

Changes in dopaminergic terminal excitability induced by amphetamine and haloperidol

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An accumulation of biochemical evidence suggests that there may be receptors located on the axon terminals of catecholaminergic neurons. In noradrenergic neurons, activation of presynaptic autoreceptors by norepinephrine appears to modulate release of the neurotransmitter^{19,20,30}. In dopaminergic neurons, activation of autoreceptors has been suggested to modulate synthesis of the amine^{18,32} in addition to its release from the terminal^{10,17,25}, although evidence for the latter is inconsistent^{9,24}. In addition to biochemical evidence, the existence of these receptors and their pharmacological properties have been inferred from neurophysiological studies of catecholaminergic nerve cells where it has been shown that the spontaneous activity of both noradrenergic and dopaminergic neurons is inhibited by their own transmitter^{1,2,6,13–15}. It is possible that activation of presynaptic receptors may alter the excitability of the synaptic terminal in a similar way.

We have developed a method for assessing the excitability of nerve terminals of single neurons in the brain using a modification of the antidromic stimulation paradigm developed by Wall to characterize the phenomenon of presynaptic inhibition³¹. This method is similar to one used successfully to measure alterations in the excitability of single afferent fibers of peripheral nerve^{26,27}. In this initial report, the method is applied to single dopaminergic neurons of the substantia nigra of the rat to reveal alterations in dopaminergic terminal excitability induced by amphetamine, a drug known to promote the extracellular accumulation of dopamine at these terminals⁷, and haloperidol, a dopamine receptor blocking agent⁵. We have found that amphetamine decreases the excitability of dopaminergic nerve terminals, while haloperidol increases the excitability of the terminal field to direct electrical stimulation. These results suggest that dopamine released from the nerve terminal could hyperpolarize the synaptic ending, while pharmacological blockade of presynaptic dopamine receptors could lead to a depolarization of the synaptic terminal.

Twenty-eight male Sprague–Dawley rats, weighing between 200 and 500 g, were used for experimentation. Subjects were anesthetized by urethane (1.3 g/kg i.p.) or by

initial ether inhalation followed by intravenous chloral hydrate (400 mg/kg), were secured in a stereotaxic instrument, immobilized with D-tubocurarine and artificially respired. Heart rate, body temperature and expired CO₂ were monitored and maintained within normal physiological limits. Microelectrodes of 3–6 MΩ impedance were lowered stereotaxically into the substantia nigra, pars compacta and a bipolar stimulating electrode was positioned within the head of the ipsilateral caudate nucleus. In 4 subjects, a second stimulating electrode was positioned to stimulate the medial forebrain bundle. Presumed dopaminergic neurons of the substantia nigra were identified electrophysiologically by criteria described below, and single unit activity was recorded extracellularly by conventional techniques and stored on magnetic tape for subsequent analysis. The antidromic nature of the response was verified by testing for collision with spontaneous action potentials. Stimulating electrode placements in the head of the caudate nucleus, and recording electrode placements in pars compacta of the substantia nigra, were verified by subsequent histological analysis. The stimulus for antidromic activation consisted of a monophasic square wave pulse approximately 0.1–0.5 ms in duration. Stimulus current, which ranged from 100 to 3000 μA, was monitored continuously. Once the identity of the activated cell had been characterized with certainty, stimulus currents sufficient to evoke antidromic responses on 0 and 100% of the non-collision trials on which they were delivered were determined. One or more values between these extremes were also chosen and several hundred stimuli, presented 1/s at each of these values, were delivered in both ascending and descending order, counterbalanced within subjects, for later analysis of the proportions of antidromic activations obtained at each current. D-Amphetamine sulfate (courtesy of Smith, Kline and French) or haloperidol (courtesy of McNeil Labs) dissolved in 0.9% NaCl were administered in incremental doses intravenously. For each increment, data were collected at each current value as in the pre-drug condition described above.

Presumed dopaminergic neurons in the pars compacta of the substantia nigra exhibited characteristic wave forms and patterns of firing that were identical to those of 'Type I' cells reported by Guyenet and Aghajanian¹⁶ and others^{1–4,11}. During extracellular recording, these neurons exhibited spontaneous action potentials of unusually long duration (>2.0 ms) with a relatively low frequency of firing (0.5–8.0 Hz) characterized by occasional episodes of bursting during which the amplitude of each successive action potential diminished progressively within the burst. A clearly delineated initial segment component of the action potential was frequently noted. All neurons used in this study fulfilled the above criteria and were antidromically activated from the ipsilateral caudate nucleus. The antidromic responses were observed to occur at relatively fixed latencies and exhibited collisions with spontaneous discharges. The antidromic latencies ranged from 9.0 to 21.0 ms with a mean value \pm S.E.M. of 13.8 ± 0.9 ms, in accordance with parameters previously described for presumed dopaminergic neurons of the nigrostriatal pathway¹⁶. As described by these authors, we found that the antidromic response typically consisted of an initial segment spike only; a full antidromic spike was observed only rarely and, when observed, showed a characteristic break between the initial segment and soma-dendritic components¹⁶. Occasionally, antidromic responses of a single, well-isolated unit were observed to

occur at two or three discrete latencies, a phenomenon that has recently been described for nigrostriatal neurons⁸. In these cases the lowest threshold, longest latency response was chosen and followed throughout the course of the experiment.

Fig. 1 illustrates the typical effects of two incrementally administered doses of D-amphetamine sulfate on the excitability of the terminal field of a dopaminergic neuron. Prior to drug administration, the stimulus current necessary to evoke antidromic potentials on 100% of stimulus deliveries was slightly less than 1900 μ A, while current levels less than this provoked antidromic potentials on proportionately fewer stimulus trials as shown. Within 1–2 min following intravenous delivery of 0.5 mg/kg D-amphetamine sulfate, the stimulus currents required to evoke similar proportions of antidromic potentials were shifted nearly uniformly to the right. A cumulative dose of 1.0 mg/kg led to a further increase in stimulus currents necessary to evoke these proportions of antidromic responses. Amphetamine was administered to 16 subjects in doses ranging from 0.25 to 2.0 mg/kg intravenously. Eleven subjects showed an increase in current necessary to evoke antidromic responses on 100% of non-collision trials. The mean percent increase in stimulus current \pm S.E.M. for this group was $64.1 \pm 6.8\%$. Two cases showed no change in excitability (i.e. less than 10% change), and three subjects exhibited a decrease in current necessary to evoke antidromic responses (mean percent decrease \pm S.E.M. $22.3 \pm 6.2\%$). Concomitant with decreased terminal excitability, the antidromic response latency sometimes increased slightly (0.5–1.0 msec) and spontaneous firing rate showed a moderate decline as previously

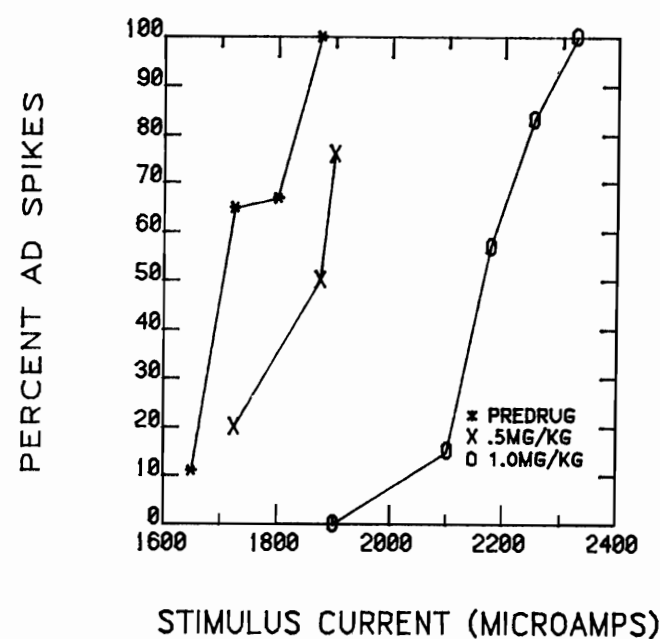


Fig. 1. Intravenous administration of amphetamine produces a decrease in the excitability of dopaminergic nerve endings to direct electrical stimulation. The percent of antidromic spikes evoked by various current values is indicated along the ordinate, while stimulus current is shown along the abscissa. Prior to drug administration, stimulus currents ranging between 1600 and 1900 μ A evoked antidromic responses between 0 and 100% of the time. Following 0.5 mg/kg D-amphetamine, the curve is shifted in a parallel fashion to the right (X) indicating that higher currents were necessary to evoke antidromic responses, reflecting a decrease in terminal excitability. Following a second injection of 0.5 mg/kg D-amphetamine, the curve is again shifted to the right (O), indicating a further decrease in terminal excitability to the higher cumulative dose. The percent increase in current above control value required to elicit antidromic responses on 100% of the stimulus trials (eliminating trials in which collision with spontaneous action potentials occurred) after a cumulative dose of 1.0 mg/kg D-amphetamine is approximately 23%.

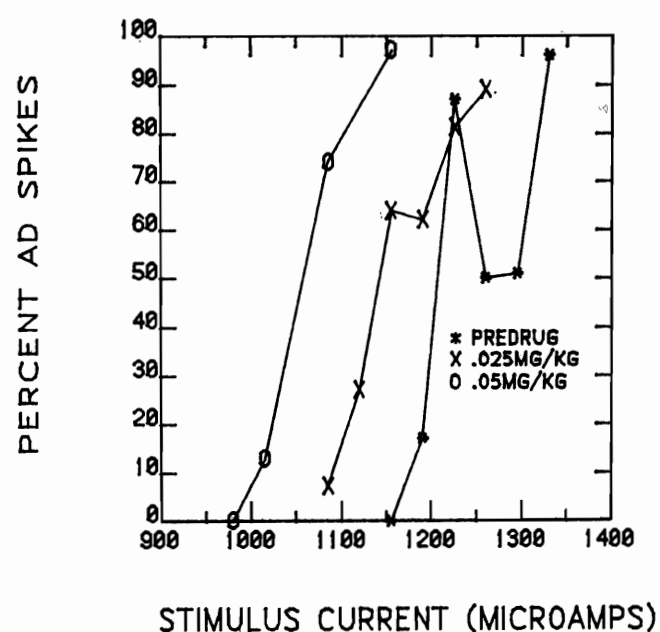


Fig. 2. Intravenous administration of haloperidol produces an increase in the excitability of dopaminergic nerve endings to direct electrical stimulation. Prior to haloperidol administration (*) currents ranging between 1150 and 1350 μA were required to evoke antidromic responses on between 0 and 100% of non-collision stimulus trials. Following 0.025 mg/kg haloperidol, the curve is shifted in a parallel fashion to the left (X), indicating an increase in terminal excitability. A second injection of 0.025 mg/kg produces a further increase in terminal excitability (O). The percent decrease in stimulus current from control value required to elicit antidromic responses on 100% of stimulus trials (eliminating those in which collision with spontaneous action potentials occurred) following a cumulative dose of 0.05 mg/kg haloperidol is approximately 14%.

reported¹³⁻¹⁵. These results show that following amphetamine administration the terminal fields of most presumed dopaminergic neurons of substantia nigra exhibit a dose-dependent decrease in excitability to direct electrical stimulation.

In contrast to amphetamine, the dopamine receptor blocking agent, haloperidol, led to a modest increase in the excitability of the ending to direct electrical stimulation in five of seven animals tested. A typical case is illustrated in Fig. 2. Prior to drug administration, stimulus currents necessary to evoke antidromic potentials on between 0 and 100% of the trials ranged from approximately 1150 to 1350 μA , with intermediate values leading to correspondingly proportionate numbers of antidromic responses. One to two minutes following intravenous administration of 0.025 mg/kg haloperidol, stimulus currents necessary to evoke similar proportions of antidromic invasions were reduced uniformly by approximately 50 microamperes. A cumulative dose of 0.05 mg/kg led to a further increase in terminal excitability reflected by a further reduction in the stimulus currents necessary to evoke antidromic responses. The mean percent decrease in stimulus current for this group was $14.4 \pm 2.37\%$. Two subjects showed no effects (less than a 10% change) to the drug. This increase in terminal excitability was at times accompanied by a decrease in antidromic response latency of from approximately 0.5 to 1.0 ms, and a modest increase in neuronal firing rate as noted in previous reports^{13,15}. These results are consistent with the view that haloperidol, by blocking dopaminergic receptors located on the terminal, leads to an increase in the excitability of the dopaminergic terminal, although of considerably smaller magnitude than the opposite effect rendered by amphetamine.

Our method suggests that dopamine released from dopaminergic nerve terminals

causes a decrease in the excitability of the nerve terminal while blockade of presynaptic receptors produces an increased terminal excitability, with concomitant changes in antidromic response latency. These changes in excitability following amphetamine or haloperidol administration do not occur when antidromic stimulation is delivered along the medial forebrain bundle ($n=4$), showing that the effects of these agents on antidromic excitability are specific for the terminal field of the dopaminergic neuron and not along the trajectory of its axon. It is unlikely that these effects are mediated by a postsynaptic interneuronal pathway since an ultrastructural analysis of synaptic endings in the neostriatum in serial sections has failed to reveal a single axo-axonic synapse¹².

The neurophysiological mechanisms underlying these modifications in excitability remain unknown at present, although it seems clear that some changes in the electrical properties of the terminal membrane, i.e. conductance and/or polarization are central to the phenomenon. These alterations in terminal excitability are consistent with the proposition that dopamine released from the terminal causes a hyperpolarization of the terminal membrane, while blockade of presynaptic dopamine receptors leads to a depolarization of the terminal. Effects similar to these are thought to occur at the level of the dopaminergic cell body consequent to dendritic release of neurotransmitter^{13,15}, and we have recently obtained similar effects following local infusion of alpha receptor agonists and antagonists into the terminal regions of noradrenergic neurons of nucleus locus coeruleus²¹.

Activation of autoreceptors by catecholamines is believed to result in feedback inhibition of catecholamine release. Our evidence argues that this process could be accompanied by a hyperpolarization of the terminal membrane. Although a reduction in impulse related, calcium dependent neurotransmitter release has been thought to occur during the sustained depolarization of the presynaptic ending^{23,33}, several chemical synapses have been described in which hyperpolarization of the presynaptic element results in reduced transmitter release while depolarization has the opposite effect^{22,23,26-29}.

Although the precise mechanisms of presynaptic inhibition are still unsettled, our evidence suggests that activation of presynaptic receptors by dopamine released from the synaptic ending has functional consequences on the biophysical properties of the terminal membrane. These may be of significance in the many receptor mediated events governing synthesis, release and other functional properties of the nerve terminal including drug and neurotransmitter actions at the synaptic interface.

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1 Aghajanian, G. K. and Bunney, B. S., Central dopaminergic neurons: neurophysiological identification and responses to drugs. In E. Usdin and S. H. Snyder (Eds.), *Frontiers in Catecholamine Research*, Pergamon Press, Oxford, 1975, pp 643-648.

2 Aghajanian, G. K. and Bunney, B. S., Dopamine 'autoreceptors:' Pharmacological characteri-

- zation by microiontophoretic single cell recording studies, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 297 (1977) 1-7.
- 3 Bunney, B. S. and Aghajanian, G. K., Electrophysiological effects of amphetamine on dopaminergic neurons. In E. Usdin and S. H. Snyder (Eds.), *Frontiers in Catecholamine Research*, Pergamon Press, Oxford, 1975, pp. 957-962.
 - 4 Bunney, B. S., Walters, J. R., Roth, R. H. and Aghajanian, G. K., Dopaminergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity, *J. Pharmacol. exp. Ther.*, 185 (1973) 560-571.
 - 5 Carlsson, A. and Lindqvist, M., Effect of chlorpromazine and haloperidol on formation of 3-methoxytryamine and normetanephrine in mouse brain, *Acta Pharmacol. Toxicol.*, 20 (1963) 140-144.
 - 6 Cedarbaum, J. M. and Aghajanian, G. K., Catecholamine receptors on locus coeruleus neurons: pharmacological characterization, *Europ. J. Pharmacol.*, 44 (1977) 375-385.
 - 7 Chiueh, C. C. and Moore, K. E., In vivo release of endogenously synthesized catecholamines from the cat brain evoked by electrical stimulation and by D-amphetamine, *J. Neurochem.*, 23 (1974) 159-168.
 - 8 Collingridge, G. K., James, T. A. and MacLeod, N. K., Antidromic latency variations of nigral compacta neurons, *Experientia*, 36 (1980) 970-971.
 - 9 Dismukes, K. and Mulder, A. H., Effects of neuroleptics on release of 3-H-dopamine from slices of rat corpus striatum, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 297 (1977) 23-29.
 - 10 Farnebo, L.O., and Hamberger, B., Catecholamine release and receptors in brain slices. In E. Usdin and S. H. Snyder (Eds.), *Frontiers in Catecholamine Research*, Pergamon Press, Oxford, 1975, pp. 589-593.
 - 11 Grace, A. A. and Bunney, B. S., Nigral dopamine neurons: intracellular recording and identification with L-dopa injection and histofluorescence, *Science*, 190 (1980) 654-656.
 - 12 Groves, P. M., Synaptic endings and their postsynaptic targets in neostriatum: synaptic specializations revealed from analysis of serial sections, *Proc. nat. Acad. Sci USA*, 77 (1980) 6926-6929.
 - 13 Groves, P. M., Wilson, C. J., Young, S. J. and Rebec, G., Self-inhibition by dopaminergic neurons, *Science*, 190 (1975) 522-529.
 - 14 Groves, P. M., Staunton, D. A., Wilson, C. J. and Young, S. J., Sites of action of amphetamine intrinsic to catecholaminergic nuclei: catecholaminergic presynaptic dendrites and axon terminals, *Prog. Neuropsychopharmacol.*, 3 (1979) 315-335.
 - 15 Groves, P. M., Young, S. J. and Wilson, C. J., Self-inhibition by dopaminergic neurons: disruption by (\pm)-alpha-methyl-tyrosine pretreatment or anterior diencephalic lesions, *Neuropharmacology*, 15 (1976) 755-762.
 - 16 Guyenet, P. G. and Aghajanian, G. K., Antidromic identification of dopaminergic and other output neurons of the rat substantia nigra, *Brain Research*, 150 (1978) 69-84.
 - 17 Hertting, G., Riemann, W., Zumstein, A., Jackisch, R. and Starke, K., Dopaminergic feedback regulation of dopamine release in slices of the caudate nucleus of the rabbit. In S.Z. Langer, K. Starke and M. L. Dubocovich (Eds.), *Presynaptic Receptors, Advanc. Biosci., Vol 18*, Pergamon Press, New York, 1978, pp. 145-150.
 - 18 Kehr, W., Carlsson, A., Lindqvist, M., Magnusson, T. and Atack, C., Evidence for a receptor mediated feedback control of striatal tyrosine hydroxylase activity, *J. Pharm. Pharmacol.*, 74 (1972) 744-747.
 - 19 Langer, S. Z., Presynaptic regulation of catecholamine release, *Biochem. Pharmacol.*, 23 (1974) 1793-1800.
 - 20 Mulder, A. H., Wemer, J. and de Langen, C. D. J., Presynaptic receptor mediated inhibition of noradrenaline release from brain slices and synaptosomes by noradrenaline and adrenaline. In S.Z. Langer, K. Starke and M. L. Dubocovich (Ed.), *Presynaptic Receptors, Advanc Biosci., Vol. 18* Pergamon Press, New York, 1978 pp. 219-224.
 - 21 Nakamura, S., Tepper, J. M., Young, S. J. and Groves, P. M., Neurophysiological consequences of presynaptic receptor activation: changes in noradrenergic terminal excitability, *Brain Research*, in press.
 - 22 Nicoll, R. A. and Alger, B. E., Presynaptic inhibition: transmitter and ionic mechanisms, *Int. Rev. Neurobiol.*, 21 (1979) 217-258.
 - 23 Nicholls, J. and Wallace, B. G., Modulation of transmission at an inhibitory synapse in the central nervous system of the leech, *J. Physiol. (Lond.)*, 281 (1978) 157-170.
 - 24 Raiteri, M., Cervoni, A. M., del Carmine, R. and Levi, G., Lack of presynaptic autoreceptors

- controlling dopamine release in striatal synaptosomes. In S. Z. Langer, K. Starke and M. L. Dubocovich (Eds.), *Presynaptic Receptors, Advanc. Biosci., Vol. 18*, Pergamon Press, New York, 1978, pp. 225–230.
- 25 Reimann, W., Zumstein, A., Jackisch, R., Starke, K. and Hertting, G., Effect of extracellular dopamine on the release of dopamine in the rabbit caudate nucleus: evidence for a dopaminergic feedback inhibition, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 306 (1979) 53–60.
 - 26 Sastry, B. R., Presynaptic effects of morphine and methionine-enkephalin in feline spinal cord, *Neuropharmacology*, 18 (1979) 367–375.
 - 27 Schmidt, R. F., Senges, J. and Zimmermann, N., Excitability measurements at the central terminals of single mechano-receptor afferents during slow potential changes, *Exp. Brain Res.*, 3 (1967) 220–233.
 - 28 Shapiro, E., Castellucci, V. F. and Kandel, E. R., Presynaptic inhibition in *Aplysia* involves a decrease in the Ca^{2+} current of the presynaptic neuron, *Proc. nat. Acad. Sci. USA*, 77 (1980) 629–633.
 - 29 Shimahara, T. and Tauc, L., Multiple interneuronal afferents to the giant cells in *Aplysia*, *J. Physiol. (Lond.)*, 247 (1975) 299–319.
 - 30 Starke, K., Taube, H. D. and Borowski, E., Presynaptic receptor systems in catecholaminergic transmission, *Biochem. Pharmacol.*, 26 (1977) 259–268.
 - 31 Wall, P. D., Excitability changes in afferent fibre terminations and their relation to slow potentials, *J. Physiol. (Lond.)*, 142 (1958) 1–21.
 - 32 Walters, J. R. and Roth, R. H., Dopaminergic neurons: drug-induced antagonism of the increase in tyrosine hydroxylase activity produced by cessation of impulse flow, *J. Pharmacol. exp. Ther.*, 191 (1976) 82–91.
 - 33 Weight, F. F., Physiological mechanisms of synaptic modulation. In F. O. Schmitt and F. G. Worden (Eds.), *The Neurosciences: Third Study Program*, M.I.T. Press, Cambridge, 1974, pp. 929–941.