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The neuropharmacology of the autoinhibition of monoamine release

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Monoamine neurons possess presynaptic autoreceptors, sensitive to the neuron's own neurotransmitter, which modulate the calcium-dependent, stimulus-evoked release of transmitter from the nerve endings. Agonists reduce, whereas antagonists enhance evoked release. What are the mechanisms underlying autoreceptor-mediated effects at monoamine nerve terminals, and does this phenomenon play a physiological role in vivo? *In the following review* James Tepper, Philip Groves *and* Stephen Young *summarize recent biochemical and electrophysiological findings on the autoinhibition of monoamine release in the mammalian CNS.*

Certain neurons within the CNS, most notably monoamine neurons, possess receptors sensitive to the neuron's own transmitter, which have been termed 'autoreceptors'. Stimulation of these autoreceptors, located in the soma-dendritic region of the neuron, leads to inhibition of spontaneous firing[1–6]. This has been shown, by intracellular recordings from dopaminergic, noradrenergic, or serotonergic neurons to result from a hyperpolarization of the soma-dendritic region of the neuron[4–6]. The autoreceptors are apparently stimulated *in situ* by transmitter released from recurrent axon collaterals[7] and/or dendro-dendritic synapses[8,9] and serve as part of a negative feedback mechanism for regulating cell firing[1].

In addition to soma-dendritic autoreceptors, the existence of presynaptic autoreceptors, located on or near the sites of transmitter release on central and peripheral monoaminergic neurons, has been inferred from numerous biochemical studies. Terminal autoreceptors regulate the calcium-dependent, impulse-related release of neurotransmitter from many different classes of synaptic endings. This is indicated by *in-vitro* studies showing that autoreceptor agonists reduce, whereas autoreceptor antagonists increase the amount of stimulus-evoked transmitter release[10–13]. This negative feedback mechanism has been termed autoinhibition. Soma-dendritic autoreceptors act to reduce the firing rate, and thus, transmitter release from the neuron as a whole, whereas terminal autoreceptors, by virtue of their location on the axon near to the sites of transmitter release, have the potential to regulate transmitter release locally, without affecting distant regions of the neuron. They may, in a sense, 'fine tune' the synaptic output at certain regions in the terminal fields of a neuron, depending on the local environment.

While the autoinhibitory effect has been well characterized phenomenologically *in vitro*, little is known about the neuronal mechanisms underlying the effect, or the role that autoinhibition plays within active neuronal systems. A basic knowledge of the mechanisms underlying autoinhibition, as well as the development of useful methods for demonstrating the operational characteristics of autoinhibition *in situ* is of great interest in the study of the sites and mechanisms of action of many different classes of neuroactive drugs, including the psychomotor stimulants and neuroleptics.

Autoreceptor-mediated changes in presynaptic terminal excitability

Although intracellular recording from central catecholamine nerve terminals is not feasible at the present time due to the small size of these terminals, electrical events occurring as a consequence of autoreceptor stimulation may be inferred by placing a stimulating electrode in the terminal field, and an extracellular recording electrode near the cell body of the neuron. Stimulating currents necessary to antidromically activate the neuron from its terminal field can be measured as a function of autoreceptor stimulation and blockade, and current-response curves of terminal excitability can be derived[2,3,14,15,16] (see box I).

When terminal excitability is measured in this manner, local infusions of appropriate autoreceptor agonists (e.g. apomorphine in the case of dopamine neurons, clonidine in the case of noradrenergic neurons, or 5-methoxy-*N*,*N*-dimethyltryptamine in the case of serotonergic neurons), lead to marked increases in the stimulating currents necessary to elicit antidromic responding from neostriatal or cortical terminal fields. These effects are observed at appropriately low concentrations (0.1–50 μM) and with small infusion volumes (300 nl). Similar decreases in terminal excitability result from i.v. administration of autoreceptor agonists. Decreases in terminal excitability produced by local or systemic administration of autoreceptor agonists can be blocked or reversed by appropriate autoreceptor blocking agents, such as haloperidol, fluphenazine or the D_2 receptor-specific dopamine antagonist sulpiride, in the case of dopaminergic neurons[15] or the α_2 adrenergic receptor antagonists phentolamine or yohimbine in the case of noradrenergic neurons[14]. Moreover, local infusions of autoreceptor antagonists by themselves lead to increases in terminal excitability[14,15]. This finding is of particular importance with respect to the issue of whether terminal autoreceptors and autoinhibition represent only pharmacological effects or are of physiological significance, an issue which is discussed in greater detail below.

However, autoreceptor agonists and antagonists do not affect all regions of the axon uniformly. Thus, when excitability is measured from regions of the axon proximal to the terminal arborizations, such as from the medial forebrain bundle for dopaminergic or serotonergic neurons or from the dorsal noradrenergic pathway for noradrenergic neur-

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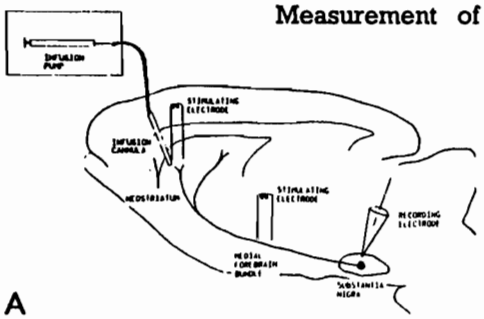
ons, neither systemic administration, nor local infusions of autoreceptor agonists or antagonists alters the excitability at these more proximal sites[3,14,15]. Furthermore, the possibility that the changes in terminal excitability seen following local infusions of drugs are due to a trans-synaptic effect can be excluded, since they still occur in dopaminergic neurons after extensive destruction of postsynaptic neostriatial neurons induced by Kainic acid[15]. Thus, the observed excitability effects appear attributable to stimulation of autoreceptors confined to the terminal regions of monoamine axons.

In catecholaminergic neurons, infusion of the depolarizing agent potassium chloride into the terminal fields produces an increase in terminal excitability[14,15]. Thus, like Wall in his classic studies of primary afferent depolarization in the spinal cord[17], we interpret autoreceptor-mediated increases in terminal excitability as reflecting a drug-induced depolarization at the site of antidromic impulse initiation. Conversely, decreases in excitability would indicate a hyperpolarization of the terminal membranes. This is consistent with results of intracellular studies on the soma-dendritic autoreceptor[4–6]. Since biochemical studies indicate that autoreceptor stimulation leads to a reduction in stimulus-evoked transmitter release *in vitro*, and we observe a decrease in terminal excitability *in vivo*, it follows that in central monoaminergic neurons, autoinhibition of transmitter release is associated with a hyperpolarization of the presynaptic terminal regions. In contrast, since autoreceptor blockade enhances release *in vitro* and increases terminal excitability *in vivo* it follows that in central monoaminergic neurons, antagonist-induced facilitation of release is associated with presynaptic depolarization.

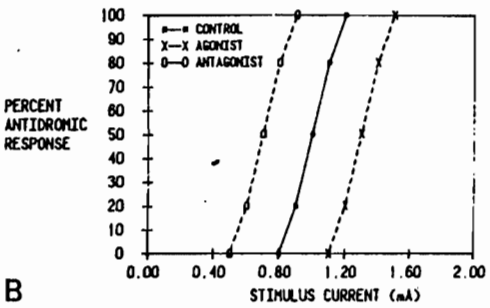
Physiological role for terminal autoreceptors?

The fact that local infusions of autoreceptor antagonists alone lead to increased terminal excitability indicates that under our experimental conditions, monoamine nerve terminals are constantly inhibited to some degree by neurotransmitter released as a consequence of on-going neuronal activity, much as the soma–dendritic regions of monoamine neurons appear to be subject to self-inhibition by dendritic or collateral release of their own neurotransmitters, mediated through soma-dendritic autoreceptors[1,2,3,7]. This interpretation is consistent with our observation that the magnitude of the increase in dopaminergic terminal excitability following neostriatal infusions of dopaminergic antagonists is directly related to the level of spontaneous firing, whereas the magnitude of the excitability decrease following infusion of dopaminergic agonists is inversely related to ongoing firing rate[14]. These data suggest that measurements of terminal excitability reflect the total amount of autoreceptor stimulation, from both endogenous and exogenous sources. Thus, it seems likely that in a rapidly firing neuron, relatively large amounts of transmitter are liberated over a short time, and extracellular concentration of transmitter is increased, resulting in a high degree of occupancy of the autoreceptors. Under these conditions, autoreceptor antagonists should exert relatively large effects since the level of autoinhibition resulting from endogenously-released neurotransmitter would be relatively high, whereas exogenously ap-

Measurement of autoreceptor-mediated terminal excitability



A



B

Measuring autoreceptor-mediated changes in terminal excitability. Autoreceptor-mediated effects at the nerve terminal can be measured electrophysiologically, *in vivo*. A: a stimulating electrode is placed in the terminal fields of the neuron under study, and an extracellular recording electrode is placed at the cell body. Stimulation of the terminal field elicits an antidromic action potential, which is propagated backwards down the axon, and is detected by the recording electrode at the soma. The stimulating current is adjusted to a value which is just sufficient to elicit an antidromic response to every stimulus. Slight decreases in current strength yield proportionately lower frequencies of antidromic responding. B: it is possible to construct a current–response curve of terminal excitability for a single neuron, similar to a dose response curve, by varying the stimulating current in small steps. Drugs are delivered either intravenously or directly into the terminal fields by microinjection through 32 gauge cannulae positioned within 100–200 μm of the tips of the stimulating electrode. An excitability curve is determined for a neuron before and after drug administration. Shifts in the curve to the right after drug delivery signify a decrease in terminal excitability, as seen with autoreceptor agonists (X--X), since higher stimulating currents are required to elicit equivalent proportions of antidromic responding. Leftward shifts in the curve, as seen following autoreceptor antagonists (O--O), indicate that the terminal fields have become more excitable.

plied agonists would exert little additional effect on excitability (many or most of the autoreceptor binding sites would already be occupied). These data are in good agreement with biochemical release studies which show that the facilitation of evoked transmitter release induced by autoreceptor antagonists *in vitro* is greatest at high frequencies of stimulation while agonist-induced reduction of release is greatest at low stimulation frequencies[18–20].

Evidence that autoinhibition occurs, *in situ*, under physiological conditions can also be demonstrated in the absence of drug application. In catecholamine neurons, terminal excitability is inversely related to *in-vivo* spontaneous firing rate, which may vary significantly over the course of seconds or minutes[14,15]. When a cell is firing rapidly, its terminal excitability is relatively depressed. However, when the neuron's firing rate slows, its terminal fields become more excitable. This phenomenon can also be demonstrated by manipulating the rate at which impulses reach the terminal fields by stimulating preterminal portions of the axon with trains of pulses of varying frequency and duration. Fig. 1 illustrates this phenomenon for a dopaminergic neuron, using trains of shocks to the medial forebrain bundle which approximate the frequency and duration of the naturally occurring bursts of action potentials in these neurons.

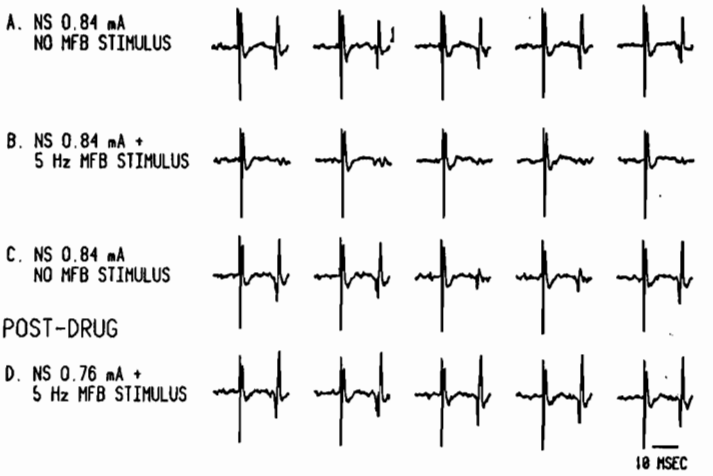


Fig. 1. When impulse flow to the terminal regions of a dopamine neuron is artificially increased by stimulation of the more proximal axon in the medial forebrain bundle terminal excitability is decreased. Each trace represents a single oscilloscope sweep. The first large deflection in each trace is the stimulus artifact, and the second is the antidromic action potential of the dopamine neuron. In A: 0.84 mA is able to antidromically activate the neuron from neostriatum on every stimulus presentation. In B: 750 ms trains of 5 Hz conditioning stimulation is applied to the medial forebrain bundle and a 0.84 mA neostriatal test pulse is delivered 225 ms after the end of the medial forebrain bundle conditioning train. The same neostriatal stimulating current that was able to reliably activate the neuron antidromically in the absence of the increased impulse flow now does not elicit any antidromic responses due to increased autoreceptor stimulation resulting from a build-up of dopamine in the synaptic regions. In C: when the medial forebrain bundle stimulation is stopped, and extracellular dopamine levels return to normal, antidromic excitability returns to the pre-stimulation level. D: a local infusion of haloperidol (0.31 μl; 1μM) directly into the neostriatal stimulating site, which prevents endogenously released dopamine from stimulating the autoreceptors, lowers the current necessary to antidromically activate the neuron to each stimulus delivery, as well as completely blocking the excitability-decreasing effects of the medial forebrain bundle conditioning, as shown in D. Note that in the 3rd and 4th traces in C, the antidromic response consists only of the initial segment spike, whereas in all other traces the response consists of a full, initial segment, soma-dendritic spike. Data taken from Ref. 16, with permission.

In the absence of any conditioning stimulation of the medial forebrain bundle, 0.84 mA was sufficient to elicit an antidromic response to every neostriatal stimulus (Fig. 1A). However, 225 ms after the medial forebrain bundle was stimulated with a 750 ms train at 5 Hz, this neostriatal stimulating current did not elicit any antidromic responses, reflecting the decreased excitability of the terminal fields (Fig. 1B). When the medial forebrain bundle conditioning stimulation was stopped, excitability returned to preconditioning levels (Fig. 1C). Both the magnitude and duration of the decrease in terminal excitability following increased impulse flow vary with the frequency and number of presentations of the conditioning train, and may last for several seconds following a 10 s train at 10 Hz. Decreases in dopaminergic terminal excitability resulting from increased impulse flow can be blocked by local infusion of the dopamine receptor antagonist, haloperidol, into the neostriatal terminal fields of dopaminergic neurons, prior to conditioning stimulation, as shown in Fig. 1D (see Ref. 16). Decreases in excitability as a consequence of increased impulse flow occur only at the terminal regions, and not along more proximal regions of the pre-terminal axons[14,16]. These observations suggest that the impulse-dependent changes in terminal excitability are mediated via autoreceptors, and are not simply due to a nonspecific effect of increased impulse traffic along the axon.

Thus, terminal autoreceptors are sufficiently sensitive to respond to changes in the level of transmitter in the region of the synapse that occur within a physiological range of firing rates *in situ*. Since reduced terminal excitability is associated with a reduction in the amount of transmitter released per impulse, and since terminal excitability is reduced at high rates of neuronal firing, it follows that one physiological effect of terminal autoreceptors may be to reduce the amount of transmitter liberated by each presynaptic impulse at sustained high rates of firing. Cubeddu and co-workers have recently examined the relationship between stimulus frequency and duration on the electrically-evoked release of dopamine from striatal slices *in vitro*. Using physiologically relevant stimulus fre-

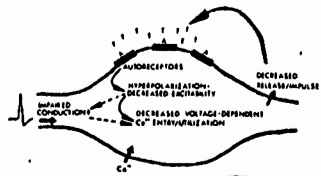
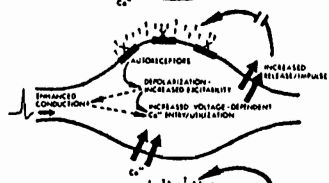
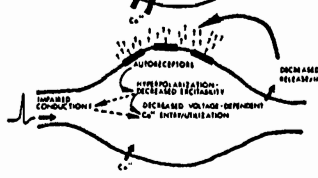
quencies (0.3–10 Hz), they found that the amount of dopamine released per stimulus was reduced when the total number of stimuli was increased, and, with longer train durations, the release per stimulus diminished with increasing stimulus frequency. Such a mechanism might help compensate for the effects of frequency dependent facilitation of transmitter release so as to maintain a more linear relationship between presynaptic activity and postsynaptic response despite alterations in overall firing rate, or the occurrence of burst firing.

Effects of terminal hyperpolarization on transmitter release

Exactly how presynaptic hyperpolarization leads to a reduction in impulse-related transmitter release remains unclear. One possibility is that the hyperpolarization acts to reduce the probability that the presynaptic impulse travelling down the axon will successfully invade the sites of transmitter release[13,21]. This hypothesis is supported by evidence showing that electrically-evoked release of catecholamines, which is dependent upon the active propagation of nerve impulses as demonstrated by its sensitivity to tetrodotoxin, is more sensitive to autoinhibition than release evoked by high potassium, which is insensitive to blockade by tetrodotoxin[13]. Impairment of conduction could arise from an increase in conductance to potassium or chloride, which would act to shunt action potential currents out through the membrane, thereby attenuating their longitudinal spread. This is an attractive possibility inasmuch as activation of soma–dendritic autoreceptors has been shown to lead to a membrane hyperpolarization in central monoamine neurons[4–6]. At least for noradrenergic and serotonergic neurons, there is good evidence to suggest that this hyperpolarization arises from a specific increase in conductance to potassium[4,5,6,22]. Indeed, we have observed an increase in antidromic latency accompanying decreased terminal excitability following terminal autoreceptor stimulation. This is consistent with impaired impulse conduction in the terminal regions of the axon. However, there is good reason for believing that there cannot be complete failure of the action potential along the main axon, since it is always possible to regain 100% antidromic responding following drug or stimulation induced decreases in terminal excitability with modest increases in stimulus current[2,3,14,15,16].

Alternatively, it may be that the hyperpolarization inhibits the voltage-dependent calcium conductance which is essential for stimulus–secretion coupling[22]. In synaptosomal preparations of cortical noradrenergic terminals, norepinephrine release can be evoked by the addition of the calcium ionophore calimycin (A23187). However, unlike potassium or electrically stimulated release, the release evoked by calimycin, which bypasses the voltage-dependent calcium conductance, is not subject to modification by presynaptic autoreceptor stimulation[23]. This is consistent with data that indicate that transmitter release evoked by calcium-independent agents (e.g. amphetamine or tyramine) is not affected by presynaptic autoreceptors[24], suggesting that control of calcium entry into the nerve terminal, or the intracellular utilization of calcium for depolarization–secretion coupling may be a primary locus for autoreceptor modulation of transmitter release. These two hypotheses are not mutually exclusive, and it is likely that both could play a role in the terminal autoreceptor-mediated modulation of transmitter release, as illustrated

TABLE I. Possible mechanisms of autoreceptor-mediated effects on monoamine release

Condition	Effects on monoamine release *(in vitro)*	Effects at nerve terminal *(in vivo)*	
Agonist directly stimulates autoreceptor	Reduced release per impulse. Effect greatest at low rates of stimulation	Hyperpolarization. Decrease in terminal excitability. Effects greatest in neurons with low firing rates	
Antagonist blocks autoreceptor stimulation by endogenous transmitter	Increased release per impulse. Effect greatest at high rates of stimulation	Depolarization. Increase in terminal excitability. Effects greatest in neurons with high firing rates	
Increased rate of stimulation or neuronal activity. Transmitter accumulates extracellularly and stimulates autoreceptors	Reduced release per impulse. Effect greatest with long durations and high frequencies of stimulation	Hyperpolarization. Decrease in terminal excitability. Effect greatest with increasing frequency and duration of increased activity	

Summary of biochemical and electrophysiological effects at the nerve terminal under conditions of autoreceptor stimulation and blockade, and increased impulse flow. To the right of each entry is a hypothetical scheme of events occurring at the nerve terminal to account for the observed effects. *T* symbolizes endogenous transmitter, *A* symbolizes exogenous autoreceptor agonist, and *X* symbolizes autoreceptor antagonist.

schematically in Table I.

Possible clinical significance of autoreceptor pharmacology

Neuropharmacological studies of the autoinhibition process offer the potential for the development of improved methods for the treatment of certain psychiatric and neurological conditions thought to involve disturbances in monoamine neurotransmission. Most current strategies for the treatment of schizophrenia, for example, rely upon the administration of neuroleptic drugs, which block dopamine receptors. Although efficacious in the relief of certain schizophrenic symptoms, prolonged use of these dopamine antagonists often leads to severe and sometimes irreversible side effects including dyskinetic and Parkinson-like symptoms, which are thought to result from changes in the sensitivity of postsynaptic dopamine receptors consequent to the chronic pharmacological blockade. A better understanding of the mechanisms underlying autoinhibition of transmitter release may eventually lead to the discovery of antischizophrenic drugs which act as specific presynaptic autoreceptor agonists to promote autoinhibition, without producing chronic postsynaptic receptor blockade and the accompanying side effects[20]. Indeed, the potential for therapy of this type is suggested by preliminary results obtained by Tamminga and associates, in which low, presumably autoreceptor-specific doses of dopamine agonists alleviated psychotic symptoms in some patients[25] . These intriguing findings await confirmation, and the continued study of autoreceptor pharmacology promises to be an exciting avenue for future research.

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