Autoreceptor Mediated Changes in Dopaminergic Terminal Excitability *in vivo*

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ABSTRACT

Electrophysiological studies were performed to examine changes in the excitability of the terminal fields of single dopaminergic nigrostriatal neurons as a consequence of the stimulation or blockade of terminal autoreceptors. Stimulation of terminal autoreceptors, by local infusion of direct- or indirect-acting agonists, or by natural or stimulation-induced increases in the rate of impulse flow at the terminals produced decreased terminal excitability, whereas dopamine receptor antagonists including the dopamine D_2 receptor specific antagonist, sulpiride, blocked these effects, and by themselves, produced increased terminal excitability. Changes in excitability were obtained only at terminal regions of the dopaminergic axon, and were not altered by kainate-induced destruction of postsynaptic neurons in the neostriatum, thus ruling out postsynaptic involvement in the phenomenon. These data indicate that nigrostriatal axons possess D_2 autoreceptors that are constrained to their terminal regions. These terminal autoreceptors are functional under normal physiological conditions, and are sensitive to physiologically occurring changes in the rate of impulse flow along dopaminergic axons *in vivo*.

KEYWORDS

autoreceptor, autoinhibition, substantia nigra, impulse flow, dopamine release, synaptic transmission

INTRODUCTION

That activation of presynaptic dopamine autoreceptors can inhibit the calcium-dependent, stimulus-evoked release of dopamine from neostriatal slices *in vitro* was first demonstrated nearly 20 years ago (Farnebo and Hamberger, 1971), and this phenomenon has since been replicated many different times in many different preparations (see Starke et al., 1989 for recent review). The fact that only calcium-dependent, stimulus evoked release of dopamine is subject to such modulation offered some clues to the mode of action of presynaptic autoreceptors (Kamal et al., 1981) but it is only relatively recently, however, that the mechanism(s) underlying the inhibition of release and the conditions under which this phenomenon obtains *in vivo* have begun to be understood. One of the major questions regarding the autoinhibition of dopamine release (and indeed, that of other neurotransmitters as well) is the extent to which autoinhibition normally plays a role in regulating synaptic transmission *in vivo*.

One useful method for exploring autoinhibition *in vivo* uses single unit extracellular recording from nigrostriatal dopaminergic neurons coupled with antidromic activation of the neuron from its terminal fields in neostriatum (Groves et al., 1981, Tepper et al., 1984a). The technique has been described in detail elsewhere (Tepper et al., 1987). Briefly, it is possible to adjust the stimulating current to a value that is the minimum current just sufficient to always evoke an antidromic response, a value termed threshold. If the current is decreased slightly, an antidromic response will occur on some trials, and not on others. Typically, 50-200 trials are presented at each current in a counterbalanced fashion in order to

ensure the stability of the measurements. By the judicious selection of stimulating currents, it is possible to construct a curve relating stimulus current to percent antidromic response, similar to a dose response curve. This curve, and in particular the threshold current, give a measure of the excitability of the terminals. Drugs (0.1-10 μM, 300 nl) are then infused through 32 g. cannulae affixed to the stimulating electrode, and the excitability measurements repeated. The results obtained with a number of dopaminergic agonists and antagonists under different *in vivo* experimental conditions are illustrated in Figure 1.

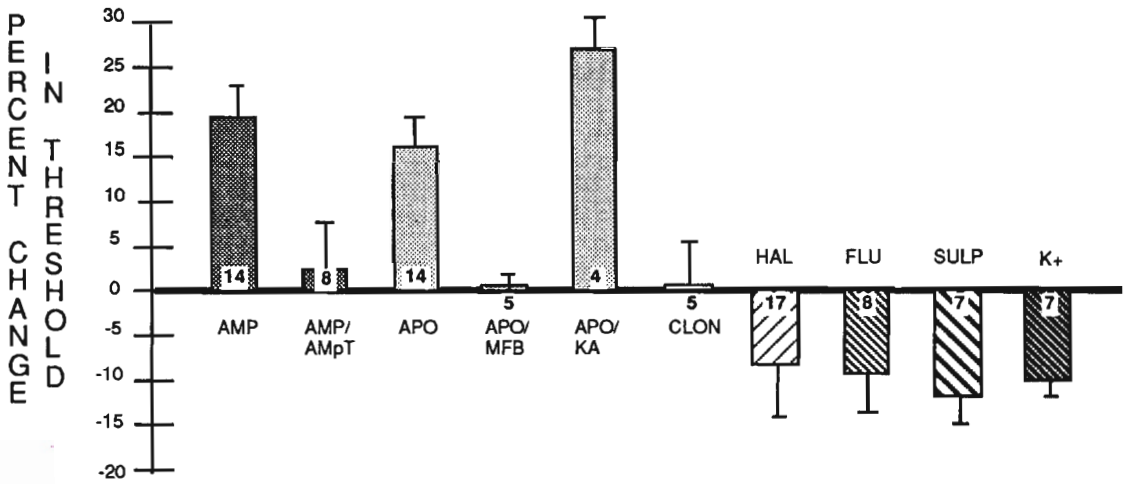

Fig. 1 Changes in threshold current following neostriatal infusion of various agents. Values represent means ± SEM from many individual experiments. AMP, amphetamine HCl, 10 μM; AMP/AMpT; amphetamine HCl following pretreatment with alpha-methyl-p-tyrosine, 200 mg/kg 18 and 3 hours prior to experiment; APO, apomorphine HCl, 10 μM; APO/MFB, apomorphine HCl, 10 μM with local infusion and excitability testing from pre-terminal axons in MFB; APO/KA, apomorphine HCl 10 μM 3-6 days after kainate lesion of striatum (0.625 mg in 0.5 μl), postsynaptic neuron loss verified histologically; CLON, clonidine HCl, 10 μM, HAL, haloperidol lactate, 0.1-1 μM, FLU, fluphenazine HCl, 10 μM, SULP, -sulpiride HCl, 10 μM, K+, KCl, 50-100 mM. Numbers within bars indicate number of cells tested.

AUTORECEPTOR-MEDIATED CHANGES IN TERMINAL EXCITABILITY

Infusion of apomorphine, a direct-acting dopamine receptor agonist leads to an increase in threshold current, corresponding to a decrease in terminal excitability. Such decreases in terminal excitability can be reversed by subsequent local or systemic administration of dopamine receptor antagonists. Conversely, a number of dopamine receptor antagonists including the D_2 receptor-specific antagonist, sulpiride, produce a decrease in threshold corresponding to an increase in terminal excitability when administered alone. It has thus far been impossible to obtain intracellular recordings from central dopaminergic nerve terminals, but the biophysical interpretation of these changes in excitability can be inferred from the effect of KCl, which is known to depolarize neuronal membranes. Since KCl produces an increase in terminal excitability, it follows that dopamine receptor agonists produce a hyperpolarization of the terminal regions whereas dopamine receptor antagonists produce a depolarization. This interpretation is strengthened by *in vitro* intracellular recordings from the cell bodies of nigral dopaminergic neurons, where dopamine, acting through a D_2 somatodendritic autoreceptor, which is pharmacologically identical to that at the nerve terminal, produces a hyperpolarization of the soma by increasing potassium conductance (Lacey et al., 1987).

Since both *in vitro* as well as *in vivo* apomorphine is known to inhibit the stimulus-evoked release of dopamine and haloperidol, fluphenazine and sulpiride have been shown to increase dopamine release, it follows that autoinhibition of dopamine release occurs when the terminals are hyperpolarized, and that antagonist-induced increases in dopamine release occur when the terminals are depolarized. The association of an autoreceptor-mediated terminal hyperpolarization with inhibition of transmitter release and an autoreceptor blockade-induced terminal depolarization with increased release is not idiosyncratic to the dopaminergic system; identical relations have been observed in both noradrenergic

(Nakamura et al., 1981) and serotonergic (Sawyer et al., 1985) terminal fields. This is not to say, however, that it is the change in membrane potential per se that is responsible for the inhibition of transmitter release by the autoreceptor; it is quite possible that the actual modulation of transmitter release might be a consequence of an increase in membrane conductance at the release sites that serve to shunt the presynaptic action currents thereby reducing the calcium influx necessary for exocytosis, as has been demonstrated directly with intraterminal recordings for presynaptic inhibition in the crayfish (Baxter and Bittner, 1981; Tepper et al., 1987).

The fact that amphetamine, which serves to increase extracellular levels of dopamine by promoting the release of catecholamines and blocking their reuptake, also produces decreased excitability indicates that the autoreceptors are sensitive to endogenously released dopamine (Tepper et al., 1984a, Mereu et al., 1985). That the receptors responsible for the decrease in terminal excitability are located on the dopamine axon terminals themselves is evidenced by the fact that destruction of postsynaptic neurons by local infusion of kainic acid several days before excitability testing does not affect the ability of agonists or antagonists to alter terminal excitability, despite a histologically verified complete absence of postsynaptic neurons for several millimeters surrounding the stimulating electrode and infusion cannulae. The effects of autoreceptor agonists and antagonists on more proximal, pre-terminal regions of the axon can also be tested by implanting a stimulating electrode and infusion cannulae in the medial forebrain bundle (MFB). Neither direct local infusion nor intravenous administration of agonists or antagonists alters excitability from MFB sites (Groves et al., 1981; Takeuchi et al., 1982; Tepper et al.,1984). Thus, the receptors responsible for changes in dopaminergic terminal excitability are located on the nigrostriatal axons themselves where they appear to be constrained to the terminal regions, and are responsive to the endogenously released transmitter, i.e., they are truly autoreceptors (Tepper et al., 1984a).

PHYSIOLOGICAL RELEVANCE

Several lines of evidence suggest that the terminal autoreceptors are physiologically functional *in vivo*. Firstly, antagonists alone produced decreases in threshold, indicating that there is sufficient endogenous dopamine in the region of the autoreceptor to maintain a basal level of inhibitory tone, extending similar observations made *in vitro* (Starke et al., 1989). Secondly, the ability of exogenous autoreceptor agonists and antagonists to modify the terminal excitability of individual dopaminergic neurons depends on the baseline rate of spontaneous activity of the neuron. Threshold currents were elevated markedly by local infusions of apomorphine or amphetamine in slowly firing neurons, but only slight increases were observed in rapidly firing neurons. Conversely, the decrease in threshold induced by haloperidol or fluphenazine was most robust in rapidly firing neurons, but only marginal in slowly firing neurons (Tepper et al, 1984a). This resembles closely the frequency dependence of autoreceptor-mediated inhibition of dopamine release previously reported *in vitro* (Cubeddu and Hoffmann, 1982), as well as the dependence of somatodendritic autoreceptor-mediated inhibition of firing of dopaminergic neurons on the baseline firing rate *in vivo* (Tepper et al., 1982). In addition, the thresholds for individual neurons that displayed a variable firing rate over a period of several minutes also varied in direct proportion to the firing rate, and even the occurrence of single bursts of action potentials, consisting of only a few spikes, produced transient decreases in terminal excitability (Tepper et al., 1984b, Gariano et al., 1989). Finally, artificially increasing the rate of impulses along dopaminergic axons by stimulating the MFB with short trains at low frequencies mimicking the natural bursting pattern of these neurons increased the threshold at the terminal, but not at pre-terminal regions of the axon. The spontaneous and stimulus-induced increases in threshold, like those induced by direct application of dopamine agonists, could be reversed or blocked by local infusions of autoreceptor antagonists and occur at terminal, but not pre-terminal regions of the axon (Tepper et al., 1984b; Gariano et al., 1989). These results indicate that the basal level of autoinhibition and thus the effects of exogenous agonists and antagonists depends on the level of autoreceptor occupancy by the endogenous ligand that is a function of the stimulus frequency *in vitro* and the spontaneous firing rate *in vivo*. Thus, under *in vivo* conditions, dopamine terminal autoreceptors are physiologically functional in a phasic manner, sensitive both to relatively prolonged (several seconds to minutes) as well as rapid (several tens of milliseconds) fluctuations in the extracellular levels of dopamine that arise as a function of alterations in neuronal activity (Tepper et al., 1985).

In summary, there is abundant electrophysiological evidence supporting the concept that autoreceptors present on the terminals of central dopaminergic neurons act under normal physiological conditions *in vivo* to regulate the excitability of dopaminergic nerve terminals, and that these receptors are not only sites of action of exogenously applied drugs, but are sensitive to changes in levels of endogenous dopamine

that occur as a function of changes in the rate and pattern of impulse flow. Furthermore, autoreceptor-mediated regulation of terminal excitability has also been demonstrated in noradrenergic and serotonergic neurons (Nakamura et al., 1981; Sawyer et al., 1985; for recent review see Tepper and Groves, 1990), and may be a ubiquitous mechanism whereby synaptic transmission is adjusted or fine-tuned locally in a phasic manner to compensate for changes in the rate or pattern of presynaptic activity.

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