19,35,41,65,71]

Although excitatory amino acid-containing afferents have been shown to play an important modulatory role in the control of dopaminergic neuron activity, (e.g., see Refs 54, 60, and for recent review see Ref. 48) as many as 90% of the afferents to substantia nigra are GABAergic, arising principally from the neostriatum and the globus pallidus.[21,28,51,52,53,59,62] Nigrostriatal neurons also receive input from GABAergic projection neurons in substantia nigra pars reticulata through axon collaterals terminating in pars compacta.[9,11,22,65]

Although the striatonigral afferents project most densely to substantia nigra pars reticulata where they appear to terminate preferentially on GABAergic neurons,[21,28] part of the striatonigral pathway has

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Abbreviations: CUSUM, cumulative sum histogram; IPSP, inhibitory postsynaptic potential; PSTH, peristimulus time histograms.

also been shown to synapse directly on dopaminergic neurons of the substantia nigra pars compacta.[2,28,62] Pharmacological studies of the monosynaptic neostriatal input to dopaminergic neurons have generated some conflicting results. Early *in vivo* recording studies showed that striatal stimulation produces monosynaptic inhibitory postsynaptic potentials (IPSPs) mediated by a $GABA_A$ receptor in substantia nigra; however the nigral neurons were not identified in these studies and appear to have been pars reticulata GABAergic neurons.[51,72] More recent *in vivo* intracellular recording studies from identified substantia nigra dopaminergic and non-dopaminergic neurons have also revealed a monosynaptic IPSP evoked by striatal stimulation that is also mediated by a $GABA_A$ receptor.[20] In contrast, activation of D_1 receptors in substantia nigra has been shown to selectively facilitate $GABA_B$ responses elicited by high frequency trains of stimuli in dopaminergic neurons *in vitro*.[4] Since only the striatonigral afferents to nigra are known to express D_1 receptors[27] these data suggest that the striatonigral inhibition is mediated via the $GABA_B$ receptor subtype.[4]

Another well established GABAergic projection to dopaminergic neurons is the pallidonigral pathway.[28,59] Stimulation of globus pallidus produces IPSPs in nigrostriatal dopaminergic neurons.[66,67] Since kainic acid lesions of globus pallidus cause a change in dopaminergic neuron firing pattern similar to local pharmacological blockade of $GABA_B$ receptors, the pallidonigral input was suggested to terminate principally on $GABA_B$ receptors,[65] although this was never demonstrated directly.

A third GABAergic input to nigral dopaminergic neurons arises from the axon collaterals of pars reticulata GABAergic projection neurons.[9,22,23] Stimulation of this pathway by antidromic activation of nigrothalamic or nigrocollicular neurons inhibits dopaminergic neurons in pars compacta, an effect which is eliminated if the recording micropipettes contain the $GABA_A$ antagonist, bicuculline, but not the selective $GABA_B$ receptor antagonist, saclofen, suggesting that this input activates selectively $GABA_A$ receptors.[65] However, the method of drug application in that study precluded consistently obtaining control, drug, and recovery data on single neurons and the conclusions were drawn from separate populations of neurons.

The present study was designed to investigate more directly the receptor subtype(s) mediating GABAergic inhibition of nigral dopaminergic neurons arising from neostriatum, globus pallidus and substantia nigra pars reticulata *in vivo*. $GABA_A$ and $GABA_B$ antagonists were applied by local microPressure application during extracellular recordings of stimulus evoked inhibition of nigrostriatal neurons. Portions of these results have been presented in abstract form.[49]

EXPERIMENTAL PROCEDURES

General surgery

Experiments were carried out on 30 adult male Sprague–Dawley rats (Zivic–Miller) weighing between 225 and 350 g at the time of recording. All animals were housed two to a cage, maintained on a 12 h light–dark cycle and allowed food and water *ad libitum*.

Rats were anesthetized with urethane (1.3 g/kg) administered intraperitoneally. The animals were installed into a stereotaxic frame and the atlanto-occipital membrane was punctured to allow some drainage of the cerebrospinal fluid. All wound margins and points of contact between the animal and the stereotaxic apparatus were infiltrated with lidocaine solution (2%) and xylocaine ointment (5%) respectively. Body temperature was maintained at $37 \pm 1°C$ and the electrocardiogram was monitored on an auxiliary oscilloscope. All animals were treated in strict accordance with guidelines set forth in the USPHS manual "Guide for the Care and Use of Laboratory Animals".

After scalp removal, small burr holes were drilled over the appropriate coordinates for one of two areas for striatum (0.5 or 0 mm anterior to bregma, 3.7 or 3.2 mm lateral to the midline), globus pallidus (1.3 mm posterior to bregma, 3.2 mm lateral to the midline) and thalamus (5.1 mm anterior to lambda, 2.0 mm lateral to the midline) for the insertion of stimulating electrodes. Two different areas of striatum were stimulated in order to determine whether the two regions evoked different responses in dopaminergic neurons. A recording hole approximately 3.0 mm in diameter was drilled above substantia nigra at coordinates 2.1 mm anterior to lambda and 2.0 mm lateral to the midline.

Stimulating electrodes

Bipolar stimulating electrodes consisting of two stainless steel enamel-coated wires (California Fine Wire) approximately 100 μm in diameter with a tip separation of approximately 150 μm were lowered to the appropriate depths below the cortical surface for striatum (4.1 mm for the more anterolateral position or 5.0 mm for the more dorsomedial position), globus pallidus (5.8 mm) and thalamus (6.1 mm) and affixed in place with cyanoacrylate glue and dental cement. The *in vitro* impedances of the stimulating electrodes averaged 10 kΩ. The ventromedial nucleus of thalamus was electrically stimulated in order to activate antidromically substantia nigra pars reticulata projection neurons.[10,24,42] The stimulation of these axons causes an antidromic action potential to spread into the axon collaterals which synapse onto the dopaminergic neurons in pars compacta.[9,65]

Hemitransections

In some cases rats were given a hemitransection three to six days prior to recording just anterior to globus pallidus (see Fig. 1) in order to ensure that responses elicited by pallidal stimulation were due to activation of pallidonigral afferents and not striatonigral fibers passing through globus pallidus. Rats were anesthetized with ketamine (80 mg/kg) and xylazine (15 mg/kg), placed in a stereotaxic frame and a long burr hole was drilled at coordinates 0.5 mm posterior to bregma, 1–5 mm from the midline. A thin metal knife (tip size 11 × 2 mm) constructed from a razor blade was lowered to 9 mm from the cortical surface and passed mediolaterally three times and then withdrawn. The wound was closed and the animals were allowed to recover for at least three days before recording in order ensure that striatonigral fibers had degenerated.

Stimulation

Constant current electrical stimuli were generated with a Winston A-65 timer and SC-100 constant current stimulus

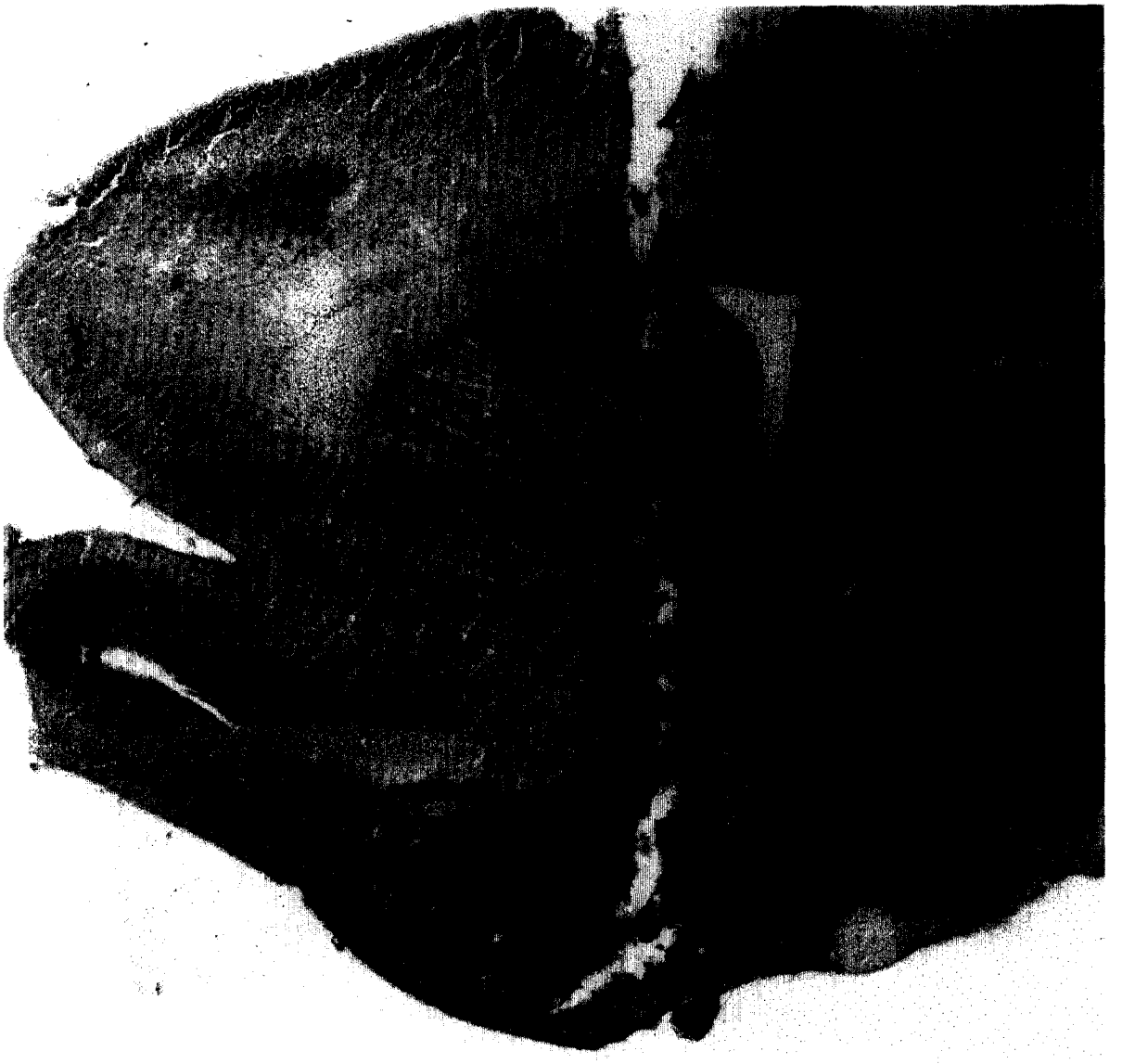

Fig. 1. Digital photomontage of a Neutral Red-stained parasagittal section illustrating the lesion of a hemitransection positioned anterior to globus pallidus.

isolators. Stimuli consisted of single monophasic square wave pulses ranging in intensity from 0.1 to 1.0 mA with a duration between 100 and 500 μs delivered at a rate of 0.67 Hz. Train stimulations consisted of 100–500 μA, 50 to 500 μs pulses delivered at 50 Hz for 150 ms. Trains were delivered at 0.1 Hz. At the end of each experiment, stimulating sites were marked with a small lesion made by passing a 1.0 mA d.c. current for 1 s through the stimulating electrode.

Recording

Recording electrodes were constructed from 2.0 mm outer diameter capillary tubing (World Precision Instruments) on a Narishige PE-2 vertical pipette puller. The electrode tips were broken back under microscopic control to yield final tip diameters between 1.0 and 2.0 μm. These electrodes typically possessed resistances between 5 and 10 MΩ *in vivo*. Micropressure ejection barrels were constructed from 1 mm outer diameter borosilicate glass tubing (Fisherbrand) on a Narishige vertical puller. The tips were then broken back to final tip diameters between 7.5 and 10 μm. These barrels were then heated and bent to approximately 30° from center at approximately 10 mm from the tip. Two bent pressure ejection barrels were glued with epoxy adhesive adjacent to the shaft of the recording electrode and displaced approximately 50–100 μm behind the tip of the recording electrode in order to minimize any artifactual effect of drug diffusing to the cell before pressure ejection. Single-unit extracellular recordings were amplified with a Neurodata IR183 preamplifier and displayed on a Tektronix 5113A storage oscilloscope. All data were recorded on magnetic tape for off-line analysis.

Drug infusions

Drugs (bicuculline methiodide, 200–400 μM; picrotoxin, 200–1000 μM; 2-OH-saclofen, 200–1000 μM; CGP-55845A, 200–400 μM; apamin, 100 μM or eticlopride, 200 μM in 0.9% saline solution, pH=7) were applied locally through the side barrels by pressure ejection for a period of 100 to 500 ms at 20 p.s.i. using a picospritzer (General Valve

Corporation). This resulted in an injected volume of 2–6 nl. The difference in volume ejected between barrel tips from 7.5 μm to 10.0 μm was 1:1.75. Drugs were applied in random order after pre-drug data was collected. All cells responded to the drugs after one or two applications.

Data analysis

Data were analysed off-line with a Macintosh computer equipped with a National Instruments MIO16L multifunction board running custom-designed data acquisition and analysis software (SpikeTrain). The responses elicited by stimulation of neostriatum, globus pallidus and thalamus were characterized by measuring the duration and magnitude of the changes in firing. These responses were measured using peristimulus time histograms (PSTH) and cumulative sum histograms (CUSUM) following the methods described by Ellaway[13] and Tepper *et al.*[65] Briefly, the onsets and offsets of stimulus evoked responses were determined by measuring the change in slope of the CUSUM with a straight line fit before the stimulus and after the slope changed. To ascertain the time of onset of a stimulus-evoked effect, a straight line is fit through the pre-stimulus portion of the CUSUM histogram, and another through the region where the slope changes. The intersection of these two lines represents the time at which the firing rate changes. The offset of stimulus-driven effects is similarly calculated by fitting a third line to the remainder of the CUSUM histogram and the intersection of this line segment with the second line segment is the point at which the effect is no longer detectable, i.e. the offset. These time-points were then superimposed on a standard PSTH constructed from the same data and a cursor driven measurement system was used to calculate the mean number of spikes per bin during the pre-stimulus baseline period and again over the duration of the stimulus effect defined by the CUSUM. The number of spikes per bin were then measured during the response period and for the same amount of time before the stimulus. The change in firing rate is reported as a percent change in firing rate between the prestimulus firing rate and the response period firing rate.[65] All data were analysed with paired *t*-tests at the $P<0.05$ level. All values are expressed as the mean ± S.E.M.

Materials

The selective $GABA_A$ receptor antagonist, bicuculline methiodide was obtained from Sigma, (St Louis, MO), and the chloride channel blocker, picrotoxin, the selective $GABA_B$ receptor antagonist, 2-hydroxysaclofen, the D_2 receptor antagonist eticlopride hydrochloride, and the calcium-activated potassium conductance blocker apamin were obtained from Research Biochemicals Inc., (Natick, MA). The highly selective $GABA_B$ antagonist, CGP-55845A, was a generous gift from Ciba-Geigy (Basel, Switzerland).

Histology

At the end of each experiment, animals were given a lethal overdose of urethane and perfused transcardially with a saline rinse followed by 10% formalin. Brains were postfixed, sectioned, and stained with Neutral Red for verification of stimulating, lesion and recording sites.

RESULTS

Neuronal identification

All 65 neurons in this report were identified as dopaminergic neurons by their spontaneous firing rates (4.70 ± 0.21 spikes/s, $n=65$), regular, random or slow bursty patterns of firing, and extracellular waveform (>2 ms) that often exhibited a notch on the initial positive component corresponding to an initial segment-somatodendritic break (last trace in Fig. 2). In addition, 24 neurons were further identified by their long-latency antidromic response from striatum (13.02 ± 0.62 ms, $n=24$) which usually consisted of an initial segment spike only.[10,24] The remaining neurons could not be antidromically driven but displayed all the other characteristics that are generally accepted as belonging to nigral dopaminergic neurons.[10,24] Both sets of neurons were identical in firing rate, firing pattern distribution, extracellular waveform, and response to striatum, globus pallidus, and thalamus stimulation. Therefore the data were combined into a single group assumed to represent nigral dopaminergic neurons. Antidromic responses from a representative nigrostriatal dopaminergic neuron are illustrated in Fig. 2.

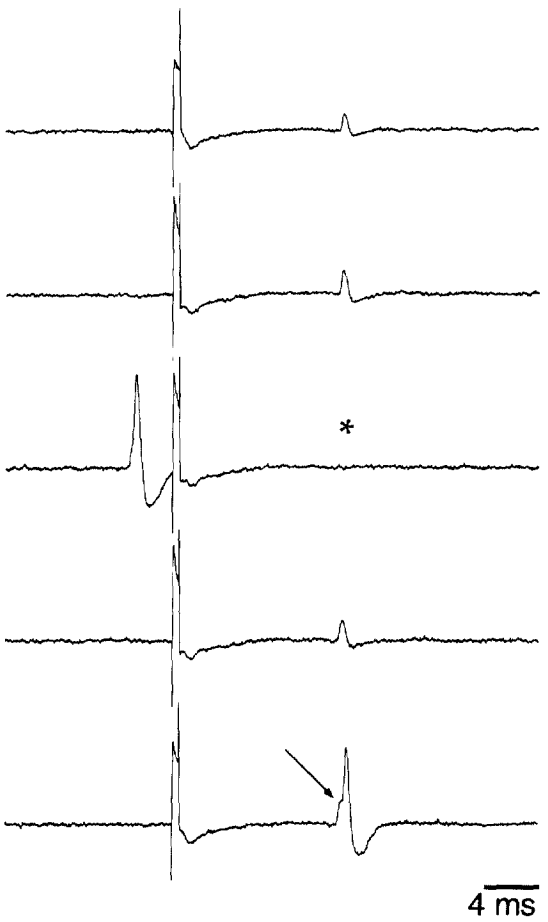

Fig. 2. Antidromic identification of a nigrostriatal neuron. Single pulse stimulation (2.0 mA) of ipsilateral striatum evokes antidromic activation of a nigrostriatal neuron at a long latency (13 ms). Most antidromic spikes consisted of an initial segment spike only as seen in the first, second and fourth traces. A spike duration of greater than 2 ms and a waveform with a notch in the initial positive component (arrow, fifth trace) also characterize the neuron as dopaminergic. Asterisk denotes collision extinction with a spontaneously occurring action potential. Positive is up.

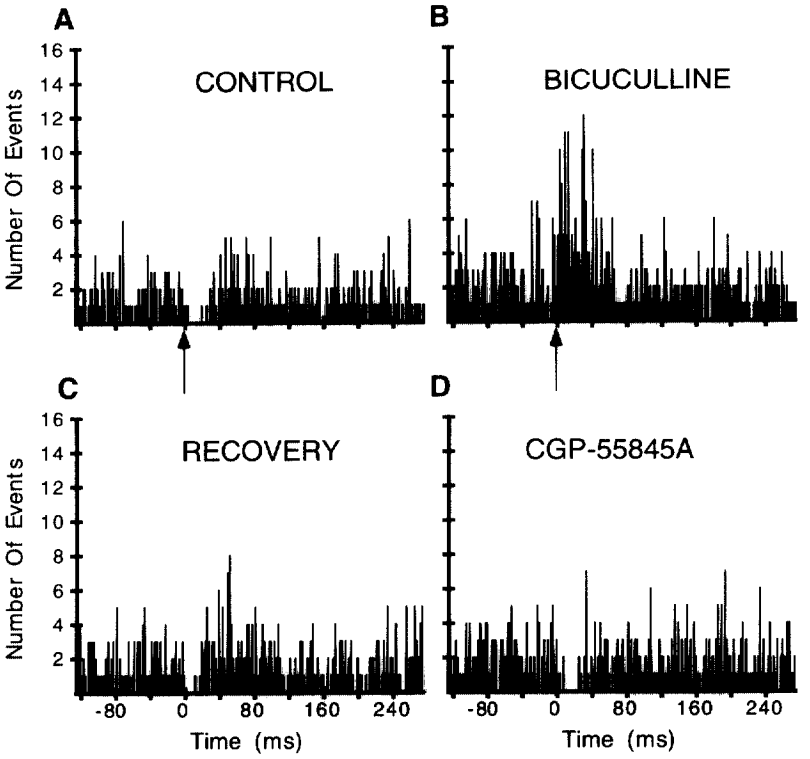

Fig. 3. Effects of striatal stimulation (0.7 mA) on a representative substantia nigra dopaminergic neuron. (A) Striatal stimulation (arrow) typically produced inhibition of dopaminergic neurons (suppression to 23% of pre-stimulus firing rate). (B) Application of the $GABA_A$ antagonist, bicuculline, completely blocks the inhibition revealing a facilitation (209% of pre-stimulus firing rate). (C) The blockade is reversible (suppression to 36% of prestimulus firing rate after waiting for 10 min). (D) The inhibition was not blocked by local pressure application of the $GABA_B$ antagonist CGP-55845A (suppression to 8% of pre-stimulus firing rate). Each PSTH consists of 200 trials with a 2-ms bin width.

Sriatal, thalamic and pallidal stimulation causes inhibition of dopaminergic neurons

Single pulse stimulation of striatum at currents ranging from 0.1 mA to 1 mA inhibited the activity of dopaminergic neurons. No differences in response were observed when either of the two areas of striatum were stimulated and therefore both sets of data were pooled. The mean onset latency for striatal-evoked inhibition was 7.78 ± 2.03 ms and the duration of the inhibition was 34.08 ± 9.29 ms ($n=18$). Identical stimulation of globus pallidus also evoked inhibition with a 5.60 ± 1.25 ms onset latency and duration of 37.36 ± 10.45 ms ($n=23$). Activation of the axon collaterals of pars reticulata neurons by stimulation of the axon terminals of the same neurons in thalamus also produced inhibition of nigrostriatal neurons (1.23 ± 1.00 ms onset latency, 19.36 ± 5.84 ms duration, $n=11$). There were no significant differences in the duration or magnitude of inhibition among any of the stimulation sites ($P>0.05$). Occasionally the inhibitory response was followed by a post-inhibitory rebound excitation which was occasionally followed by a secondary inhibitory period. Initial excitation was never observed following stimulation of striatum, globus pallidus, or thalamus.

Bicuculline and picrotoxin block the responses of dopaminergic neurons to striatal, thalamic and pallidal stimulation

Application of 200–400 μM bicuculline or 200–1000 μM picrotoxin blocked the inhibitory responses to striatal stimulation as shown for one representative neuron in Fig. 3. In all cases, bicuculline and picrotoxin ($n=16$) attenuated or completely blocked the striatal-evoked inhibition. Under control conditions, striatal stimulation depressed firing to $22.19 \pm 4.32\%$ of pre-stimulus baseline levels. After bicuculline or picrotoxin administration, striatal stimulation resulted in an *increase* to $152.26 \pm 34.22\%$ of pre-stimulus values ($t=-3.99$, d.f.$=15$, $P<0.05$; Fig. 3B). $GABA_A$ antagonist application also blocked the inhibitory responses produced by stimulation of globus pallidus (Fig. 4). Under pre-drug control conditions pallidal stimulation depressed firing to $16.08 \pm 2.38\%$ of pre-stimulus values. After bicuculline application pallidal stimulation resulted in an *increase* to $111.79 \pm 85.52\%$ of the prestimulus firing rate ($t=2.53$, d.f.$=14$, $P<0.05$; see Fig. 4). Antidromic activation of pars reticulata projection neurons depressed dopaminergic neuron firing to $28.62 \pm 8.68\%$ of pre-stimulus values. This inhibition was similarly significantly blocked by

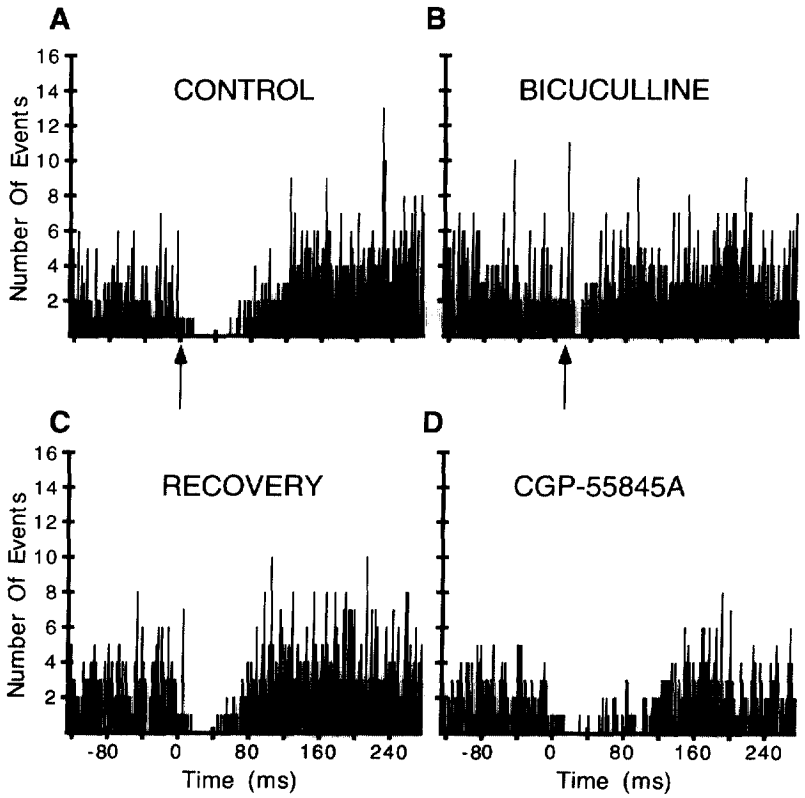

Fig. 4. Effects of globus pallidus stimulation (1.0 mA) on a representative substantia nigra dopaminergic neuron. (A) Pallidal stimulation reliably induces firing inhibition of dopaminergic neurons (suppression to 10% of pre-stimulus firing rate, 59 ms duration) which is completely and reversibly blocked by bicuculline administration (B and C, respectively). (D) Application of the $GABA_B$ antagonist CGP-55845A not only fails to attenuate the evoked inhibition (suppression to 18% of prestimulus firing rate) but instead *increases* the duration (82 ms duration) of inhibition relative to recovery (13% inhibition, 46 ms duration in C) and control (A). Each PSTH consists of 200 trials with a 2-ms bin width.

$GABA_A$ antagonists (90.27 ± 8.50% of basal values; $t=6.14$, d.f.$=6$, $P<0.05$; see Fig. 5).

After $GABA_A$ antagonist administration, five out of 14 neurons exhibited an excitatory response to striatal stimulation (Fig. 3B). Prior to bicuculline administration striatal stimulation depressed activity to 34.6 ± 7.32% of control values. After bicuculline striatal stimulation facilitated firing to 333.90 ± 33.18% of basal levels. Similarly, four out of 15 neurons exhibited facilitation following pallidal stimulation after bicuculline administration. In these four neurons pallidal stimulation depressed firing to 16.35 ± 3.83% of pre-stimulus values. After bicuculline, the firing of these four neurons was facilitated by pallidal stimulation to 447.59 ± 153.83% of baseline values (data not shown). However, stimulation of thalamus after bicuculline or picrotoxin application never revealed an excitatory response.

Bicuculline and picrotoxin application also significantly modified the firing pattern and rate of dopaminergic neurons. The firing rate increased from 4.60 ± 1.44 to 5.77 ± 1.31 spikes/s after bicuculline application while picrotoxin had no effect on firing rate. Both drugs caused dopaminergic neurons to exhibit a dramatic shift from regular or random firing to a bursty firing pattern. These data are discussed more fully elsewhere.[50]

2-Hydroxysaclofen and CGP-55845A do not block the responses of dopaminergic neurons to striatal, thalamic and pallidal stimulation

In contrast to bicuculline or picrotoxin, pressure application of 200–1000 μM 2-hydroxysaclofen or 200–400 μM CGP-55845A did not attenuate the inhibitory response of dopaminergic neurons to stimulation of striatum ($n=10$, Fig. 3), globus pallidus ($n=15$, Fig. 4), or thalamus ($n=9$, Fig. 5).

In some instances, application of a $GABA_B$ antagonist produced an increase in the magnitude and/or duration of the inhibition evoked from stimulation of striatum, globus pallidus, and the projection neurons of substantia nigra pars reticulata presumably due to the antagonistic effects on presynaptic $GABA_B$ receptors. Of the neurons which showed an increase in the stimulus evoked inhibition after application of a $GABA_B$ antagonist, the increase was observed to be significant (striatum: 28.28 ± 6.70% of baseline firing

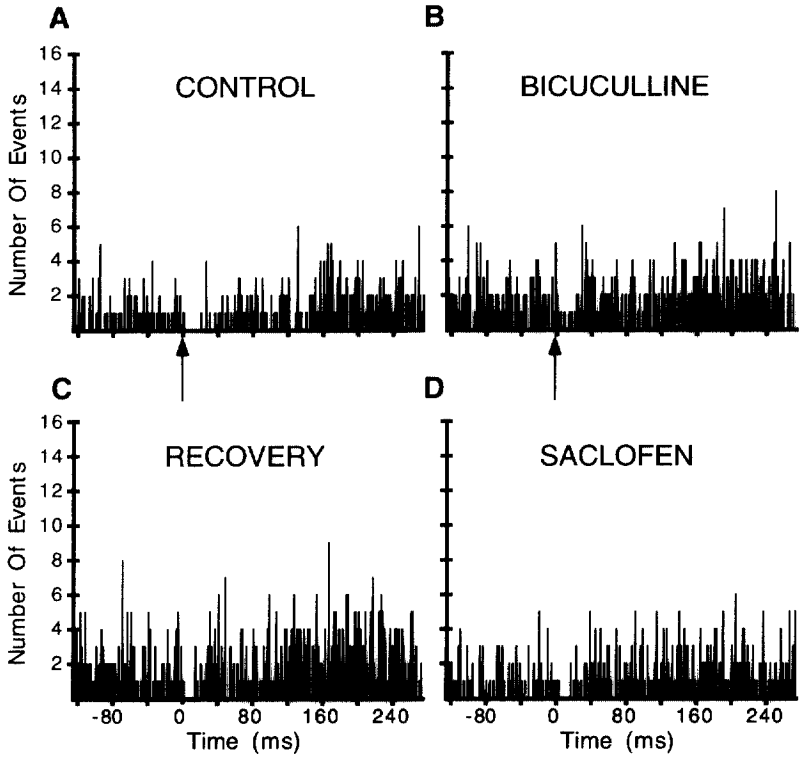

Fig. 5. Effects of antidromic activation of pars reticulata projection neurons on a representative substantia nigra dopaminergic neuron. (A) Antidromic activation of substantia nigra pars reticulata GABAergic projection neurons by thalamic stimulation (1.0 mA) induces a 13% of pre-stimulus firing rate inhibition after stimulation at time=0 with a duration of 27 ms. (B) Application of bicuculline blocks the evoked inhibition reversibly (C). (D) This evoked inhibition is not blocked by application of the $GABA_B$ antagonist saclofen. Each PSTH consists of 200 trials with a 2-ms bin width.

before drug to $14.54 \pm 6.08\%$ after drug application, $t = -3.74$, d.f.=3, $P<0.05$; globus pallidus: from $21.18 \pm 3.61\%$ to $4.61 \pm 3.41\%$, $t = -4.21$, d.f.=5, $P<0.05$). The measured increase in inhibition due to application of a $GABA_B$ antagonist during thalamic stimulation did not turn out to be statistically significant because of the great variability in the percentage of inhibition, but showed the greatest increase of all three stimulation sites (from $55.22 \pm 17.26\%$ of baseline firing before drug to $18.54 \pm 11.45\%$ after drug application, $t=2.29$, d.f.=4, $P>0.05$) and occurred in the highest percentage of neurons than all three stimulation sites (thalamus: 55.6%, striatum: 40.0%, globus pallidus: 46.7%). Administration of a $GABA_B$ antagonist was also at times able to reveal an inhibition previously unnoticed without application of the $GABA_B$ antagonist, as shown for a response of a dopaminergic neuron to thalamic stimulation in Fig. 6. This unmasked inhibition could then be blocked by the subsequent application of the $GABA_A$ antagonist, bicuculline, together with a $GABA_B$ antagonist (Fig. 6C).

Although the inhibition elicited from neostriatum, globus pallidus or substantia nigra pars reticulata was not blocked, application of the $GABA_B$ antagonists 2-hydroxysaclofen or CGP-55845A exerted modest effects on dopaminergic neuron spontaneous activity tending to cause a regularization in firing pattern. The $GABA_B$ antagonists also had a slight effect in decreasing the firing rate of these neurons (from 4.43 ± 0.281 to 4.22 ± 0.27 spikes/s). These data are reported in more detail elsewhere.[50]

Globus pallidus evoked responses in hemitransected rats are the same as in intact rats

Pallidal stimulation in hemitransected rats resulted in inhibition of dopaminergic neurons that was indistinguishable from that in intact rats. Stimulation of globus pallidus suppressed firing to $17.02 \pm 7.38\%$ of pre-stimulus values ($n=4$). Subsequent local application of bicuculline significantly attenuated the inhibition ($73.56 \pm 9.94\%$; $t=4.67$, d.f.=4, $P<0.05$, see Fig. 7). There was no significant difference in the inhibitory effects of pallidal stimulation in intact versus hemitransected rats (suppression to $15.62 \pm 2.38\%$ versus $17.02 \pm 7.38\%$, respectively, $P>0.05$).

There was also no difference in the ability of bicuculline to block the evoked inhibition between hemitransected and intact rats. Bicuculline effectively attenuated the inhibition due to pallidal stimulation

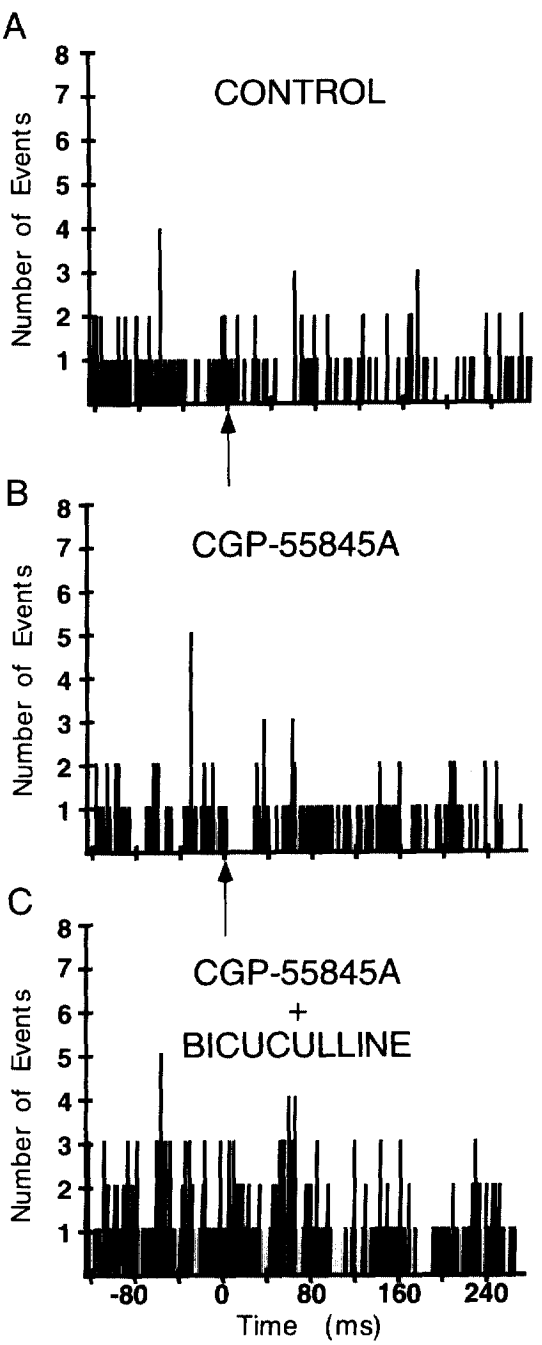

Fig. 6. Unmasking of $GABA_A$-mediated inhibition by CGP-55845A in an antidromically identified (latency= 9.12 ms) nigral dopaminergic neuron. (A) Stimulation of thalamus (1.0 mA) fails to affect the firing of a dopaminergic neuron. (B) Application of CGP-55845A reveals an inhibition (suppression to 0% of control for 24 ms duration). (C) Application of bicuculline together with CGP-55845A abolishes the unmasked inhibition. PSTH consists of 100 trials with a 2-ms bin width.

in hemitransected rats (73.56 ± 9.94% of control values after bicuculline administration) as well as intact rats (111.79 ± 85.52% facilitation after bicuculline, $P<0.05$).

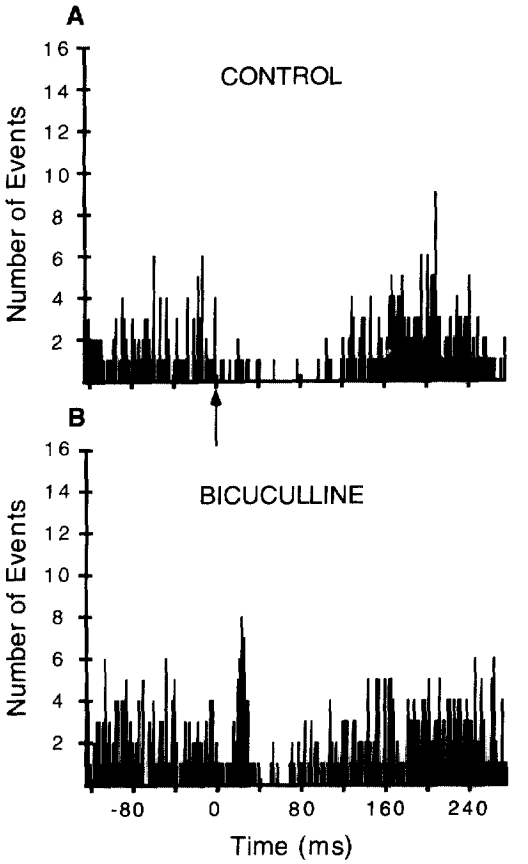

Fig. 7. PSTH constructed from a spike train recorded from a dopaminergic neuron in a rat hemitransected anterior to globus pallidus and posterior to striatum. (A) Stimulation of globus pallidus (1.0 mA) at time=0 produces an inhibitory period (suppression to 20% of pre-stimulus firing rate, 91 ms duration) which is blocked by the $GABA_A$ antagonist bicuculline (B) unmasking a short latency facilitation. Each PSTH consists of 200 trials with a 2-ms bin width.

Train stimulation

Because a number of *in vitro* studies have reported a $GABA_B$-mediated IPSP in midbrain dopaminergic neurons following local train stimulation in the presence of bicuculline[4,34] the effects of similar trains applied to the neostriatum and globus pallidus *in vivo* were examined. Stimulation of globus pallidus and striatum with a train of pulses evoked an inhibition of firing in dopaminergic neurons. The duration of inhibition induced by striatal stimulation lasted for 320.69 ± 31.76 ms with a 100.84 ± 23.24 ms latency of onset. This inhibition reduced the firing of dopaminergic neurons to 17.38 ± 4.54% of pre-stimulus firing rate ($n=8$ for all measures). Pallidal stimulation produced a similar inhibition to 14.72 ± 3.89% of pre-stimulus firing with a 309.18 ± 65.08 ms duration and 84.18 ± 15.30 ms latency of onset ($n=11$ for all measures).

In two cases, application of picrotoxin (250 or 500 μM) completely abolished the train stimulus induced inhibition (see Fig. 8C, D). Before drug

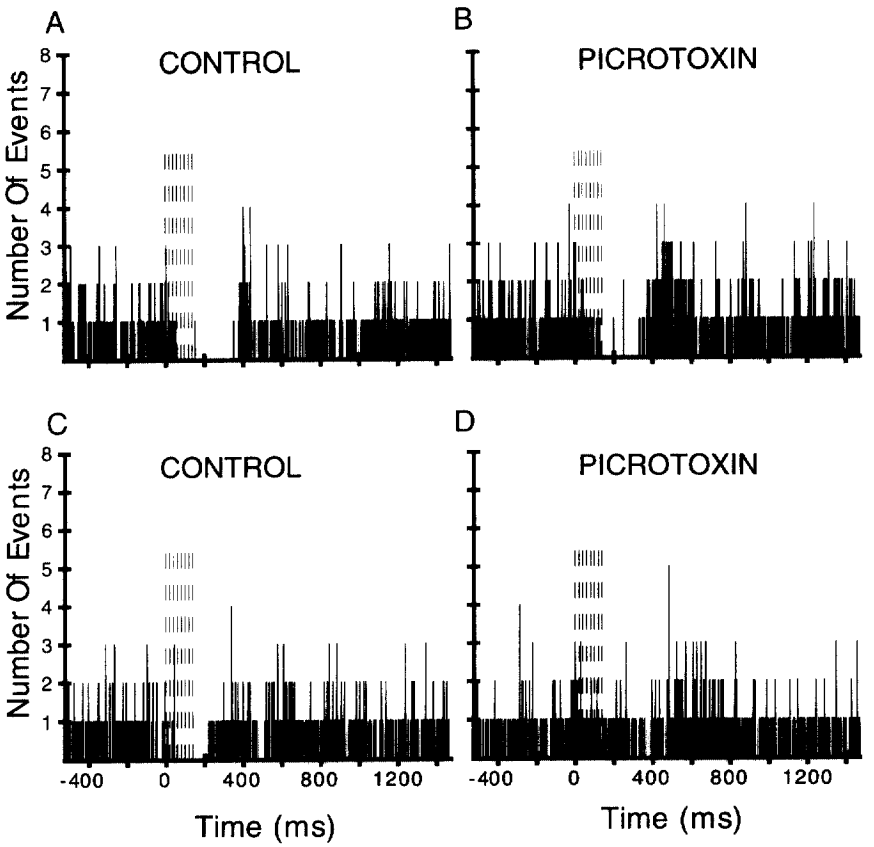

Fig. 8. Train stimulation (0.5 mA, 250 μs pulses at 50 Hz for 150 ms repeated every 10 s) of striatum (A) or globus pallidus (C) at time=0 (dashed lines represent each pulse in train) evokes a longer duration (320 ms in A and 189 ms in C) and onset latency (61 ms delay in A and 46 ms in C) inhibition of firing of dopaminergic neurons than single pulse stimulation. (B) In the majority of cases this inhibition was not attenuated by application of picrotoxin or bicuculline although the early part of the inhibitory period was consistently blocked (onset latency increased to 129 ms from A to B). (D) However, in two cases the electrically evoked inhibition was completely abolished by picrotoxin. Each PSTH consists of 50 trials with a 2-ms bin width.

application, stimulation of globus pallidus or striatum produced an inhibition to 6.14 ± 6.14% of pre-stimulus firing rate with a latency of onset of 106.00 ± 60.00 ms and a duration of 301.00 ± 112.50 ms. After drug application the inhibition was completely blocked.

Application of 200–400 μM bicuculline or 250–500 μM picrotoxin also significantly increased the latency of onset of inhibition due to pallidal stimulation (78.86 ± 18.08 ms before drug application to 116.63 ± 25.95 ms after antagonist application. $t = -3.64$, d.f. = 5, $P < 0.05$. See Fig. 8A, B). No differences were observed between the effects of bicuculline or picrotoxin on the train stimulation induced inhibition of firing of pars compacta dopaminergic neurons ($P > 0.05$). However, $GABA_A$ antagonist application had no effect on the overall duration (331 ± 91.76 ms before drug application to 302.11 ± 86.51 ms after drug; $P > 0.05$) or percentage of pre-stimulus firing inhibition (control = 12.85 ± 4.76%; drug = 21.04 ± 9.33%, $P > 0.05$).

Application of either saclofen or CGP-55845A also had no observable effect on the train stimulation induced inhibition of dopaminergic neurons. Stimulation of striatum after $GABA_B$ antagonist application still evoked an inhibition of firing to 29.26 ± 5.15% of pre-stimulus firing rate with a duration of 384.12 ± 51.54 ms and an onset latency of 146.12 ± 28.55 ms ($P > 0.05$, $n = 4$). Pallidal stimulation after application of the $GABA_B$ antagonists produced similar results. Dopaminergic neurons were inhibited to 12.09 ± 3.73% of pre-stimulus firing for a duration of 156.83 ± 13.10 ms and an onset latency of 93.92 ± 46.86 ms ($P > 0.05$, $n = 3$).

In a few cases apamin ($n = 3$) or eticlopride ($n = 2$) was applied to a cell in order to determine the sensitivity of the train stimulation induced inhibition. Similar to the $GABA_B$ antagonists, these drugs had no effect on the evoked inhibition.

DISCUSSION

GABAergic afferents

The main purpose of this study was to determine the GABA receptor subtype that mediates the

inhibition of dopaminergic neurons produced by electrical stimulation of striatum, globus pallidus and substantia nigra pars reticulata. Although dopaminergic neurons receive excitatory inputs[15] from pedunculopontine nucleus,[8,16] subthalamic nucleus[8,37] and prefrontal cortex,[56] disinhibition appears to be a major mechanism for activation of neurons in the basal ganglia.[6] The majority (up to 90%) of the afferents to dopaminergic neurons appear to be inhibitory and GABAergic due to the symmetric morphology of the synapses and immunostaining for glutamic acid decarboxylase.[28,47,52,53,59,61]

Both $GABA_A$ and $GABA_B$ responses can be evoked by local electrical stimulation in dopaminergic neurons *in vitro*.[4,25,34] In *in vitro* intracellular recording experiments dopamine neurons respond to selective $GABA_B$ receptor agonists with robust hyperpolarizing responses, (for review see Ref. 40) and *in vitro* whole-cell recordings in slices from juvenile rats reveal spontaneous IPSPs that appear to be $GABA_B$-mediated.[39] In addition, dopaminergic neurons change their firing pattern in response to $GABA_B$ agonist application *in vivo*.[14] However, studies investigating the effects of $GABA_B$ receptor stimulation or blockade on the spontaneous activity of dopaminergic neurons show that $GABA_B$ agonists have a significant effect on firing pattern and rate whereas $GABA_B$ antagonists have only modest or no effect.[14,50] This suggests the lack of a significant tonic GABAergic input affecting postsynaptic $GABA_B$ receptors on dopaminergic neurons *in vivo*, consistent with our data showing that the inhibition of substantia nigra pars compacta dopaminergic neurons due to single pulse stimulation of striatum, globus pallidus, and pars reticulata projection neurons *in vivo* is due to $GABA_A$ receptor stimulation.

Although it is possible that afferent inhibition is mediated simultaneously through both receptor subtypes, Sugita *et al.*[64] demonstrated that muscarine acts presynaptically to affect specifically $GABA_A$ receptor mediated inhibition whereas 5-hydroxytryptamine acts presynaptically affecting only $GABA_B$ receptor mediated inhibition. This suggests that the inputs terminating on either $GABA_A$ or $GABA_B$ receptors originate from separate populations of cells, one possessing presynaptic muscarinic receptors and the other possessing presynaptic 5-hydroxytryptamine receptors.[64]

The present results show that inhibitory effects resulting from single pulse stimulation of striatum, globus pallidus and pars reticulata projection neurons were attenuated or abolished by the $GABA_A$ antagonists, bicuculline and picrotoxin. The initial portion of the longer inhibitory periods evoked by train stimulation was also blocked by $GABA_A$ antagonists. Conversely none of the inhibitory responses was attenuated at all by the selective $GABA_B$ antagonists, saclofen or CGP-55845A. Thus, all three pathways are proposed to form synapses acting, at least *in vivo*, predominantly or exclusively via the $GABA_A$ receptor subtype. The onset latencies for the inhibitions obtained from stimulating these pathways (mean latency of inhibition from globus pallidus = 5.60 ms, striatum = 7.78 ms, reticulata = 1.23 ms) were consistent with the shortest antidromic latencies recorded from striatum,[55] globus pallidus[38] or the projection neurons from substantia nigra pars reticulata to pars compacta[10,24] strongly suggesting that they were all mediated monosynaptically.

The input from striatum to substantia nigra has been well studied and is generally thought to exert some control over dopaminergic neuron activity due to its direct monosynaptic connections on dopaminergic neurons.[62] However, striatal-evoked IPSPs are smaller in dopaminergic neurons than in non-dopaminergic nigral neurons[20,66,67] and the effects of striatal lesions on dopamine neuron activity are transient and relatively minor.[3,12]

Pallidal afferents to substantia nigra were suggested in an early study to synapse primarily on dopaminergic neurons[28] and stimulation of globus pallidus reliably induced inhibition of dopaminergic neurons in the present study. However, even if pallidal afferents synapse mostly onto non-dopaminergic neurons as suggested by Smith and Bolam,[61] globus pallidus still appears to play a major role in controlling dopaminergic neuron firing activity. When pallidal neuron activity is pharmacologically altered, dopaminergic neurons reliably shift their firing pattern as a consequence of altered pallidal firing rate.[5] However, this does not appear to be due to a monosynaptic input but rather to the powerful effects of globus pallidus on GABAergic neurons in substantia nigra pars reticulata which in turn can affect dopaminergic neuron activity.[5,20,26,65]

Dopaminergic and GABAergic neurons in pars compacta and pars reticulata, respectively, show a reciprocal relationship in firing activity[17,20,71] suggesting a strong connection between these two populations of neurons. Direct electrical stimulation within discrete regions of substantia nigra pars reticulata produces inhibitory postsynaptic potentials in dopaminergic neurons in pars compacta *in vitro* reversing at potentials close to the chloride Nernst potential[26] suggesting $GABA_A$ mediation. Antidromic activation of pars reticulata GABAergic projection neurons produces inhibition of dopaminergic neurons *in vivo*,[65 and present results] and this inhibition is also $GABA_A$ mediated.

Hemitransections

In order to exclude any possible effects of stimulating the fibers of passage traveling through globus pallidus while stimulating globus pallidus, some rats were hemitransected just anterior to globus pallidus three to six days prior to recording. Hemitransections anterior to globus pallidus do not eliminate the possibility that subthalamic axon terminals could be antidromically activated by pallidal stimulation

which could then excite substantia nigra pars reticulata neurons orthodromically and lead to a $GABA_A$-mediated inhibition dopaminergic neurons in pars compacta.[44,54] Nevertheless, when globus pallidus was stimulated in hemitransected rats the evoked inhibition was attenuated by application of bicuculline, just as in intact rats. Thus the globus pallidus-evoked inhibition appears to be mediated by the $GABA_A$ receptor subtype.

GABAergic inhibition from train stimulation of striatum, globus pallidus and substantia nigra pars reticulata

A number of *in vitro* studies have demonstrated a $GABA_B$ receptor mediated hyperpolarization of dopaminergic neurons after train stimulation of the descending fibers to ventral tegmental area in the presence of bicuculline.[4,34] Because these IPSPs were sensitive to presynaptic modulation by D_1 agonists, they were considered to have arisen from stimulation of striatonigral fibers since of the descending GABAergic afferents, only the striatonigral fibers are known to possess presynaptic D_1 receptors.[4] Train stimulation *in vivo* in this study produced an inhibition of firing of longer duration and latency of onset, (these trains, modeled after those used by other *in vitro* experiments,[4,34] consisted of pulses that were of lower intensity and shorter duration than those necessary to consistently elicit inhibition in our single pulse stimulation experiments) than that following single pulse stimulation. In two cases, the inhibition was completely abolished by application of picrotoxin. However, in most cases this inhibition was insensitive to either $GABA_A$ or $GABA_B$ antagonists indicating that the inhibition was not mediated entirely through GABAergic receptors. Although only a few cases were tested, the inhibition produced due to train stimulation of striatum or globus pallidus also appeared to be insensitive to eticlopride and apamin application ruling out both D_2 receptor mediation and a calcium-activated potassium conductance as possible mechanisms. Although it is difficult to determine the exact cause of this inhibition it does seem to have a $GABA_A$ receptor mediated component since the onset latency was consistently increased after $GABA_A$ antagonist application (see Fig. 8A, B). It is possible that this inhibitory period could be adenosine mediated.[29,45]

Although stimulating afferent nuclei using a train of pulses might simulate the firing pattern of an individual neuron, electrical stimulation would drive almost all neurons under the influence of the stimulating electrode simultaneously. The synchronized activation of a large population of neurons at high frequency might allow for a larger extracellular GABA accumulation than would be seen after single pulse stimulation or during the asynchronous firing of striatum and globus pallidus, that probably is normally encountered *in vivo*, due to increased release and/or saturation of the re-uptake mechanism.[43] This could result in activation of a population of $GABA_B$ receptors, perhaps extrasynaptic, that are not normally activated *in vivo*.[31,33,68]

Excitatory responses after bicuculline administration

In several cases application of bicuculline or picrotoxin to a dopaminergic neuron not only blocked the inhibitory response induced by stimulation of globus pallidus and striatum but also unmasked an excitatory response. A similar facilitation of dopaminergic neurons to stimulation of thalamus after bicuculline application was previously reported.[65] These excitatory responses are likely due to co-activation of excitatory subthalamic and/or cortical pathways which project to the dopaminergic neurons in substantia nigra, but whose effects are normally masked by coincident activation of GABAergic inputs under normal conditions. For example, striatal stimulation activates descending excitatory corticofugal fibers and application of bicuculline unmasks excitatory responses to both cortical and striatal stimulation in nigral dopaminergic neurons.[46] Thalamic stimulation could also facilitate subthalamic activation through projections from the median-parafascicular complex to subthalamic nucleus.[63] Direct stimulation of subthalamic nucleus *in vivo*, which consists of only glutamatergic output neurons also results in mixed inhibitory and excitatory effects in nigral dopaminergic neurons.[54,60] The inhibitory responses were suggested to be due, at least in part, to activation of GABAergic neurons in pars reticulata,[54] an hypothesis verified by recent *in vitro* intracellular recordings.[44]

$GABA_B$ receptor mediated effects

The inhibitory response of dopaminergic neurons to single pulse stimulation of the striatum, globus pallidus, and substantia nigra pars reticulata was consistently blocked by the selective $GABA_A$ antagonists, bicuculline and picrotoxin, but not by the selective $GABA_B$ antagonists, 2-hydroxysaclofen and CGP-55845A. Furthermore application of $GABA_B$ antagonists increased the inhibition evoked from stimulation of all three stimulation sites in about 45% of the cases. At times the $GABA_B$ antagonist even revealed an occult inhibition which could be blocked when a $GABA_A$ antagonist was subsequently administered. These data suggest that the three GABAergic afferents studied possess inhibitory presynaptic $GABA_B$ receptors which are occupied to some degree by endogenous GABA *in vivo*. Although some early studies argued against the existence of $GABA_B$ autoreceptor-mediated inhibition of GABA release in substantia nigra,[69,70] more recent electrophysiological studies clearly indicate the existence of $GABA_B$ autoreceptor-mediated inhibition of $GABA_A$-mediated inhibitory postsynaptic currents

on nigral dopaminergic neurons.[57] We suggest that local application of $GABA_B$ receptor antagonists increases the evoked inhibition by blocking the presynaptic inhibitory GABA autoreceptors[30] thereby increasing GABA release on postsynaptic $GABA_A$ receptors. These data also suggest an explanation for previous results in which application of saclofen or lesion of globus pallidus produced a regularization of dopamine neuron firing pattern.[65] Although those data were originally interpreted to suggest that the effects of pallidonigral afferents were mediated by postsynaptic $GABA_B$ receptors, the results from the present study indicate that this is not correct. Rather, the data suggest that the previous results in which the $GABA_B$ receptor antagonist, saclofen, produced regularization of firing was due to blockade of presynaptic $GABA_B$ receptors which led to increased GABA release and consequent activation of postsynaptic $GABA_A$ receptors, thereby inhibiting burst firing while promoting pacemaker-like firing.[5,50,65]

CONCLUSION

Direct electrical stimulation of neostriatum or globus pallidus or antidromic activation of substantia nigra pars reticulata projection neurons produces monosynaptic inhibition of nigral dopaminergic neurons *in vivo*. In each case, the inhibition caused by single pulse stimulation can be completely blocked by the $GABA_A$ antagonists, bicuculline or picrotoxin, suggesting that in all three cases inhibition is mediated predominantly or exclusively by a $GABA_A$ receptor. In addition, facilitation of evoked inhibitory responses was observed with local application of the selective $GABA_B$ antagonists, 2-hydroxysaclofen or CGP-55845A, indicating that each of the GABAergic afferents are modulated by inhibitory presynaptic $GABA_B$ autoreceptors. Train stimulation of striatum and globus pallidus also inhibits the firing of dopaminergic neurons and this inhibition may be partly mediated through $GABA_A$ receptors since this longer duration inhibition can be partly blocked by $GABA_A$ antagonists.

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