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D_1 Agonist–induced Excitation of Substantia Nigra Pars Reticulata Neurons: Mediation by D_1 Receptors on Striatonigral Terminals via a Pertussis Toxin–Sensitive Coupling Pathway

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Iontophoresis of dopamine or the D_1 agonist SKF 38393 has been shown to elicit current-dependent increases in the firing of rat substantia nigra pars reticulata neurons, suggesting a discrete physiological role for the D_1 dopamine receptor population in the substantia nigra. The effects of SKF 38393 differed from those of dopamine, however, in that the D_1 agonist also augmented inhibitory responses to applied GABA, whereas dopamine and D_2-like agonists were previously found to attenuate responses to GABA. The present studies involved various manipulations of the nigral D_1 receptors in order to examine the pharmacological specificity, receptor localization, and second messenger coupling underlying the D_1 agonist response. The excitatory and GABA-potentiating effects of SKF 38393 were found to be attributable to D_1 receptor stimulation, rather than a nonspecific action, since (1) the effect was mimicked by iontophoresis of A-68930, a D_1 agonist of a different structural class than SKF 38393, and (2) the response to SKF 38393 was prevented by intranigral injection of the receptor inactivator *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; 50 nmol/0.5 μl) 1 d before, or the D_1 antagonist SCH 23390 (1 μg/μl) 1 hr before electrophysiological testing. Additional studies revealed that the involved D_1 receptors were located presynaptically on striatonigral terminals. For instance, in rats given ipsilateral striatal kainic acid lesions 1 week earlier, application of SKF 38393 failed to elicit the usual increases in cell firing, but loss of the response was observed only among the group of pars reticulata neurons that were shown to be unresponsive to striatal stimulation (i.e., those whose striatonigral inputs had been terminated by the lesion). Finally, to examine the second messenger coupling characteristics of the involved D_1 receptors, several membrane-permeable analogs of cAMP were tested iontophoretically in place of SKF 38393. Surprisingly, none of these compounds gave a pattern of response typical of the D_1 agonist, raising questions about the involvement of cAMP. Even more suggestive of an unconventional D_1 coupling pathway, the excitatory and GABA-potentiating effects of applied SKF 38393 were completely abolished by prior intranigral injection of the G_i/G_o protein inactivator, pertussis toxin. Collectively, these results suggest that stimulation of D_1 receptors on striatonigral terminals causes an excitation of substantia nigra pars reticulata neurons with an exaggerated responsiveness to GABA, and the effects appear to be mediated by a pertussis toxin–sensitive (i.e., a non-G_s-like) G-protein and possibly a second messenger other than cAMP.

[Key words: substantia nigra pars reticulata, D_1 dopamine receptor, pertussis toxin, EEDQ, extracellular single-unit recording, iontophoresis]

Dopamine can act at various locations within the basal ganglia nuclei, and through multiple receptor subtypes, to influence motor output. Most studies attempting to decipher dopamine's role in regulating movement have focused on its effects postsynaptically, within the striatum, or presynaptically, on the dopamine neurons themselves. Less intensely studied has been the direct effect of dopamine on the neurons of the substantia nigra pars reticulata. The pars reticulata is located just ventral to the pars compacta dopamine cells, and it constitutes one of the two major output pathways of the basal ganglia. Neurons of the substantia nigra pars reticulata are targets of a major portion of the striatonigral GABA projection (Graybiel and Ragsdale, 1977) and are also directly exposed to dopamine released from dendrites of the pars compacta neurons (Geffen et al., 1976; Korf et al., 1976; Nieoullon et al., 1977; Tagerud and Cuello, 1979; Cheramy et al., 1981; Robertson et al., 1991; Santiago and Westerink, 1991), which extend ventrally and densely infiltrate the pars reticulata (Bjorklund and Lindvall, 1975). The dendritic release of dopamine, together with the presence of a dense network of dopamine receptors in the nigra (Richfield et al., 1987; Beckstead et al., 1988), suggests a direct role for nigral dopamine in regulating basal ganglia output function.

Electrophysiological studies support such a role for dendritically released dopamine. Previous studies revealed that dopamine elicits a dual response from pars reticulata neurons (Waszczak and Walters, 1983, 1986). Specifically, when dopamine was iontophoretically applied, or released indirectly by iontophoresis or intravenous administration of amphetamine, these neurons exhibited both an increase in their firing rates and a diminished inhibitory response to applied GABA. Similarly, dopamine and amphetamine attenuated the GABA-mediated inhibition of pars reticulata neurons evoked by electrical stimulation of the striatonigral pathway (Waszczak and Walters,

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1986). Further studies revealed that the excitatory and GABA-attenuating effects of dopamine occurred independently, and that they were pharmacologically distinguishable. For instance, it was shown (Waszczak, 1990) that the rate-increasing effect of dopamine was mimicked by SKF 38393, a D_1-selective agonist (Setler et al., 1978), whereas the GABA-modulatory response was reproduced by quinpirole, a D_2/D_3-selective agonist (Tsuruta et al., 1981; Sokoloff et al., 1990). Thus, the two effects of dopamine appeared to be mediated by separate dopamine receptor subtypes localized within the substantia nigra.

The objective of the present study was to characterize the D_1 agonist-induced excitatory response of pars reticulata neurons to confirm that the effect is indeed D_1 receptor mediated, to identify the location of the involved receptors, and to begin to examine the second messenger coupling pathway underlying the response. *In vivo* electrophysiological methods were used since intact preparations provide the advantage of preserving the afferent–efferent circuitry of the substantia nigra, a condition essential for assessing the contribution of D_1 receptors located presynaptically on the terminals of striatonigral neurons. Several experimental approaches were taken to determine the pharmacological and site selectivity of the D_1 agonist action, including (1) substitution of a non-benzazepine D_1 agonist in place of SKF 38393; (2) evaluations of a D_1-selective antagonist, a dopamine receptor inactivator, and striatonigral lesions for their abilities to prevent the agonist response; (3) evaluation of cAMP analogs for their abilities to mimic the D_1 agonist effect; and (4) evaluation of pertussis toxin for its ability to prevent the agonist-induced excitatory response. We report that the excitatory response of pars reticulata neurons to D_1 agonists is mediated by a D_1 receptor population localized to striatonigral terminals. Unexpectedly, however, the agonist response was found not to be mimicked by cAMP analogs, but was pertussis toxin sensitive. These findings suggest unconventional coupling characteristics for the nigral D_1 receptors mediating the electrophysiological response.

Materials and Methods

Techniques for extracellular single-unit recording and microiontophoresis. Male Sprague–Dawley albino rats (Charles River, Wilmington, MA) weighing 250–350 gm were anesthetized with chloral hydrate (400 mg/kg, i.p.) and then placed in a stereotaxic apparatus. A needle was inserted into a lateral tail vein so that additional chloral hydrate could be administered intravenously to maintain an adequate level of anesthesia. Body temperature was monitored throughout the experiment by a rectal probe and was maintained between 36°C and 38°C by a feedback-controlled heating pad. All aspects of the animals' care, use, and sacrifice were carried out with strict adherence to the NIH *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication 85-23, revised 1985). Next, a 3.0 mm burr hole, 2.0 mm lateral to lambda and 3.0 mm anterior to the lambdoid suture, was drilled through the skull and an electrode lowered into the brain to the level of the substantia nigra with the aid of a hydraulic microdrive.

The electrodes used in these experiments were five-barrel glass microiontophoresis pipettes that had been prefilled with glass fibers, and then pulled on a Narashige PE-2 microelectrode puller. The central (recording) barrel was filled with 1% pontamine sky blue (Bio/Medical Specialties, Santa Monica, CA) in 2 M NaCl. One of the outer barrels was filled with 4 M NaCl and was used for current balancing to maintain electrical neutrality at the electrode tip. The remaining three outer barrels were filled with the drug solutions to be delivered iontophoretically, including GABA (0.001 M in 0.2 M NaCl, pH 4), (±)-SKF 38393 (0.01 M, pH 4), A-68930 (0.01 M, pH 4), dibutyryl cyclic AMP (0.2 M, pH 6), 8-bromo-cyclic AMP (0.2 M, pH 6), chlorophenylthio-cyclic AMP (0.05 M, pH 6), and cyclic AMP (0.2 M, pH 6). The electrodes were then broken back under microscopic control to a tip diameter of 4–5 μm. Resistances for the central recording barrel ranged from 3 to 4 MΩ, and those for outer drug-containing barrels ranged from 50 to 100 MΩ. During periods of iontophoresis, SKF 38393 and GABA were ejected by passing a positive current of varying intensities, and cAMP analogs were ejected using negative currents. Between periods of drug delivery, a 10 nA retaining current of opposite polarity was passed through drug channels to prevent diffusion of the drug from the pipette.

The neurons recorded in these studies were located within the pars reticulata region of the substantia nigra, just ventral to pars compacta dopamine neurons. They were located stereotaxically within the following coordinates, according to the atlas of Konig and Klippel (1970): anterior 1760–2580 μm, lateral 2.0–2.4 mm, ventral −1.5 to −2.5 mm. Neurons of the pars reticulata have previously been well characterized electrophysiologically (Bunney et al., 1973; Waszczak et al., 1980) and were easily distinguished based upon their extracellular wave forms, firing rates, and location ventral to the pars compacta dopamine neurons. These neurons displayed smooth, sharp, biphasic action potentials with an average duration of <1 msec, and firing rates between 10 and 40 spikes/sec.

Standard extracellular, single-unit recording methods (Bunney et al., 1973; Waszczak et al., 1980) were used to monitor neuronal firing. Briefly, electrical potentials were passed from the electrode through a high-input impedance microprobe amplifier whose output was then sent to an amplitude/time discriminator. The amplified signal was monitored on both an oscilloscope screen and an audio monitor. The slope–height window discriminator was set so that each action potential triggered a rate counter. Firing rates were summed over successive 5 sec periods and printed by a Datel digital strip printer. Rate histograms of firing were also generated and printed by a Gould physiological recorder.

Drug testing paradigm. Studies were designed to test the effects of various drugs both on the basal firing rates of pars reticulata neurons, and on their responses to iontophoretically applied GABA. The drug testing paradigm followed the protocols of Waszczak and Walters (1983, 1984, 1986) and Waszczak (1990). After locating a pars reticulata neuron, GABA was pulsed iontophoretically for repeated 30 sec intervals, separated by 30 sec periods of basal activity. An ejection current for GABA was chosen that was sufficient to inhibit cell firing by at least 50%, but not totally. After achieving a baseline response (i.e., three consecutive applications of GABA at the same ejection current that elicited similar degrees of inhibition), the test compound was iontophoresed simultaneously with GABA for at least three additional GABA pulses. Current–response curves for D_1 agonists were generated by increasing the ejection current in 2 nA increments after every three or four GABA pulses until an ejection current of 10 nA was attained. For cyclic AMP analogs, currents were increased in 5 nA increments to a total of −25 to −30 nA. The effect of the test compound on baseline firing rate was calculated by comparing the 30 sec periods of spontaneous firing (basal firing between GABA pulses) both before and during iontophoresis of the test drug. Evaluation of the effect of the test drug on responses to GABA was determined by comparing the average percentage inhibition elicited by GABA before and during application of the test compound. Current–response curves were plotted for each test agent illustrating both changes in baseline firing rate and the degree of inhibition elicited by GABA (indicated in figures as the percentage change in GABA potency). Points and error bars on graphs represent the mean value ± the standard error of the mean. Statistical comparisons between values referred to in the text were determined by a two-tailed Student's *t* test for unpaired data.

Intracerebral injections. Some experiments required localized intracerebral microinjections to inactivate receptors or G-proteins in the substantia nigra, or to lesion the striatonigral pathway. Agents injected intracerebrally were not assumed to be pharmacologically or regionally selective for the intended neuronal or receptor populations since under *in vivo* conditions it is not possible to control spread accurately, or to assess concentrations of an injected drug at the surface of targeted neurons. It was therefore likely that local tissue concentrations varied considerably with distance from the site of injection and, in some areas, exceeded those at which the drugs are known to be selective under *in vitro* applications. However, none of the areas or receptors likely to have been inadvertently affected by these injections appear to be directly or indirectly involved in the effects of D_1 agonists on pars reticulata neurons. Nevertheless, given these constraints, a D_1-mediated mechanism was supported only if a collection of drugs known to inactivate, block, or destroy nigral D_1 receptors could prevent the response of pars reticulata neurons to the D_1-selective agonist SKF 38393.

For intracerebral injections, rats were anesthetized with pentobarbital

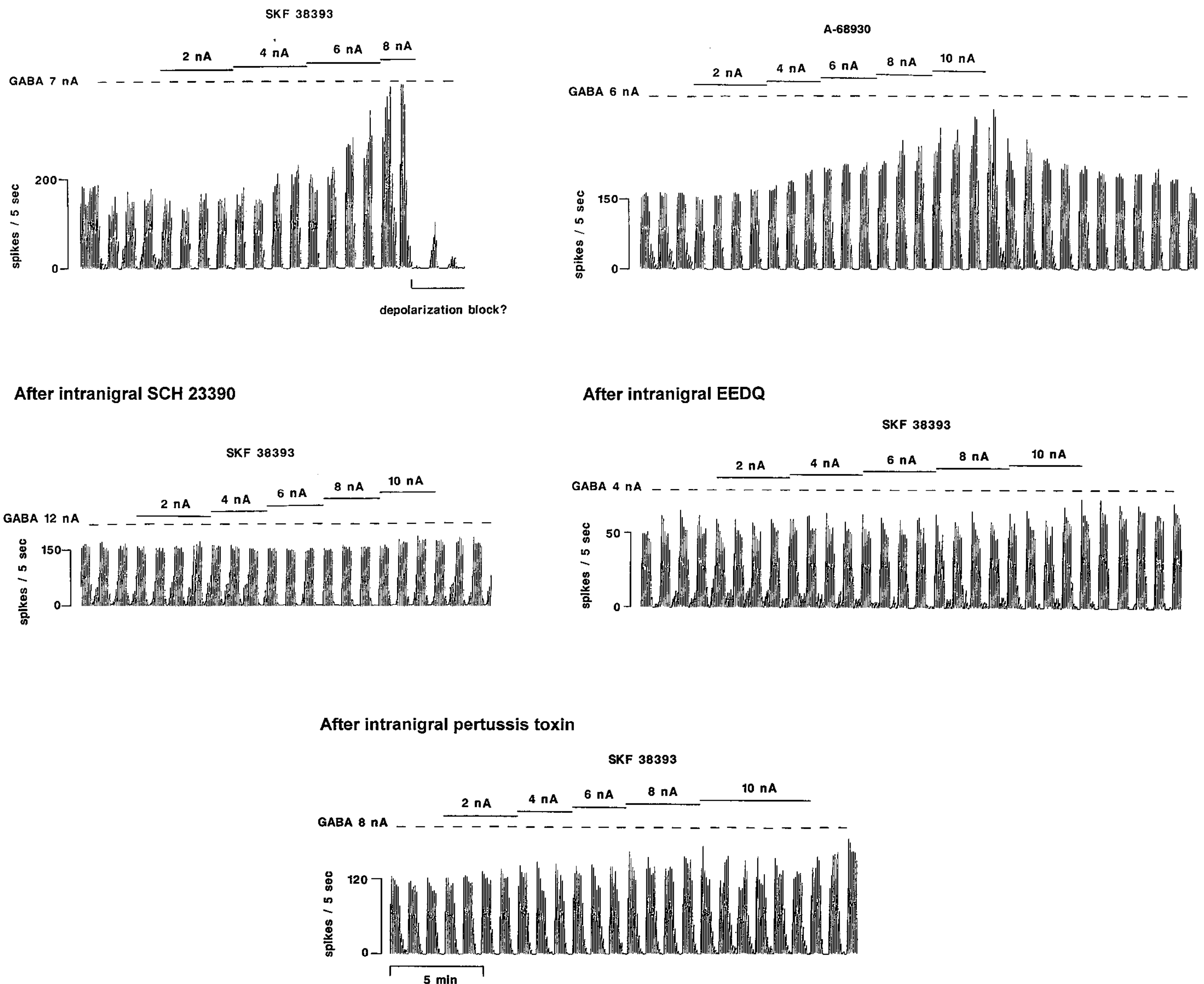

Figure 1. The excitatory response of substantia nigra pars reticulata neurons to iontophoretically applied D_1 agonists is lost after intranigral injections of agents that inactivate or pharmacologically block D_1 receptors or G_i/G_o-proteins. *Top traces,* SKF 38393 and A-68930, both D_1-selective agonists, cause similar current-related increases in pars reticulata cell firing and an apparent increase in the inhibitory potency of applied GABA. In the example shown, SKF 38393 caused an increase in firing sufficient to cause failure of spike formation at 8 nA (likened to depolarization block). In such cases, firing typically recovered several minutes after termination of SKF 38393 application. *Middle and bottom traces,* SKF 38393 fails to increase pars reticulata cell firing (or alter responsiveness to GABA) after intranigral injections of the D_1 antagonist SCH 23390, the dopamine receptor inactivator EEDQ, or the G_i/G_o-protein inactivator pertussis toxin (see Materials and Methods).

sodium (60 mg/kg) and positioned in a stereotaxic apparatus using special nonpenetrating ear bars to minimize trauma to the animal's ear drums. All injections were made on the left side of the brain, ipsilateral to the recording site, using a 1 or 2 μl Hamilton syringe controlled by a Kopf microinjection apparatus attached to the stereotaxic. Injections were made at a rate of approximately 0.1 μl/min, and the needle was withdrawn slowly to minimize backflow of solutions up the needle track. For rats receiving intranigral injections of either EEDQ, pertussis toxin, or SCH 23390, the stereotaxic measurements were 2.0 mm lateral, 3.0 mm anterior to the lambdoid suture, and 7.0 mm ventral to the surface of the brain. Injection volumes and concentrations were, for EEDQ, 50 nmol/0.5 μl of 22.5% hydroxypropyl-β-cyclodextrin; pertussis toxin and SCH 23390, 1 μg/μl of 0.9% NaCl.

Lesions of the striatonigral pathway were accomplished by injecting kainic acid (1 μg/0.5 μl of 0.9% NaCl) at two sites in the striatum. Coordinates were 2.3 mm lateral, 1 mm anterior to bregma, 4.5 mm ventral; and 3.8 mm lateral, 0 mm anterior to bregma, 4.5 mm ventral. Kainic acid was selected for these experiments despite the fact that it is more neurotoxic and produces more widespread lesions than two alternative excitotoxin lesioning tools, ibotenic and quinolinic acids. The rationale for use of kainic acid was to produce more complete rather than discrete lesions of the striatal inputs to the pars reticulata. Although more restricted striatal lesions might have minimized damage to adjacent structures, they would have resulted in a yield of fewer reticulata neurons that were devoid of striatonigral inputs. Electrophysiological experiments took place 7–14 d after kainic acid injections, approximately 24 hr after treatments with EEDQ or pertussis toxin, and 1 hr after intranigral injections of SCH 23390.

Striatal stimulation. Striatal stimulation was used as a means for prescreening individual pars reticulata neurons to determine whether striatal kainic acid lesions had interrupted their inhibitory GABAergic inputs. This approach for assessing effectiveness of the lesions was considered preferable to biochemical and histological methods because absence of a normally observed electrophysiological response provides a more discrete indicator that striatal inputs to a particular pars reticulata neuron had been terminated by the lesion. Since biochemical and his-

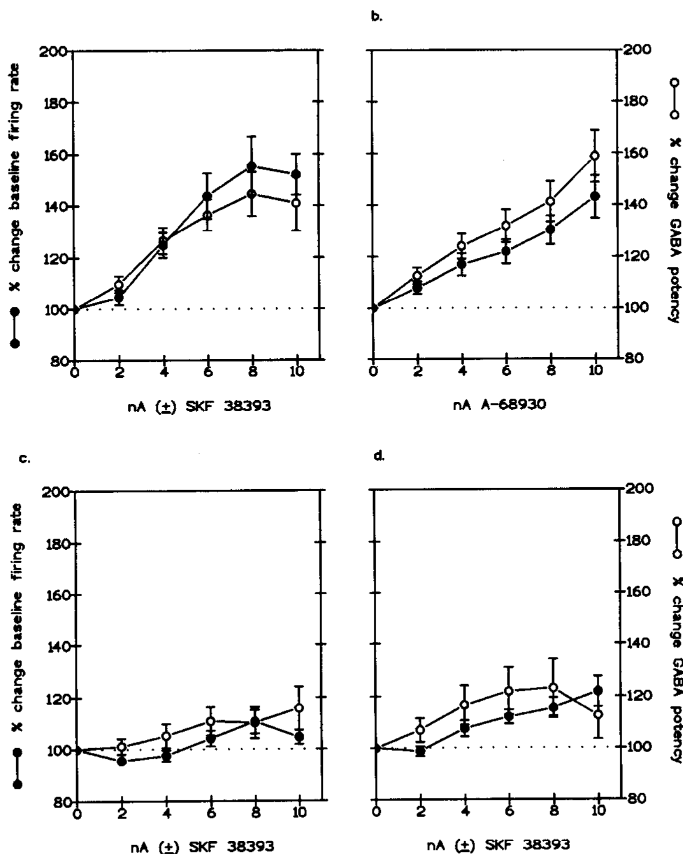

Figure 2. Current–response curves for the effects of D_1 agonists on the baseline firing rates (*solid circles*) and responses of pars reticulata neurons to applied GABA (*open circles*). *a,* Racemic SKF 38393 caused current-related increases in both baseline firing and responsiveness to GABA, with maximal increases observed at 8–10 nA. *b,* A-68930, a second D_1-selective agonist, caused increases in baseline firing and GABA potency similar to those seen with SKF 38393. *c,* Intranigral injection of receptor inactivator EEDQ on the previous day prevented the increases in firing and GABA potency caused by applications of SKF 38393. *d,* Intranigral injection of the D_1 antagonist SCH 23390 1 hr earlier also attenuated the increases in firing and GABA potency due to SKF 38393.

tological verification were not done, it was not possible to assess whether overall completeness of lesions correlated with the numbers of stimulation-responsive and nonresponsive pars reticulata neurons found in individual animals. However, in lesioned animals in which multiple pars reticulata neurons were encountered, it was typical that about half of cells did respond to striatal stimulation, while the others did not. Those reticulata neurons that remained responsive to striatal stimulation despite the lesion served essentially as a sham-lesioned control group. For this reason, a separate group of sham-lesioned animals was not included.

The stimulating electrode was a 2 × 2 array of four electrodes with tip separation of approximately 2 mm, and insulation to within 0.5 mm of the tips. The electrode was positioned at 2.0 and 4.0 mm lateral, 9.0 and 11.0 mm anterior to the lambdoid suture, and 4.5 mm ventral. The stimuli consisted of square pulses, 100–300 μA in intensity and 300 μsec in duration, delivered at a frequency of 1 Hz for 1 min to the left striatum. Stimuli were applied to three of the electrodes and returned through the fourth. Oscilloscope traces of the neuronal activity during 1 min periods of striatal stimulation were stored and photographed for each reticulata neuron recorded in lesioned rats. Failure to observe an inhibitory response to striatal stimulation was regarded as evidence of a successful lesion of the striatal inputs to that pars reticulata neuron.

Measurement of ADP-ribosylation. The ability of pertussis toxin to inactivate nigral G-proteins was determined by assaying punches of substantia nigra taken from both the right (control) and left (pertussis toxin injected) sides of each rat brain from which electrophysiological data were gathered. The tissue punches were homogenized in 100 μl of membrane buffer (10 mM thymidine; 10 mM HEPES, pH 8; 1 mM EDTA; 5 mM dithiothreitol), and brought up to a final volume of 600 μl. The homogenates were centrifuged in an Eppendorf centrifuge for 10 min at maximum speed, and the pellet was resuspended in 80 μl of membrane buffer. Aliquots of the resuspended pellets were assayed for their ability to incorporate ADP-ribose in the presence of activated pertussis toxin. These reactions were carried out in 1.5 ml microfuge tubes that contained 5 μl of membrane buffer, 5 μl of ^{32}P-NAD (30 Ci/ml; New England Nuclear, Boston, MA), 30 μl of membrane homogenate, and 10 μl of activated pertussis toxin. Pertussis toxin was activated just prior to the assay by reconstituting the toxin with activation buffer (50 mM HEPES, pH 8; 20 mM dithiothreitol; 0.001% BSA; 0.125% SDS) to a final concentration of 100 μg/μl, and then incubating the toxin in a water bath for 30 min at 30°C. After addition of the activated toxin to the samples, reaction vials were incubated in a water bath for 30 min at 30°C, and then centrifuged for 10 min. The supernatant (which contains the G-proteins) was transferred to a new microfuge tube to which was added 50 μl of sample buffer (Tris, pH 8.6; glycerol, β-mercaptoethanol, bromphenol blue, and SDS), and the samples boiled for 5 min to stop the reaction. The supernatants were then subjected to one-dimensional SDS-polyacrylamide gel electrophoresis (Bio-Rad Protean II xi vertical electrophoresis unit), along with appropriate molecular weight markers. Gels were run at 50 V for 3–4 hr at constant current, and were then removed and dried overnight on a Tut's Tomb drying frame (Idea Scientific Company). The dried gels were autoradiographed, and the individual G-proteins bands were excised from the gels. The amount of radioactivity contained in each band was then quantitated using a liquid scintillation counter. The effectiveness of intranigral pertussis toxin injections was determined as the percentage decrease of ^{32}P-labeling in the left (injected) nigra relative to the right (control) substantia nigra.

Drugs. *R*-(−)-*N*-*n*-propylnorapomorphine, SKF 38393, SCH 23390, and hydroxypropyl-β-cyclodextrin were purchased from Research Biochemicals, Natick, MA. *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was purchased from Aldrich Chemical Co., Milwaukee, WI. Chloral hydrate, GABA, 8-bromo-cyclic AMP, dibutyryl cyclic AMP, chlorophenylthio-cyclic AMP, and cyclic AMP were all purchased from Sigma Chemical Co., St. Louis, MO. Pertussis toxin was purchased from List Biologicals, Campbell, CA. ^{32}P-NAD was purchased from New England Nuclear, Boston, MA. A-68930 was a gift of Dr. John Kebabian of Abbott Laboratories, North Chicago, IL. All other chemicals were of the highest reagent grade available from commercial sources.

Results

Effects SKF 38393 and A-68930 on pars reticulata neurons

SKF 38393 caused current-related increases in the firing rates of pars reticulata neurons, as was previously reported (Waszczak, 1990). At 8 nA SKF 38393, the average firing rate was 55 ± 11% above baseline (Figs. 1, 2), and 12 of the 14 cells tested showed a 20% or greater increase in rate. Although SKF 38393 had a marked excitatory effect on basal firing, it did not cause an attenuation of GABA's inhibitory potency, a response previously observed with the D_2/D_3 agonist quinpirole (Waszczak, 1990). In fact, absolute inhibitions of firing by GABA were usually exaggerated by SKF 38393; that is, as basal firing rates increased, the degree of inhibition during GABA applications was greater than that observed before SKF 38393 iontophoresis (Figs. 1, 2). At 8 nA SKF 38393, responses to GABA were 45 ± 9% greater than that observed in the period before the D_1 agonist.

For comparison, a second D_1 agonist was also evaluated. A-68930, a new D_1-selective agonist (DeNinno et al., 1990; Britton et al., 1991) of a different structural class than SKF 38393, was tested for its effects on pars reticulata neurons. Like SKF 38393, iontophoresis of A-68930 caused current-dependent increases in the firing of pars reticulata neurons (Figs. 1, 2). At 10 nA, the highest current tested, the average firing rate was 43 ± 8% above baseline, and 10 of the 11 neurons tested

showed increases in firing rate of 20% or more. There were no significant differences between the increases in firing elicited by A-68930 and SKF 38393 at any of the ejection currents tested. In the presence of A-68930, GABA inhibitions were again potentiated and were not different from those observed during iontophoresis of SKF 38393 (Figs. 1, 2).

Effect of intranigral EEDQ on the rate-increasing effect of SKF 38393

Regional inactivation of nigral dopamine receptors was accomplished by injecting EEDQ, an irreversible receptor alkylator, into the left substantia nigra 24 hr prior to recording. EEDQ has been shown to inactivate a number of receptors in addition to D_1 dopamine receptors, including D_2, α-adrenergic, and serotonergic receptors (Meller et al., 1985). The intent of these experiments was to determine if inactivation of an EEDQ-sensitive receptor could prevent the response to SKF 38393. In previous autoradiographic studies (Cox and Waszczak, 1993), EEDQ pretreatments identical to those used in the present studies were shown to cause an 85–95% decrease in nigral D_1 binding sites, relative to the uninjected nigra (data not shown). Autoradiograms generated from rats that had received intranigral vehicle (hydroxypropyl-β-cyclodextrin) injections alone revealed no loss of D_1 binding sites in the injected nigra. Moreover, the vehicle caused no gross histological or electrophysiological changes in the injected nigra, aside from slight damage at the site of the needle track. Population sampling studies including both substantia nigra pars compacta (dopamine) and pars reticulata neurons indicated that neither the number of neurons nor their average baseline firing rates were altered by the vehicle injections (Cox and Waszczak, 1993).

Twenty-four hours after intranigral EEDQ injections, the ability of SKF 38393 to stimulate pars reticulata neurons was greatly reduced or completely abolished (Figs. 1, 2; Waszczak and Martin, 1989). At 8 nA, a current that elicited maximal (55 ± 11%) increases in firing in normal rats, the average increase in firing rate for the EEDQ-treated rats was only 11 ± 5% (n = 11; p < 0.005 relative to response in control rats). Similarly, inhibitory responses to GABA were unchanged by SKF 38393 for the EEDQ-treated rats (Figs. 1, 2).

Effect of intranigral SCH 23390 on the rate-increasing effect of SKF 38393

To assess whether the responses to SKF 38393 were due to an action at D_1 dopamine receptors, efforts were made to block the effects with the D_1-selective antagonist SCH 23390. In initial studies, iontophoresis of the antagonist was carried out during simultaneous application of the agonist. However, at the antagonist concentration and ejection currents tested (0.2 M pH 4; 10–15 nA), SCH 23390 applications were without effect and failed to block the response to SKF 38393 (data not shown). Attempts to increase the amount of antagonist ejected were limited by the solubility of the drug and the amount of current that could be passed through the drug channel. Thus, it was not clear if an adequate concentration of antagonist was achieved at receptors to block the agonist effect. Intravenous administration of the antagonist just prior to iontophoresis of the agonist was also examined in a limited number of trails, but systemic SCH 23390 caused variable changes in the firing rates of pars reticulata neurons, and this confounded efforts to assess whether it could block both the rate-increasing and GABA-potentiating effects of locally applied SKF 38393.

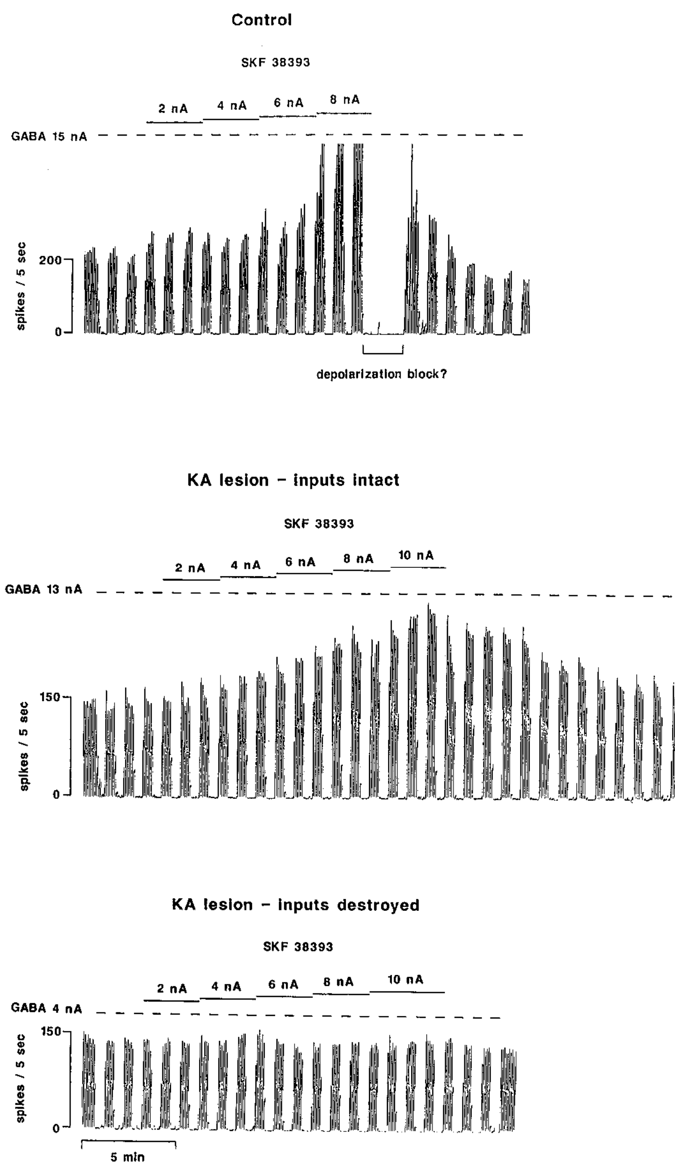

Figure 3. The excitatory response of pars reticulata neurons to iontophoretically applied SKF 38393 is lost after striatal kainic acid lesions that effectively destroyed striatonigral inputs to the recorded neuron. *Top trace,* Control response to SKF 38393 in an unlesioned rat. Baseline firing rates and responses to applied GABA were increased in a current-dependent manner during SKF 38393 iontophoresis. *Middle trace,* SKF 38393 causes a typical increase in baseline firing and responsiveness to GABA for a pars reticulata neuron whose striatonigral inputs remained intact despite the lesion. *Bottom trace,* Application of SKF 38393 has no effect on baseline firing or responsiveness to GABA for a pars reticulata neuron whose striatonigral inputs were terminated by the lesion (see Materials and Methods for electrophysiological screening criteria).

To circumvent problems encountered by the above methods, SCH 23390 (1 μg/μl) was injected into the substantia nigra 1 hr prior to electrophysiological experiments. Intranigral injections of the D_1 antagonist were found to attenuate greatly the ability of SKF 38393 to stimulate the firing of pars reticulata neurons (Figs. 1, 2). At 10 nA, SKF 38393 increased firing rates by only 22 ± 6% (n = 9), a significant (p < 0.01) reduction from that observed in control rats not pretreated with the D_1 antagonist. Similarly, responses to GABA were only slightly enhanced, that is, 13 ± 9% above baseline at 10 nA SKF 38393 (Figs. 1, 2).

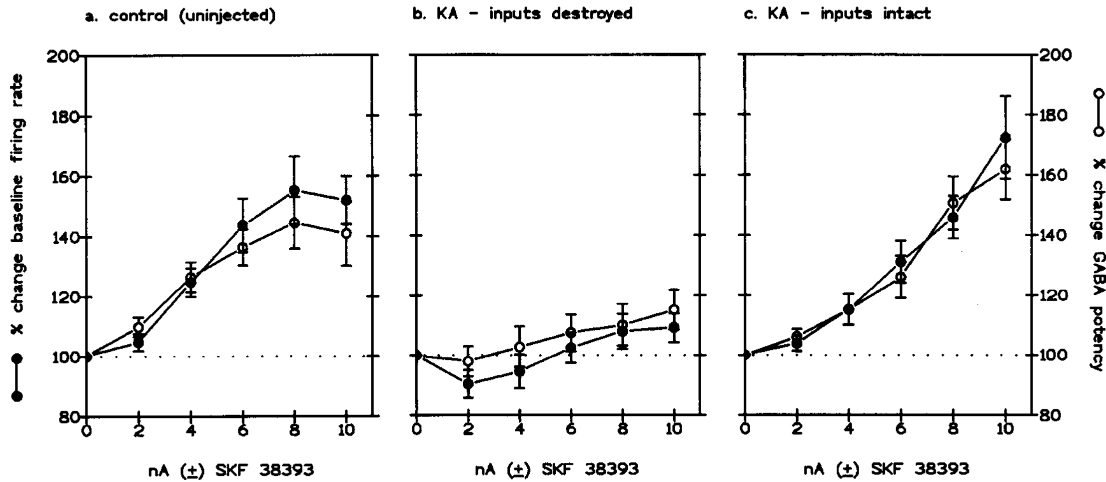

Figure 4. Current–response curves for the effects of SKF 38393 on the baseline firing rates (*solid circles*) and responses of pars reticulata neurons to applied GABA (*open circles*) in control rats and those with striatal kainic acid lesions that either did, or did not, terminate inputs to the recorded neurons. *a,* Control response to SKF 38393 in unlesioned rats (same as in Fig. 2). *b,* Lesions that destroyed striatonigral inputs to the recorded neuron resulted in loss of the excitatory response to SKF 38393. *c,* Lesions that left striatonigral inputs to the recorded cell intact did *not* result in loss of the excitatory response to SKF 38393.

Effect of striatal kainic acid injections on the rate-increasing effect of SKF 38393

To determine if the lesions had successfully destroyed the inhibitory influence of the striatonigral pathway, each reticulata cell was prescreened by monitoring its electrophysiological response after striatal stimulation. Pars reticulata cells were then separated into two groups: those not inhibited by striatal stimulation (i.e., striatal inputs were destroyed) and those that could still be inhibited by striatal stimulation (i.e., striatal inputs to that neuron remained intact).

Pars reticulata neurons whose striatal inputs had been lesioned showed only slight changes in firing rate during iontophoretic application of SKF 38393 (Figs. 3, 4; Waszczak and Martin, 1989). At 10 nA, the average firing rate of these cells was not significantly increased above basal (9 ± 5%; $n = 8$). Responses to GABA were also unaffected by application of SKF 38393 (Figs. 3, 4). The second group of neurons included those that were inhibited by striatal stimulation, and therefore still retained a functional striatonigral input. These cells responded to SKF 38393 with current-dependent increases in firing that were similar to the responses seen in unlesioned rats. The av-

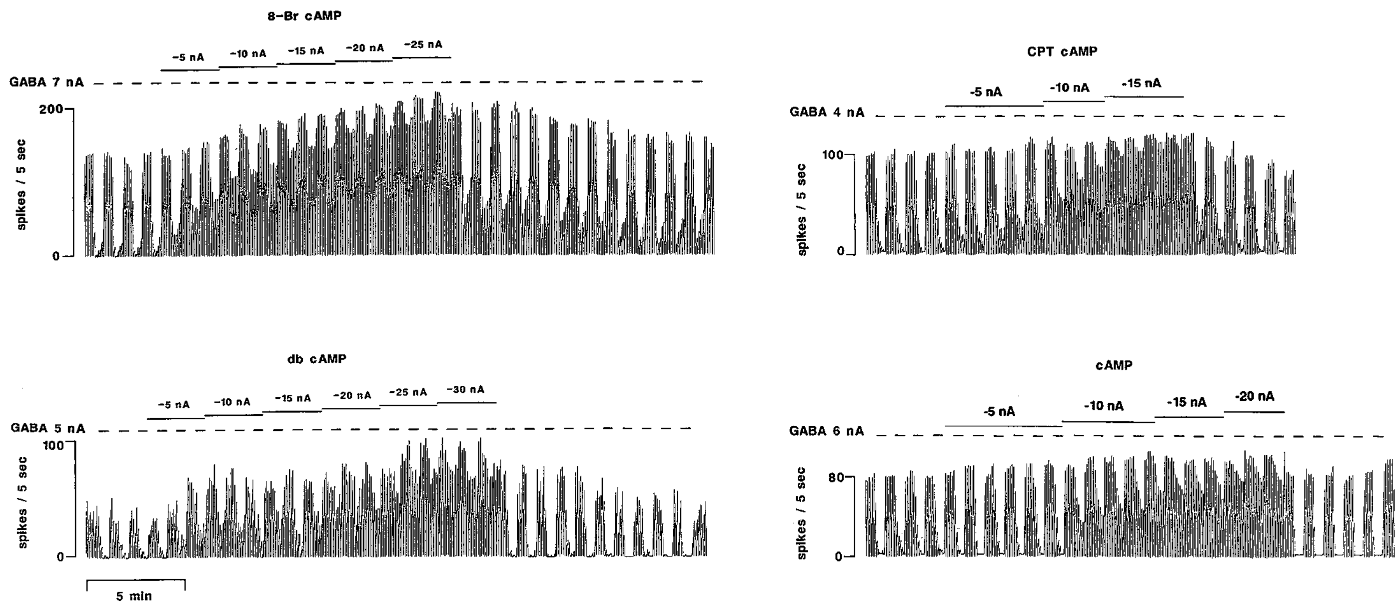

Figure 5. Iontophoretic application of cAMP analogs onto pars reticulata neurons does not produce a pattern of response typical of D_1 agonists. Several membrane-permeable analogs [8-bromo-cAMP (*8-Br cAMP*), dibutyryl cAMP (*db cAMP*), and 8-chlorophenylthio-cAMP (*CPT cAMP*)], as well as cAMP itself, were iontophoretically applied in place of the D_1 agonist in the test paradigm. 8-Br cAMP and db cAMP caused moderate increases in cell firing similar to SKF 38393, but CPT cAMP and cAMP had little effect on cell firing. Conversely, each of the analogs markedly attenuated the inhibitory responses to applied GABA, a finding opposite that typically observed with D_1 agonists.

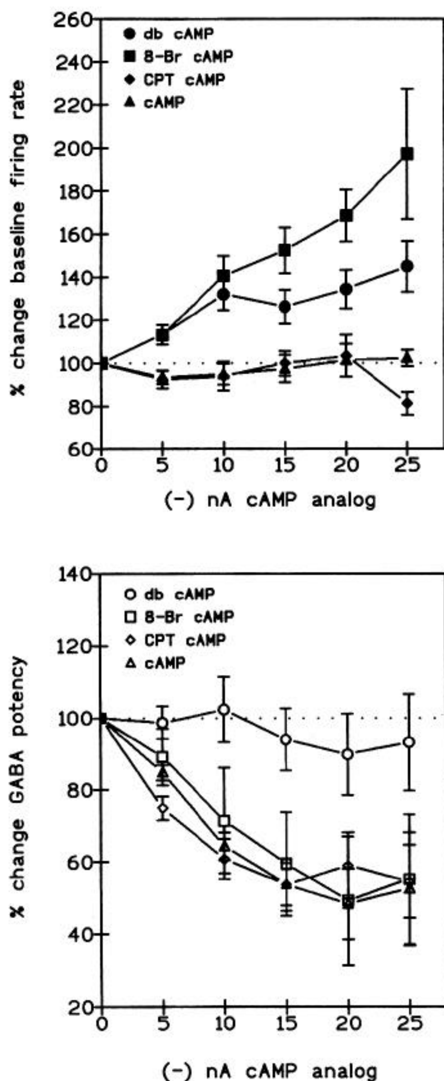

Figure 6. Current–response curves for the effects of cAMP analogs on the baseline firing rates (*top graph*) and responses of pars reticulata neurons to GABA (*bottom graph*). Only two cAMP analogs, 8-bromo-cAMP (*8-Br cAMP*) and dibutyryl cAMP (*db cAMP*), produced increases in baseline firing similar to those observed with D_1 agonists. The remaining analogs, chlorophenylthio-cAMP (*CPT cAMP*) and cAMP, had no significant effects on baseline firing rates. Distinctly *un*like the effects of D_1 agonists, however, each of the four analogs caused attenuations of GABA's inhibitory potency, and for three of the four analogs the effect was pronounced.

erage increase in firing rate at 10 nA was 73 ± 14% above baseline ($n = 9$). Responses to GABA were again exaggerated by SKF 38393, like those seen in the unlesioned rats.

Effects of cAMP analogs on pars reticulata neurons

Analogs of cAMP were iontophoresed onto pars reticulata neurons to assess their ability to mimic the rate-increasing effects of the D_1 agonists. Two membrane-permeable analogs, 8-bromo-cAMP and dibutyryl cAMP, caused significant, current-related increases in firing similar to those seen with SKF 38393 (Figs. 5, 6). At −20 nA, 8-bromo-cAMP and dibutyryl cAMP increased the average firing rates to 69 ± 12% ($n = 10$; $p < 0.05$) and 34 ± 9% ($n = 10$; $p < 0.05$) above baseline, respectively. However, iontophoresis of neither cAMP itself ($n = 8$) nor the membrane-permeable analog CPT-cAMP ($n = 7$) had any significant influence on the firing rates of pars reticulata cells (Figs. 5, 6; Martin and Waszczak, 1991).

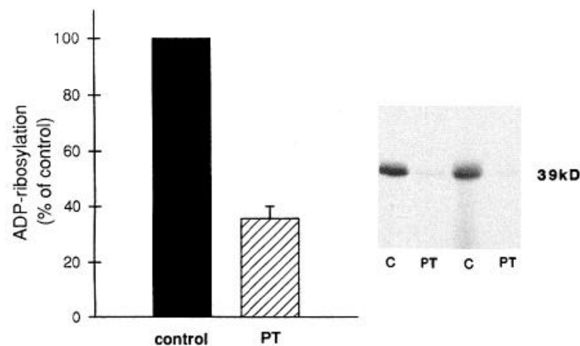

Figure 7. Intranigral injections of pertussis toxin (1 μg/μl) on the previous day ADP-ribosylated (inactivated) more than 60% of the substrate G-proteins in the injected nigra relative to the uninjected control side. Also shown are representative autoradiograms of gels from two brains showing dense ^{32}P-NAD incorporation (ADP-ribosylation) into G-protein(s) in the control (*C*) nigra, and the light incorporation on the pertussis toxin (*PT*) injected side.

Although their effects on basal firing were mixed, three of the four the cAMP analogs also caused significant, current-related attenuations in the inhibitory response to applied GABA (Figs. 5, 6). At −20 nA, the inhibitory potency of GABA was reduced by 51 ± 18% for 8-bromo-cAMP ($p < 0.05$), by 41 ± 9% for CPT-cAMP ($p < 0.05$), and by 52 ± 10% ($p < 0.05$) for cAMP. Dibutyryl cAMP also slightly reduced GABA potency, but the change was not significant relative to the basal response. The observed decreases in GABA potency were unusual in that they were opposite the exaggerated responses to GABA usually seen during the iontophoresis of D_1 agonists. In fact, the attenuation of GABA responses caused by the cAMP analogs was similar to the effects seen during coiontophoresis of a D_2 agonist (Waszczak, 1990).

Effects of intranigral pertussis toxin on the rate-increasing effect of SKF 38393

To investigate further the second messenger pathway involved in the excitatory response of pars reticulata cells to D_1 agonists, pertussis toxin was injected into the substantia nigra of rats 24 hr prior to electrophysiological testing. Verification of G-protein inactivation was determined by assessing the ability of the left (pertussis toxin–injected) nigra to further incorporate ADP-ribose in the presence of ^{32}P-NAD and pertussis toxin, compared to the right (untreated) side. Intranigral injections of pertussis toxin reduced the ^{32}P-labeling (ADP-ribosylation) in the treated nigra by 64 ± 6% relative to the control nigra ($p < 0.01$; Fig. 7).

Electrophysiology experiments performed in these rats revealed that the rate-increasing effect of SKF 38393 on pars reticulata neurons was completely abolished (Figs. 1, 8; Martin and Waszczak, 1991). At 10 nA SKF 38393, average firing rates were unchanged relative to baseline activities (97 ± 8% of basal; $n = 7$), and significantly reduced relative to the excitatory response to SKF 38393 at 10 nA in control rats ($p < 0.01$). Responses to GABA were also not altered from the baseline response. To verify further that intranigral pertussis toxin injections had successfully abolished G_i-protein–mediated pharmacological responses, the ability of intravenous *R*-(−)-*N*-*n*-propylnorapomorphine (NPA) to inhibit the firing of substantia

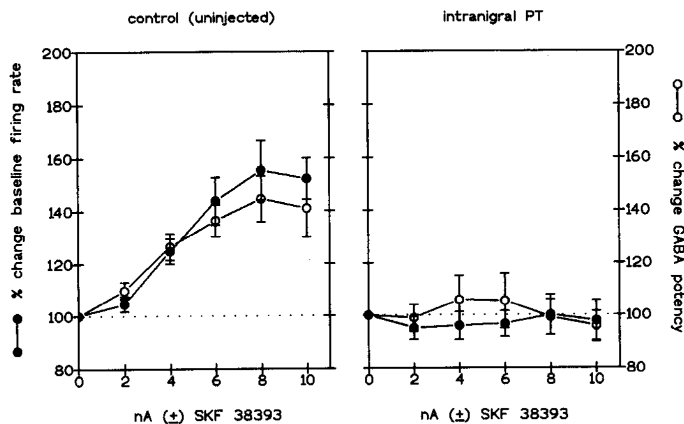

Figure 8. Current–response curves for the effects of SKF 38393 on the baseline firing rates (*solid circles*) and responses of pars reticulata neurons to applied GABA (*open circles*) in control rats (*left*), and in rats that received an intranigral injection of pertussis toxin (*PT*; 1 μg/μl) on the previous day (*right*). Pertussis toxin treatments completely abolished the rate-increasing effect of SKF 38393 on pars reticulata neurons.

nigra pars compacta dopamine neurons was also evaluated. A 5 mg/kg dose of NPA, which would typically fully inhibit the firing of dopamine neurons (Martin et al., 1990), was essentially without effect (inhibition by only 6 ± 3%) in rats given nigral injections of pertussis toxin on the previous day (data not shown). The loss of responsiveness of dopamine neurons to intravenous dopamine agonists after nigral pertussis toxin treatments is in agreement with the findings of Innis and Aghajanian (1987).

Discussion

The striatum is generally regarded as the primary site at which dopamine acts to regulate motor output from the basal ganglia. Indeed, the first stage of striatal output involves differential effects of dopamine on the two striatal efferent populations, the striatonigral and striatopallidal neurons (Albin et al., 1989; Alexander and Crutcher, 1990; Gerfen et al., 1990). The substantia nigra is a second site at which the nigrostriatal dopamine neurons could act to influence motor output from the basal ganglia, in this case by directly altering the activities of pars reticulata projection neurons. Our previous studies have shown that dopamine, applied by iontophoresis or released from dendrites, does influence the electrophysiological activities of substantia nigra pars reticulata neurons: it both stimulates their firing and attenuates their responsiveness to GABA (Waszczak and Walters, 1983, 1984, 1986). The two effects of dopamine were later shown to be pharmacologically distinguishable and appeared to be separately mediated by dopamine D_1 and D_2 receptors, respectively. For instance, the excitatory effect was reproduced by iontophoresis of a D_1 agonist, SKF 38393, which elicited current-related increases in pars reticulata neuronal firing similar to dopamine (Waszczak, 1990). This response was observed during iontophoresis of the (+)-enantiomer of SKF 38393 but not the inactive (−)-form. While the stereoselectivity of the D_1 agonist effect was consistent with a receptor-mediated action, other approaches for confirming a D_1 receptor involvement had not been attempted.

For instance, it was conceivable that the excitatory response to SKF 38393 was due either to a nonspecific action of the drug, or to a pharmacological effect of the agonist at another receptor type. To address these possibilities, a second D_1-selective agonist of a different chemical class was evaluated in place of SKF 38393. A-68930, a phenylisochroman rather than a benzazepine D_1 agonist (Britton et al., 1991; DeNinno et al., 1991), was found to share the ability of SKF 38393 to stimulate firing of pars reticulata neurons while potentiating their responses to applied GABA. Thus, the response to SKF 38393 was apparently not a nonspecific action attributable the benzazepine family of D_1 agonists, nor was it likely to be due an action at a non-D_1 receptor.

To ascertain further if D_1 receptors play a necessary role in mediating this response, various manipulations of the D_1 receptor population in the nigra were performed prior to evaluation of iontophoretically applied SKF 38393. In separate studies, D_1 receptors were either irreversibly inactivated by intranigral injections of EEDQ, pharmacologically blocked by intranigral injections of the D_1 antagonist SCH 23390, or destroyed by lesioning the striatonigral pathway. First, the role of an EEDQ-sensitive receptor in the agonist response was assessed 24 hr after intranigral injection of the receptor inactivator. EEDQ inactivates certain receptor types by first "activating" peptide carboxyl groups located at or near the binding site, and then forming an internal cross-link with a nearby α-amino group (Belleau, 1969). D_1 and D_2 dopamine receptors, α-adrenergic receptors, and serotonin receptors have all been shown to be sensitive to inactivation by EEDQ (Meller et al., 1985). Indeed, a substantial literature exists on the use of systemic EEDQ to inactivate dopamine receptors *in vivo* (Hamblin and Creese, 1983; Meller et al., 1985, 1987; Cox and Waszczak, 1989, 1990; Enz et al., 1990). Moreover, we and others have shown that intracerebral injections of EEDQ can produce a regional inactivation of dopamine receptors in restricted brain areas while allowing neurons in the injected area to survive (Cameron and Crocker, 1989; Giorgi and Biggio, 1990; Cox and Waszczak, 1993). Intranigral injections of EEDQ, which have been shown to produce a greater than 90% reduction in nigral D_1 binding sites (Cox and Waszczak, 1993), also caused a similar loss of the ability of SKF 38393 to stimulate pars reticulata cell firing. Thus, a population of nigral receptors sensitive to EEDQ appears to be necessary for the D_1 agonist to elicit increases in reticulata neuronal activity.

The ability of a D_1 antagonist to block the response was also confirmed by a similar approach. SCH 23390, injected into the substantia nigra an hour prior to electrophysiological experiments, greatly attenuated the ability of SKF 38393 to increase firing rates of pars reticulata neurons. In previous studies we had found that the rate-increasing effect of applied SKF 38393 was not blocked by iontophoresis of the D_1 antagonist SCH 23390. Attempts to demonstrate blockade by systemic administration of the antagonist were not pursued because intravenous SCH 23390 alone produced variable changes in the basal firing rates of pars reticulata cells, which complicated determination of the drug's ability to block agonist responses. The changes in reticulata cell firing seen after systemic SCH 23390 may have been due in part to actions at sites other than nigral D_1 receptors (such as striatal D_1 receptors on afferents to pars reticulata neurons), and may be reflective of a reversal of processes that cause variable responses of pars reticulata neurons to systemically administered dopamine agonists (Waszczak et al., 1984a,b). Nevertheless, it is noteworthy that our experience with both iontophoretic and systemic SCH 23390 contrasts with the report of Hu and Wang (1988), who succeeded in antagonizing effects

of applied SKF 38393 on rat caudate-putamen neurons with iontophoretically or systemically administered SCH 23390.

The apparent discrepancy between our former inability to demonstrate blockade by iontophoresis of SCH 23390 and our present data is likely due to the route of drug administration. Limitations inherent in the iontophoresis technique may have been responsible since it is difficult to control and quantitate the amount of drug ejected, its spread in tissue, and its concentration at the site of action. Thus, it is possible that the failure to observe blockade in our earlier study using iontophoresis of SCH 23390 was due to an inability to achieve sufficient concentrations of antagonist at receptors. This limitation was apparently overcome by direct injection of the antagonist into the nigra, since the latter method did result in pharmacological blockade. A disadvantage of this method, however, was an inability to assess the concentration, and ensure the D_1 selectivity, of SCH 23390 at its site of action. Despite this, the ability of a D_1 antagonist to prevent the excitatory response to the D_1 agonist directly supports involvement of a D_1 receptor in the agonist effect.

Finally, the location of the nigral D_1 receptors involved in this response was assessed after kainic acid lesions of the striatonigral pathway. The dense population of D_1 receptors located within the pars reticulata (Richfield et al., 1987; Beckstead et al., 1988) has been shown to be localized largely, perhaps exclusively, on the axon terminals of the striatonigral pathway. Lesioning the striatum virtually eliminates the dopamine-stimulated adenylate cyclase (Gale et al., 1977; Phillipson et al., 1977; Spano et al., 1977) and D_1 binding sites (Porceddu et al., 1986; Altar and Hauser, 1987; Altar and Marien, 1987; Beckstead et al., 1988) in substantia nigra. Thus, physiological responses to D_1 agonists, if indeed receptor mediated, would likely involve activation of these terminal D_1 receptors. Moreover, since a large part of striatonigral pathway impinges directly on pars reticulata neurons (Graybiel and Ragsdale, 1979; Albin et al., 1989; Alexander and Crutcher, 1990) it might be expected that these presynaptic D_1 receptors play a significant role in regulating striatonigral transmission, and thus the activity of nigral efferent neurons. Our studies confirmed that an intact striatonigral input to pars reticulata neurons was required for the excitatory response to SKF 38393. Striatal lesions that effectively terminated inputs to individual pars reticulata neurons caused loss of the excitatory response to the D_1 agonist, whereas lesions that failed to destroy striatonigral inputs to the recorded cell did not prevent the agonist-induced increase in firing. Collectively, these results show that the excitatory response of pars reticulata neurons to SKF 38393 is abolished by treatments that destroy, inactivate, or pharmacologically block the population of nigral D_1 receptors. Thus, it can now be concluded that the response is mediated presynaptically, by activation of D_1 receptors on impinging striatonigral terminals.

A second feature of the response to SKF 38393, the potentiation of GABA-mediated inhibitions, was also prevented by each of the treatments that abolished the rate-increasing effect. It appeared that responses to GABA were exaggerated only in cases where SKF 38393 also caused increases in basal firing, and that the magnitudes of the two effects were always closely correlated. Accordingly, when increases in firing were prevented by removal of nigral D_1 receptors, GABA responsiveness also appeared unchanged. The correlation of the two effects makes it unclear whether the potentiation of GABA responsiveness represents a phenomenon mechanistically distinct from the D_1-induced increase in firing, or simply reflects retention of near-complete inhibitions of firing by GABA as firing rates increased during the coiontophoresis of SKF 38393. If the two responses are mechanistically distinct, the nature of the GABA-potentiating response implies that the D_1 agonist increases the intrinsic ability of pars reticulata neurons to respond to GABA. Such a mechanism would be conceivable if pars reticulata neurons express some D_1 receptors that modulate GABA receptor sensitivity through an intracellular mechanism. In accordance with such a possibility, a modest expression of D_1 receptors on intrinsic nigral neurons has been suggested (Huang et al., 1992). However, the physiological importance of such a D_1/GABA modulatory interaction would appear questionable in view of the fact that dopamine attenuates effects of GABA on pars reticulata neurons via D_2 receptors, and this effect consistently exceeds any D_1-mediated GABA potentiation (Waszczak and Walters, 1983; Waszczak, 1990).

The mechanism by which D_1 agonists exert the excitatory effect on pars reticulata neurons is also unclear. It can be speculated that activation of D_1 receptors on striatonigral neurons regulates the release of a transmitter, which in turn regulates pars reticulata cell activities. Since the striatonigral pathway is GABAergic (Kim et al., 1971; Fonnum et al., 1974; Kataoka et al., 1974), it is tempting to postulate that D_1 receptor stimulation might inhibit GABA release, relieving the inhibitory influence of the striatonigral pathway, and allowing reticulata cells to increase in their firing rates. Although an attractive hypothesis, *in vitro* and *in vivo* studies that have examined effects of dopamine agonists on GABA release in the substantia nigra are conflicting. Various studies have found that ^{3}H-GABA release from nigral slices is either increased or unchanged by dopamine or different agonists (Reubi et al., 1977; Arbilla et al., 1981; Kelly et al., 1985), whereas endogenous GABA release measured *in vivo* by push–pull cannula techniques was found to be altered in a biphasic manner by dopamine and apomorphine (van der Heyden et al., 1980a). More recently, selective dopamine agonists and antagonists have been used in an attempt to clarify whether D_1 and D_2 receptor subtypes differentially modulate GABA release in the nigra. Several of these studies concluded that stimulation of D_1 receptors increased potassium-stimulated ^{3}H-GABA release in slices from the rat substantia nigra (Starr et al., 1987; Floran et al., 1990; Aceves et al., 1991). However, in contrast, an *in vivo* microdialysis study found that endogenous GABA release in the substantia nigra pars reticulata was increased after probe perfusion of a D_1 *antagonist*, suggesting that D_1 stimulation inhibits nigral GABA release (Yamamoto, 1989). The inconsistent results obtained from different GABA release studies might be attributable to the varied experimental conditions employed. For instance, there may be differences in dopamine regulation of basal, endogenous GABA release and depolarization-evoked release of ^{3}H-GABA from preloaded slices. In this regard, it might be noted that in studies where endogenous GABA release was evaluated in *in vivo* preparations, an inhibitory regulation by D_1 agonists could be observed, whereas in *in vitro* studies measuring ^{3}H-GABA release from nigral slices evoked by high K^+, electrical stimulation, or Ca^{2+}, a dopamine-mediated stimulation of GABA release was more often observed. Conflicting results concerning dopaminergic regulation of GABA release have also been reported in other brain areas (Brase, 1980; van der Heyden et al., 1980b; Stoof et al., 1982; Kuriyama et al., 1984; Kontro and Oja, 1988; Umeda and Sumi, 1989). Given the contradictory literature on

the regulation of GABA release in substantia nigra, the possibility that D_1 agonists excite pars reticulata cells by attenuating GABA release from striatonigral terminals remains an open question. Alternatively, the response may be elicited by another transmitter. Dynorphin and substance P are both colocalized with GABA in striatonigral fibers (Reiner and Anderson, 1990). Changes in release of either of these peptides, via activation of the D_1 receptors on striatonigral terminals, may be responsible for the stimulation of reticulata cell firing.

The most intriguing and unanticipated findings were those obtained from a series of experiments intended to confirm that the D_1 agonist response was mediated by cAMP. D_1 receptor activation has traditionally been linked to stimulation of cAMP formation (Kebabian and Calne, 1979), and a dopamine-sensitive adenylate cyclase has been identified in the substantia nigra pars reticulata (Phillipson and Horn, 1976; Spano et al., 1976, 1977; Gale et al., 1977; Phillipson et al., 1977). Iontophoretic application of membrane-permeable analogs of cAMP was expected to reproduce the effect of the D_1 agonists, that is, to increase cell firing and cause an exaggerated inhibitory response to applied GABA. However, the effects of these compounds were complex, and none of the cAMP analogs tested possessed a pharmacological profile identical to that of the D_1 agonists (Martin and Waszczak, 1988). First, their effects on basal firing were mixed. While 8-bromo-cAMP and dibutyryl cAMP caused characteristic D_1 agonist-like increases in firing, CPT-cAMP had no effect on reticulata cell firing. The inconsistent occurrence and magnitude of the excitatory effect of the three membrane-permeable analogs is inexplicable if cAMP is indeed the second messenger mediating the D_1 response. Second, two of the membrane-permeable cAMP analogs, as well as the nonpermeable cAMP, attenuated inhibitory responses to applied GABA. An attenuation of GABA's inhibitory effect was opposite the response observed with the D_1 agonists SKF 38393 and A-68930.

While these findings appear at least superficially inconsistent with a role for cAMP as mediator of D_1 the electrophysiological response, the results should be interpreted with caution. It is possible that iontophoretically applied cAMP analogs act at multiple sites, besides striatonigral terminals, and that the complex responses represent a composite of effects at different cellular locations. The rate-increasing effect of two of the analogs may reflect the expected D_1-like action on striatonigral terminals, although it is not clear why CPT-cAMP did not share this effect. The attenuation of GABA responses seen with three of the analogs may reflect direct effects of cAMP on the $GABA_A$ receptor of pars reticulata neurons themselves. For instance, intracellular phosphorylation of the $GABA_A$ receptor by cAMP-dependent protein kinase A has been shown to accelerate desensitization of the receptor (Heuschneider et al., 1989; Porter et al., 1990; Leidenheimer et al., 1991a,b; Moss et al., 1992). Additionally, the nonpermeable form (cAMP itself) has been shown to reduce GABA receptor function, presumably by a non-phosphorylation-dependent mechanism involving an action at the Cl^- channel or some other extracellular site on the GABA–Cl^- ionophore complex (Lambert and Harrison, 1990; Leidenheimer et al, 1990, 1991b). The ability of cAMP to act both intra- and extracellularly to attenuate GABA receptor function may explain why both permeable and nonpermeable cAMP analogs attenuated GABA responses. It remains unclear, however, why dibutyryl cAMP did not share this ability. In view of the many possible sites of action and effects of cAMP in intact preparations, it might be premature to conclude on the basis of these studies alone that cAMP does not mediate the D_1 agonist–induced excitatory effect.

Further attempts to evaluate the receptor coupling mechanism underlying the D_1 response did, however, provide a basis for questioning the involvement of cAMP. Specifically, nigral injections of pertussis toxin 1 d before electrophysiological studies completely abolished the D_1 agonist–induced increase in pars reticulata cell firing. Pertussis toxin ADP-ribosylates G_i- and G_o-proteins (Katada and Ui, 1982; Sternweiss and Robishaw, 1984) and, when injected into the brain, has been shown to functionally uncouple receptor transduction sequences that utilize these G-proteins (Innis and Aghajanian, 1987a,b). Subsequent assay confirmed that our pretreatments had ADP-ribosylated about 70% of the substrate G-proteins in the injected nigras (Martin and Waszczak, 1992). The ability of pertussis toxin injections to prevent the excitatory response to SKF 38393 was surprising since D_1 receptors have been defined as activating adenylate cyclase through interaction with a pertussis toxin–*in*sensitive G-protein. Although it was initially assumed that G_s coupled the D_1 receptor to adenylate cyclase, recent studies have suggested that G_{olf}, a homologous G_s-like protein, might be the more likely coupling protein in substantia nigra due to its greater abundance in striatonigral neurons (Herve et al., 1993). In either case, the lack of a cysteine residue four amino acids from their carboxy-terminal end would prevent both G_{olf} and G_s from serving as substrates for ADP-ribosylation by pertussis toxin (Milligan, 1988; Jones and Reed, 1989). Thus, the nigral D_1 receptor mediating the excitatory response to D_1 agonists in our studies might be unconventional by coupling to a non-G_s-like protein and possibly an effector other than adenylate cyclase.

These findings raise the question of whether another receptor with D_1-like pharmacology exists in striatonigral neurons but is not coupled to stimulation of adenylate cyclase, or whether the known D_1 receptor might be able to couple to a second messenger pathway other than, or in addition to, adenylate cyclase. The concepts of receptor multiplicity and/or coupling promiscuity are not unprecedented (Milligan, 1993). In other systems, a single receptor subtype has been shown to couple to multiple effector pathways within the same cell, or in different cell types (Ashkenanzi et al., 1987; Vallar et al., 1990; Milligan, 1993). Such alternate coupling might depend upon cellular location, or more specifically, the type and availability of G-proteins and effector elements in the neurons expressing the receptor.

There are multiple lines of evidence favoring existence of either a non-adenylate cyclase-linked D_1 receptor, or one with alternate second messenger coupling potential. First, a D_1 agonist, SKF 82526, can independently stimulate phospholipase C and adenylate cyclase activities in renal tubular membranes (Felder et al., 1989; Vyas et al., 1992), a tissue known to possess a D_1-like receptor. Others have shown that a D_1 receptor in the proximal convoluted tubules of the kidney is linked to Na^+-K^+ ATPase via a pertussis toxin–sensitive G-protein (Bertorello and Aperia, 1989). In addition, D_1 binding sites, labeled by ^{3}H-SCH 23390, have been identified both in the amygdala and on pituitary lactotrophs, but there is no dopamine-stimulated adenylate cyclase in these tissues (Dawson et al., 1986; Mailman et al., 1986; Schoors et al., 1991; Scibilia et al., 1992). There is also growing evidence that a D_1 receptor exists in basal ganglia that is either non-cyclase-linked, or capable of coupling to other second messenger pathways. For instance, solubilized striatal D_1 receptors, when reconstituted with exogenous G-proteins,

were found to couple with both stimulatory (G_s) and inhibitory (G_i) G-proteins (Sidhu et al., 1991). In another study, Mahan et al. (1990) expressed a receptor from rat striatal mRNA in *Xenopus* oocytes that exhibited typical D_1 pharmacology, but was coupled to inositol phosphate (IP) production and mobilization of intracellular calcium. This finding suggests the presence in striatum of a D_1 receptor capable of coupling to phospholipase C. More direct evidence for the existence of such coupling in striatonigral neurons derives from the work of Undie and Friedman (1990a, 1990b, 1992) and our studies (Martin and Waszczak, 1992, 1993) showing that dopamine and the D_1 agonist SKF 38393 stimulate ^{3}H-IP formation in both striatal and nigral slices prelabeled with ^{3}H-*myo*-inositol. In both cases, the time course and agonist concentrations were similar, and the responses were antagonized by SCH 23390. Our finding that the excitatory response of pars reticulata neurons to the D_1 agonist involves a pertussis toxin–sensitive G-protein (i.e., a G_i/G_o- but not a G_s-like protein) is also consistent with possible coupling of the nigral D_1 receptor to a phospholipase second messenger pathway.

Finally, there is intriguing circumstantial evidence suggesting that striatonigral terminals, the locus of D_1 receptors in substantia nigra, are enriched in transduction elements associated with coupling both to a phospholipase and to adenylate cyclase. For instance, the pars reticulata neuropil exhibits very dense immunohistochemical staining for both $G_{i\alpha}$ (Aronin and DiFiglia, 1992) and $G_{o\alpha}$ (Worley et al., 1986b), as well as two subspecies of protein kinase C (PKC) and the inositol 1,4,5-trisphosphate (IP_3) receptor (Yoshihara et al., 1991; Fotuhi et al., 1993). In addition, the nigra contains dense autoradiographic labeling by ^{3}H-phorbol dibutyrate, a marker for PKC (Worley et al., 1986a). These markers were found to be associated with striatonigral terminals since labeling was lost after striatal lesions. Conversely, striatonigral neurons apparently possess low levels of $G_{s\alpha}$ mRNA and protein, although they do contain high levels of $G_{olf\alpha}$, which may mediate the stimulation of adenylate cyclase in the striatonigral terminals (Largent et al., 1988; Hevre et al., 1993). High levels of adenylate cyclase also exist in both striatum and substantia nigra (Urosevic and Grundlach, 1988), but cAMP binding proteins, indicated by the density of ^{3}H-cAMP binding, are low in the nigra (Grundlach and Urosevic, 1989). To some extent, these mismatches between components of the stimulatory adenylate cyclase system leave open the possibility that it is not the sole coupling pathway for D_1 receptors in substantia nigra. Activation of nigral D_1 receptors might stimulate adenylate cyclase as well as a phospholipase or some other pertussis toxin–sensitive pathway to yield different physiological responses. While our present data are suggestive of such possibilities, they do not permit the conclusion that nigral D_1 receptors necessarily interact with more than one coupling pathway under physiological conditions, nor do they reveal which second messenger system is responsible for the excitatory effect of D_1 agonists on pars reticulata neurons.

A physiological role for dendritically released dopamine and the large population of D_1 receptors in the substantia nigra has been long anticipated. Our results suggest that locally released dopamine may act at D_1 receptors on striatal terminals to regulate the efficiency of striatonigral transmission, and thus the excitability of pars reticulata output neurons. Moreover, the excitatory response of pars reticulata neurons to D_1 stimulation appears to be mediated by a pertussis toxin–sensitive (non-G_s-like) G-protein and possibly a second messenger other than cAMP. A discrete physiological role for dendritically released dopamine on substantia nigra pars reticulata neurons confers upon this region an important processing function beyond the level of a simple basal ganglia relay station. Indeed, the direct excitatory effect of nigral dopamine can be viewed as counteracting an inhibitory influence normally exerted via dopaminergic activation of the striatonigral pathway. Thus, contrary to the simplest models of basal ganglia processing, which emphasize predominance of striatonigral transmission, output from the pars reticulata may actually be governed by a dynamic balance between two opposing dopaminergic influences. Such a revised model may help to explain why generalized dopamine system activation, as seen after systemic administration of dopamine agonists, causes highly variable and fluctuating changes in the firing of pars reticulata neurons (Waszczak et al., 1984a,b).

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