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Autoreceptor-Mediated Changes in Dopaminergic Terminal Excitability: Effects of Striatal Drug Infusions

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The neurophysiological correlates of autoinhibition at the terminals of nigrostriatal dopaminergic neurons were studied by measuring the changes in antidromic excitability of nigrostriatal neurons following local infusions of various catecholamine agonists and antagonists into the neostriatum. Infusions of apomorphine or amphetamine reduced terminal excitability whereas the dopamine antagonists, haloperidol, fluphenazine or sulpiride, led to increases in terminal excitability. Alterations in antidromic excitability were constrained to the terminal regions and were not observed when infusions and excitability testing were performed in the medial forebrain bundle. The alpha-2 agonist, clonidine, did not alter dopaminergic terminal excitability. Our results indicate that pharmacological manipulations which have been shown to reduce the amount of stimulation-induced transmitter release from dopaminergic terminals are associated with a dopamine autoreceptor-mediated hyperpolarization and/or alteration in ionic conductance of the terminal membranes. These results are discussed with respect to mechanisms of autoinhibition in the central nervous system.

INTRODUCTION

The amount of dopamine released from striatal slices or synaptosomes, or in vivo from cat striata following electrical or potassium-induced stimulation, has been shown to be reduced by perfusion of the nerve terminals by dopaminergic agonists, and facilitated by the addition of dopamine antagonists[4,19,20,29,33,48]. Similar effects are obtained with respect to the release of norepinephrine[12,20,35] and serotonin[8,20,21] from cortical tissue. These data have suggested that monoaminergic nerve terminals possess presynaptic receptors, commonly termed autoreceptors, located at or near to the sites of transmitter release, which are receptive to neurotransmitter released from the nerve terminal. These autoreceptors appear to participate in a negative feedback regulation of monoaminergic neurotransmitter release.

Despite the fact that autoinhibition of monoamine release has been demonstrated many times, in several different preparations, the physiological mechanisms underlying the autoinhibition phenomenon have not yet been elucidated. Although intracellular recording from central monoaminergic nerve terminals is at present impossible, information concerning the effects of catecholamine agonists and antagonists on biophysical parameters of nerve terminals such as polarization and conductance may be inferred from changes in the antidromic excitability of the terminal regions of these neurons, similar to the approach used by Wall[56] to study presynaptic inhibition in the spinal cord.

In a preliminary article, we reported that systemic injection of D-amphetamine reduced the excitability of dopamine terminals in rat striatum to direct electrical stimulation[24]. In complementary fashion, intravenous administration of haloperidol led to increases in dopamine terminal excitability. These changes in

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terminal excitability were interpreted to be due to an indirect pharmacological stimulation and blockade of terminal autoreceptors by amphetamine and haloperidol, respectively. The decreased excitability following amphetamine administration implied an increase in terminal membrane potential and/or an alteration in ionic conductance consequent to increased autoreceptor stimulation resulting from increases in the extracellular dopamine concentration caused by amphetamine.

Consistent with these results, the local infusion of D-amphetamine or the alpha-2 adrenergic agonist clonidine into the cerebral cortex reduced the antidromic excitability of locus coeruleus neurons in rat[40,41]. Thus, we have proposed that the reduction in catecholamine release consequent to autoreceptor stimulation is associated with a decrease in electrical excitability of the nerve terminal region.

In the present study, we describe the effects on striatal dopaminergic terminal excitability of the local infusion of various catecholaminergic agents into the striatum. Our results indicate that the decrease in terminal excitability following local or systemic administration of dopaminergic agonists is due to stimulation of autoreceptors constrained to the terminal regions of dopaminergic nigrostriatal efferents, and that the receptor mediating this effect displays a pharmacological profile similar or identical to that of the soma-dendritic autoreceptor on dopaminergic neurons which acts to inhibit the spontaneous firing of these neurons[6,25].

METHODS

Experiments were carried out on male Sprague–Dawley rats weighing between 235 and 500 g at the time of recording. All animals were housed 2 or 3 to a cage and allowed ad libitum access to Wayne Lab-Blox F4 and tap water, and maintained on a 12 h light–dark cycle.

Animals were anesthetized with 1.3 g/kg urethane (ethyl carbamate) administered intraperitoneally. A tracheotomy and intubation were performed and the left femoral vein was cannulated. Animals were then mounted in a stereotaxic apparatus using blunt, atraumatic earbars (Kopf Instruments), oriented according to the atlas of König and Klippel[34]. All wound margins and points of contact between the animal and the stereotaxic apparatus were thoroughly infiltrated with a long-acting local anesthetic ointment (5% xylocaine). Body temperature was maintained at 37 ± 1 °C, and the electrocardiogram was monitored on an auxilliary oscilloscope.

To reduce pulsation and to increase the stability of the recording and stimulating electrodes, the cisterna magna was punctured and some cerebrospinal fluid allowed to drain. The coordinates for stimulation/infusions and recordings were as follows: striatum 1.0 mm anterior to bregma, 3.6 mm lateral to the midline and 3.4–3.7 mm from the cortical surface; medial forebrain bundle (MFB) 4.2 mm anterior to lambda, 1.75 mm lateral, and 7.8 mm ventral; substantia nigra 2.1 mm anterior to lambda, 1.9 lateral, and 6.9–7.5 ventral.

At the completion of surgery, 15 mg/kg gallamine triethiodide was administered intravenously, followed by 40 mg/kg gallamine triethiodide (Sigma) intraperitoneally. During the course of the experiments, gallamine was supplemented as necessary. Artificial ventilation through a partially open system was initiated and maintained at 75–80 strokes/min, using a Harvard Apparatus rodent respirator.

Electrical stimulation

Electrical stimulation for testing of terminal and axonal excitability was delivered through bipolar electrodes consisting of two stainless steel enamel-coated wires approximately 200 μm in diameter with a tip separation of approximately 100 μm. The in vitro impedance of these electrodes was typically 30 KΩ at 500 Hz. The electrical stimuli consisted of single monophasic pulses of durations ranging from 10 to 750 μs at current intensities between 0.2 and 4.0 mA. Each channel of the stimulator (Grass S-88) was coupled to the desired stimulating electrode through a stimulus isolation unit (Grass SIU-5). Stimulating electrodes were individually calibrated before each experiment, in situ, and were monitored periodically throughout all experiments to control for possible changes in conductance.

Drugs

All drugs used for local infusion into the neostriatum or medial forebrain bundle were dissolved in 0.9% saline with the exception of apomorphine, which was prepared in 0.9% saline in 0.1% ascorbate

buffer to retard oxidation of the drug. The drugs used in these experiments and their concentrations were as follows: D-amphetamine sulphate, 1, 5, 10 and 50 μM (Smith, Kline and French); apomorphine hydrochloride, 1 and 10 μM (Sigma); haloperidol lactate, 0.1, 1, 5, 10 and 50 μM (McNeil Pharmaceutical); fluphenazine hydrochloride, 10 μM (E.R. Squibb); sulpiride hydrochloride, 10 μM (Rauizza); clonidine hydrochloride, 10 μM (Ingleheim-Boeringher); isoproterenol bitartrate, 10 μM (Breon) and potassium chloride, 50 and 100 mM.

Kainic acid lesions

Four rats were anesthetized with sodium pentobarbital, 50 mg/kg, administered intraperitoneally. Kainic acid (0.5 μl, 1.25 mg/ml) was infused bilaterally through 32-gauge cannulae at coordinates 1.0 mm anterior to bregma, 3.7 mm lateral to the midline at a depth of 3.4 mm from the cortical surface over the course of 5 min. Cannulae were left in place for 10 min following the completion of the infusion and then slowly withdrawn at the rate of 2 mm/min. Following the infusions, the scalp wound was infiltrated with 5% xylocaine, the incision closed, and the animals returned to their cages. Rats were administered 100,000 units of penicillin intramuscularly, repeated once daily for the first 3 days postoperatively. Animals were also given 20 mg/kg diazepam intraperitoneally as they recovered from anesthesia as a prophylactic against kainic acid-induced convulsions. Electrophysiological experiments were conducted 3–6 days following kainate infusion.

Dopamine depletion

Six rats were administered 200 mg/kg, i.p., of a racemic mixture of α-methyl-*p*-tyrosine on the day before the experiment, 15–20 h before the start of surgery. On the following morning, rats were administered an additional 200 mg/kg α-methyl-*p*-tyrosine approximately 2–3 h prior to the start of the electrophysiological experiments.

Recording techniques

Extracellular single unit recordings of neurons in substantia nigra pars compacta were obtained with glass micropipettes, filled with 3 M sodium chloride and possessing in vitro impedances of from 4 to 10 MΩ measured at 500 Hz. Throughout all experiments, the ipsilateral neostriatum was electrically stimulated (1.0–2.0 mA; 100–500 μs duration) once per second.

When a well-isolated single unit displaying the electrophysiological characteristics of a dopaminergic neuron was encountered in the region of the pars compacta[6,59], the stimulus current and/or duration were varied in order to determine whether the cell could be driven antidromically. Responses evoked by neostriatal or MFB stimulation were classified as antidromic if the response exhibited collision extinction with appropriately timed spontaneous action potentials. When spontaneous activity was so low as to preclude reliable collision testing, neurons were tested for their ability to follow paired pulses corresponding to a stimulus frequency of 250 Hz.

After encountering an antidromically driven cell, the 'threshold' for neostriatal or MFB stimulation was determined. Threshold was defined as the minimum stimulating current, delivered at a constant duration that was sufficient to elicit the antidromic response on 100% of the non-collision trials. Thresholds were determined in a counterbalanced fashion from ascending and descending series of current increments and decrements, with steps approximately equal to 10% of the threshold value. In a similar way, a zero point was determined as the maximum stimulating current that failed to evoke any antidromic responses. In addition to these values, intermediate currents which yielded intermediate frequencies of antidromic response were presented. A minimum of between 25 and 150 stimuli were presented at each current setting in a series.

Local infusion of test substances

Drugs were applied to the terminal fields of nigrostriatal dopaminergic neurons through dual 32-gauge cannulae connected by short lengths of 28-gauge teflon tubing to two 10-μl syringes (Hamilton Model no. 701) seated in two infusion pumps (Harvard Apparatus Co. Model no. 975). Both the stimulating electrode and the two cannulae were held in place with a small Narashige micromanipulator.

Each infusion consisted of a total volume of 0.3125 μl delivered at a rate of 0.0625 μl/min over the course of 5 min. The total number of infusions was limited in all cases to 3 per cell, and the terminal

field of only one cell per neostriatum was explored.

Excitability was monitored continuously during all drug infusions. At the termination of the infusion, the threshold, zero point and currents evoking intermediate response frequencies were re-determined. When the post-drug threshold became stable, if conditions were suitable, a second infusion was attempted. In some cases the second infusion was of the same substance as the first infusion; in other cases effort was made to reverse the effects of the first infusion by the application of an appropriate antagonist.

Data analysis

The threshold current was the most consistent and convenient measure of changes in terminal excitability for the purpose of tabulation and statistical analysis. In practice, currents eliciting antidromic responses of 95% or greater were considered to be at threshold. Due to the theoretical limits of resolution and experimental error, changes in threshold of 5% or less were classified as 'no effect', and remaining cases were divided between 'threshold increases' and 'threshold decreases'.

In order to stabilize the variances of the binomial distributions, angular (arcsine) transformations were used on all data expressed as percentages, and Student's *t*-test was performed on the transformed data.

Histology

For the purposes of verifying the location and extent of the kainate lesions, at the end of the electrophysiological experiments, animals were deeply anesthetized with urethane, 200 mg/kg (i.v.), and perfused transcardially with normal saline followed by 10% formalin. Brains were dissected free and submerged in formalin for at least 24 h, then blocked and 100 μm frozen sections cut and mounted on gelatin-subbed slides. Sections were stained with neutral red and Luxol blue. After dehydration and clearing in ethanol, butynol and xylene, cover slips were mounted and the sections examined.

RESULTS

Characteristics of spontaneous activity

All data reported were obtained from neurons which exhibited the characteristic extracellular waveform and firing rate that have previously been used to identify nigral dopaminergic neurons electrophysiologically[6,22,27,59]. These cells exhibited spontaneous firing rates in the range of 0–8.4 spikes/s, and were typified by triphasic waveforms of unusually wide duration (2.5–6.0 ms) when measured from the onset of the initial positive component to the point at which the late positive component crossed the baseline. Neurons typically displayed a notch on the initial positive component of the action potential, although this was not always the case. Many of the neurons exhibited a firing pattern consisting of slow random firing, interspersed with bursts of from 2 to 8 spikes occurring in rapid succession, in which the amplitude of each succeeding spike in the burst grew progressively smaller, consistent with previous reports[5].

In the present study, 179 dopaminergic neurons were recorded. Setting a spontaneous firing rate of less than 0.1 spikes/s as a criterion of an 'inactive' or 'silent' neuron, only 9 such cells or 5% of the cells sampled were found not to be spontaneously active.

Responses to neostriatal stimulation

Stimulation of the ipsilateral neostriatum in normal, untreated animals resulted in antidromic responses in approximately 30–50% of the nigral dopaminergic neurons encountered. Antidromic responses most often consisted of the initial segment (IS) spike only. In a small number of neurons, stimulation of the neostriatum usually (70–100% of total responses) resulted in the appearance of a full initial segment-soma-dendritic (IS-SD) antidromic spike. The cells responding in this manner were usually found to exhibit relatively low thresholds for antidromic activation, consistent with previous reports[27]. These cells also tended to exhibit below average firing rates.

Although verified as antidromic due to collision with orthodromic action potentials, neostriatal stimulation often gave rise to from 2 to 4 antidromic responses which occurred at multiple and discrete latencies. Multiple antidromic latencies for a single neuron were never observed on any given trial, but appeared intermixed, in different trials. As illustrated in Fig. 1, which consists of the superimposition of 5 consecutive single sweeps, following a constant current stimulus there are two distinct antidromic responses, separated by 1.75 ms, each of which was

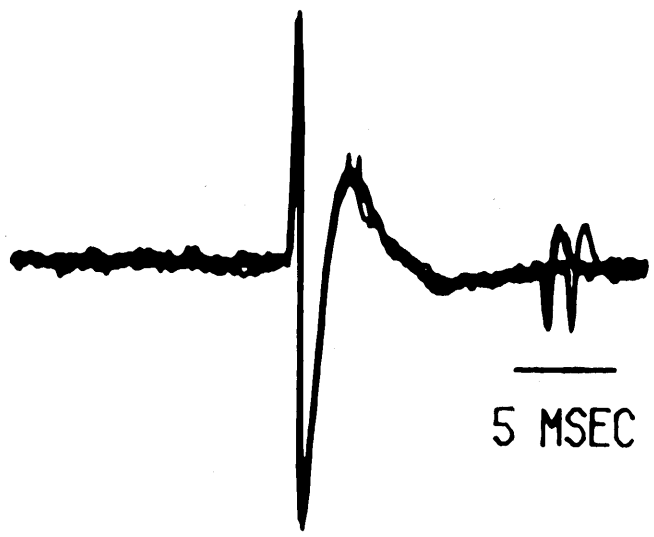

Fig. 1. Illustration of a multiple latency antidromic response in a nigral dopaminergic neuron resulting from neostriatal stimulation. The illustration consists of the superimposition of 5 consecutive single sweeps, at a constant stimulating current. Only one response occurs to each stimulus. Three responses occur at the shorter latency and two at the longer. All responses consist of the IS spike only. Note that each response displays the very constant latency characteristic of an antidromic response.

seen to collide with a spontaneous spike occurring within the appropriate collision interval. Three responses occur at the shorter latency and two at the longer. Note that each response displays the constant latency characteristic of an antidromic response. In most cases, latency to the IS component of the antidromic response was constant to within 0.3 ms or less, although there was often greater variability in the latency to the SD spike. Multiple latencies were usually separated from each other by 0.5–2.0 ms. In some extreme cases the separation reached 6 ms.

Most often, a secondary antidromic latency appeared as the stimulating current was altered. Without exception, the shorter latency responses were found to have higher thresholds for antidromic activation than the longer latency responses, and thus abolished the latter by collision. For this reason, if the thresholds for the multiple responses were close to one another, the shortest latency response was chosen as the response to be studied. Multiple latency responses also appeared at constant current, however, as shown in Fig. 1. Out of a total of 154 neurons antidromically activated from the neostriatum, 51 or 33.1% exhibited multiple latencies.

Responses to MFB stimulation

The characteristics of the antidromic responses of nigral dopaminergic neurons to stimulation of the MFB were virtually identical to those observed after neostriatal stimulation except that antidromic responses elicited by stimulation of the MFB generally exhibited only one latency, despite alterations in the strength of the stimulus. However, out of a total of 44 nigral dopaminergic neurons antidromically activated from the MFB, 5 were found that did respond with a multiple latency corresponding to 11.4% of all neurons studied. In 4 of these cases, only one additional latency was observed, and in one case two additional latencies were detected.

Estimates of nigrostriatal conduction velocity

Antidromic response latencies following neostriatal stimulation ranged from 9.0 to 25.0 ms, with a mean ± S.E.M. of 15.06 ± 0.25 ms (n = 154). Following stimulation of the MFB, antidromic response latencies ranged from 2.75 ms to 7.6 ms, with a mean ± S.E.M. of 4.6 ± 0.16 ms (n = 25). These values correspond to a point-to-point conduction velocity of 0.58 m/s. This value is in good agreement with that previously reported for dopaminergic nigrostriatal neurons in the rat[14,27]. Since the path of the axon is typically not linear, it can be assumed that this estimate of the conduction velocity is an underestimate of the true value.

Effects of striatal infusions on terminal excitability

The effects of the local striatal infusion of various catecholaminergic agonists and antagonists and potassium on striatal dopaminergic terminal excitability are summarized in Table I. The numbers in each category refer to the number of cells responding as indicated. The numbers in parentheses behind each entry refer to the mean ± S.E.M. change in threshold current.

Dopaminergic agonists

The local infusion of amphetamine produced a dose-dependent decrease in the excitability of dopaminergic nigrostriatal terminals. Fig. 2 illustrates the response of a typical cell to the infusion of 10 μM amphetamine. In Fig. 2A it can be seen that the predrug threshold current, 1.07 mA, elicits antidromic responses on 100% of the stimulus deliveries. Following a local infusion of 0.3125 μl of 10 μM D-amphetamine, the terminal excitability is decreased, seen in Fig. 2B by the absence of any antidromic responses, even with the increased stimulating current. The overall excitability change is illustrated in

TABLE I

Effects of the local Infusions of various drugs on antidromic excitability of nigrostriatal dopaminergic neurons

Threshold is defined as the minimum current sufficient to elicit antidromic responses on 100% of the non-collision trials. No effect is defined as a change in threshold of 5% or less. The numbers in each column represent the number of cases. The numbers in parentheses are the mean ± S.E.M. of the threshold changes expressed as a percentage of the pre-infusion control values.

Drug	*Threshold*			
	Increase	*Decrease*	*No effect*	*Total*
Amphetamine (1–50 μM)	13 (24.4 ± 3.5)	1 (10.0)	2	16
Apomorphine (1–10 μM)	11 (24.3 ± 3.9)	2 (13.2 ± 6.9)	2	15
Clonidine (10 μM)	0	2 (10.2 ± 3.5)	5	7
Isoproterenol (10 μM)	2 (54.8 ± 21.6)	0	1	3
Haloperidol (0.1–1 μM)	2 (13.8 ± 6.2)	11 (15.3 ± 2.7)	4	17
Haloperidol (5–50 μM)	4 (12.6 ± 4.9)	3 (8.0 ± 2.6)	2	9
Fluphenazine (10 μM)	1 (8.6)	5 (16.1 ± 3.3)	2	8
Sulpiride (10 μM)	1 (7.1)	6 (15.8 ± 5.1)	0	7
Potassium (50–100 mM)	1*	4 (14.7 ± 1.3)	2	7
Saline (0.9%)	2 (14.5 ± 7.5)	0	6	8
Ascorbate (0.1%)	0	0	3	3
Pretreated with α-methyl-p-tyrosine (200 mg/kg i.p.)				
Amphetamine (10 μM)	2 (17.2 ± 9.1)	1 (7.7)	5	8
Pretreated with striatal injections of kainic acid (1.25 mg/ml)				
Apomorphine (10 μM)	4 (27.7 ± 6.1)	0	0	4
Local infusion and excitability testing from MFB				
Apomorphine (10 μM)	0	0	5	5

* Depolarization block.

Fig. 2C where the post-drug threshold is 1.57 mA, reflecting an increase of almost 50% in the threshold from the pre-drug control condition.

Increases in the antidromic latency and latency variability often accompanied drug-induced decreases in excitability. Fig. 2D and E are each the superimposition of 5 single sweeps, at the same stimulus current. In the post-drug sweeps shown in Fig. 2E, it can be seen that the antidromic response is slightly delayed, and its variability greater compared to the pre-drug examples in Fig. 2D. Such latency increases were commonly observed following infusion of amphetamine, and varied in magnitude from about 0.2 ms up to a maximum of about 1.0 ms. Typical latency increases were on the order of 0.3–0.75 ms.

The decreases in terminal excitability following neostriatal infusion of amphetamine were dose-related, as illustrated for another typical cell in Fig. 3. Here the data are expressed by plotting the proportion of trials on which an antidromic response was elicited as a function of stimulating current. The baseline level of excitability is indicated by the solid curve. Following a local infusion of 0.3125 μl of 5.0 μM amphetamine, the terminal excitability is decreased, as evidenced by the uniform shift on the right of the excitability curve. A second infusion of 5.0 μM amphetamine leads to a further shift to the right in the current–response curve and the final post-drug threshold can be seen to be approximately 20% greater than the corresponding pre-drug value.

The effects of the local infusion of amphetamine on dopaminergic terminal excitability were almost totally prevented by pretreatment with 200 mg/kg α-methyl-*p*-tyrosine (Table I). In contrast to the effects of the drug in non-depleted rats in which infusions of amphetamine (1–50 μM) induced a threshold increase in 14 of 18 attempts (78%), the local application of 50 μM amphetamine to rats depleted of dopamine resulted in no change in excitability in 5 of 7 cases (71%) and small to moderate threshold increases in the remaining two cases.

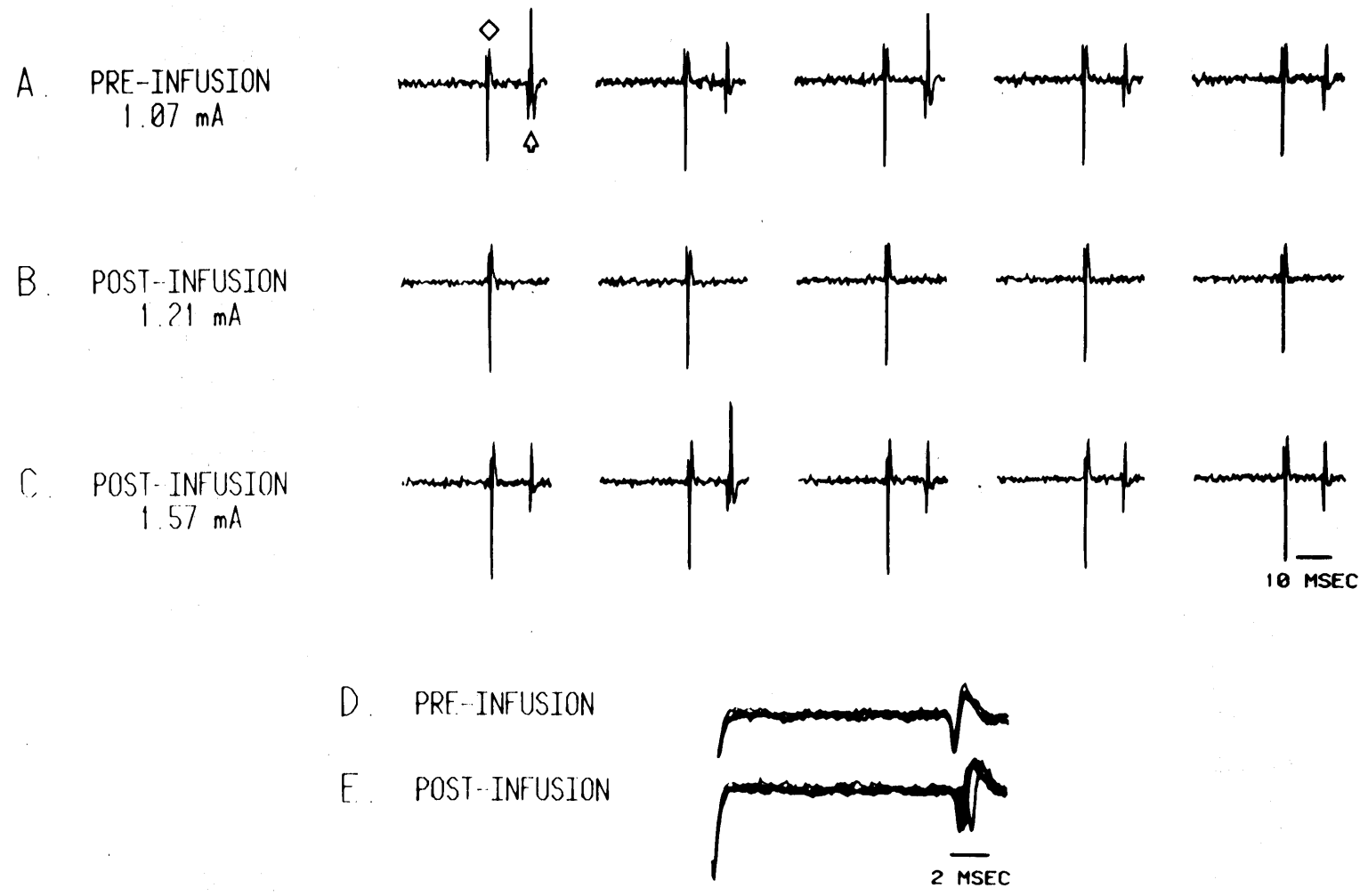

Fig. 2. Illustration of the effect of striatal infusions of amphetamine on dopaminergic terminal excitability. A: pre-infusion threshold for this neuron was 1.07 mA. The diamond denotes the stimulus artifact and the arrow indicates the antidromic response. B: following infusion of 0.31 μl D-amphetamine (10 μM), excitability was decreased and even an increased stimulus current of 1.21 mA was unable to evoke any antidromic responses. C: the final post-drug threshold was 1.57 mA, reflecting an increase of over 50%. Drug-induced decreases in excitability were often accompanied by increases in antidromic latency, and latency variability, seen by comparing the traces in E (post-drug) with those in D (pre-drug), each of which consists of the superimposition of 5 single sweeps.

The effects of apomorphine infusion on terminal excitability were found to be similar to those induced by amphetamine, and led to decreased terminal excitability in 11 of 15 attempts (Table I). As with the amphetamine infusions, increases in threshold following apomorphine infusion were often accompanied by slightly prolonged antidromic conduction times.

Decreases in terminal excitability induced by the local infusion of amphetamine were reversible upon subsequent local infusion (n = 5) or intravenous administration (n = 3) of the specific dopamine antagonists, haloperidol or sulpiride. Similarly, decreased terminal excitability induced by apomorphine infusion was subject to reversal by subsequent local infusions (n = 4) or systemic administration (n = 3) of haloperidol, as illustrated in Fig. 4. The pre-drug control level of excitability is indicated by the solid curve. Threshold is approximately 0.8 mA. Following a striatal infusion of 10 μM apomorphine, the curve is shifted uniformly to the right, indicating decreased excitability at all currents tested. Five min-

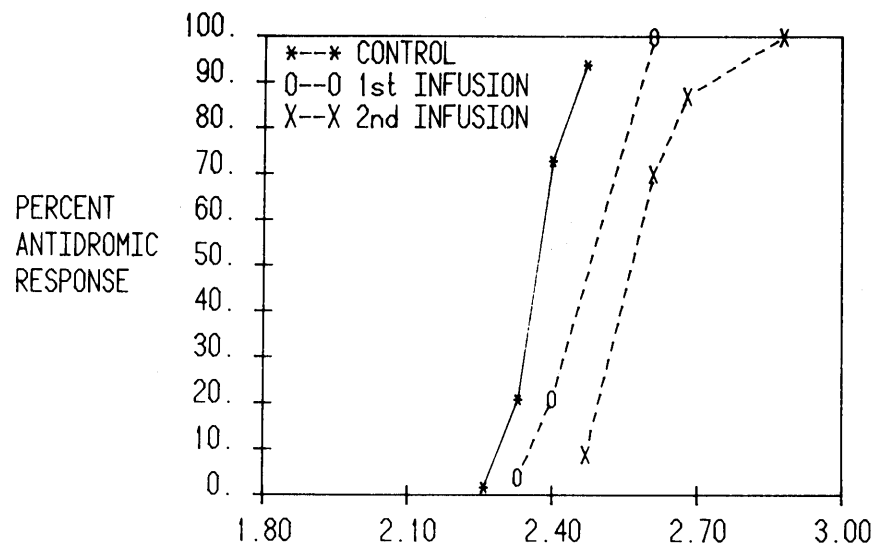

Fig. 3. Typical terminal excitability curves before and after local neostriatal infusion of 5 μM D-amphetamine. The proportion of antidromic responses obtained was plotted against stimulating current. Pre-drug control, (*— — *). Following infusion of amphetamine (○— — ○), excitability was decreased as shown by the shift to the right in the excitability curve, indicating that higher currents were required to achieve the same proportions of antidromic responses obtained prior to the infusion. A second infusion of amphetamine shifts the curve further to the right (×— — ×), illustrating the dose-dependence of the effect.

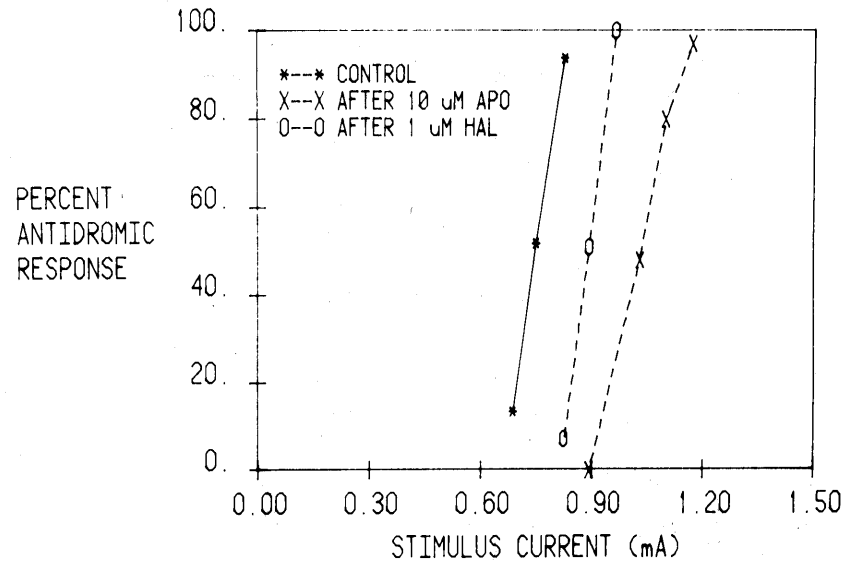

Fig. 4. Local neostriatal infusions of haloperidol can reverse excitability decreases induced by apomorphine infusion. Pre-infusion control (*— — *). Following infusion of apomorphine (10 μM) excitability is markedly reduced (×— — ×). A subsequent infusion of haloperidol (1 μM) partially reverses the decrease in excitability (○– – ○).

utes following the completion of the apomorphine infusion, the new threshold was stable at 1.20 mA and 1 μM haloperidol was infused through a second cannula. The dopamine antagonist partially reversed the decreased terminal excitability induced by apomorphine, reducing the threshold to approximately 0.96 mA and eliciting a uniform shift to the left in the current response curve.

Striatal infusions of catecholaminergic agonists and antagonists exerted effects on spontaneous nigral firing rates, consistent with previous data[25,26]. In addition, neostriatal infusions of apomorphine or amphetamine resulted in an increase in the proportion of antidromic responses consisting of an IS spike only, while infusions of haloperidol or fluphenazine resulted in an increase in the proportion of responses consisting of full IS-SD spikes. These data will be reported elsewhere[54].

Dopamine antagonists

The local infusion of haloperidol at concentrations ranging from 0.1 to 50 μM led to effects on dopaminergic terminal excitability that displayed a relatively unusual dose dependence, as summarized in Table I. The usual response of a dopaminergic nerve terminal to local infusion of low concentrations of haloperidol (0.1–1.0 μM) was an increase in terminal excitability, illustrated for one typical neuron in Fig. 5. As was the case for both amphetamine and apomorphine, the effects of haloperidol first became apparent within 2–4 min from the start of the infusion. In Fig. 5A, depicting the pre-drug control, the neostriatal stimulating current is 2.50 mA, just below threshold as evidenced by one 'miss' occurring on the third trace. In Fig. 5B, a neostriatal stimulating current of 2.06 mA is shown to be far below threshold, and fails to elicit any antidromic responses. Following a local infusion of 1.0 μM haloperidol, terminal excitability is markedly increased, and 2.06 mA is now at threshold for this neuron, eliciting antidromic responses 100% of the time as shown in Fig. 5C.

Decreases in threshold induced by haloperidol were often accompanied by a reduction in antidromic latency, and a reduction in the latency variability, as demonstrated by comparing Fig. 5E with 5D. Each is the superimposition of 5 single sweep records of the same neuron as in A and B at the same stimulus strength (2.50 mA), D recorded prior to and E after the infusion of the drug.

In contrast to the effects seen after infusions in the lower concentration range, local infusions of haloperidol of 5, 10 or 50 μM led to equivocal changes in terminal excitability. As summarized in Table I, the proportion of experiments which resulted in no effect, or in a decrease in excitability, was much greater than those obtained with the lower concentrations of the drug. These changes in threshold were difficult to quantify because in many cases the effects were biphasic, often with an initial increase in excitability that began within 2 or 3 min of the start of infusion which subsequently decayed into a marked decrease in excitability. In other cases the haloperidol infusion initially seemed to be without effect, but led to a gradual decrease in excitability over the course of the next 10 or 15 min.

The local infusion of two other representative dopamine receptor antagonists led to effects on dopamine terminal excitability which were similar to those induced by the lower concentrations of haloperidol. Both fluphenazine and sulpiride were effective at increasing the excitability of dopaminergic nerve terminals at concentrations of 10 μM. The effects of a striatal infusion of fluphenazine on the terminal excitability of a single dopaminergic neuron is illustrated in Fig. 6. The control level of excitability is represented by the solid curve. Following infusion of 0.3125 μl of 10 μM fluphenazine, the current–res-

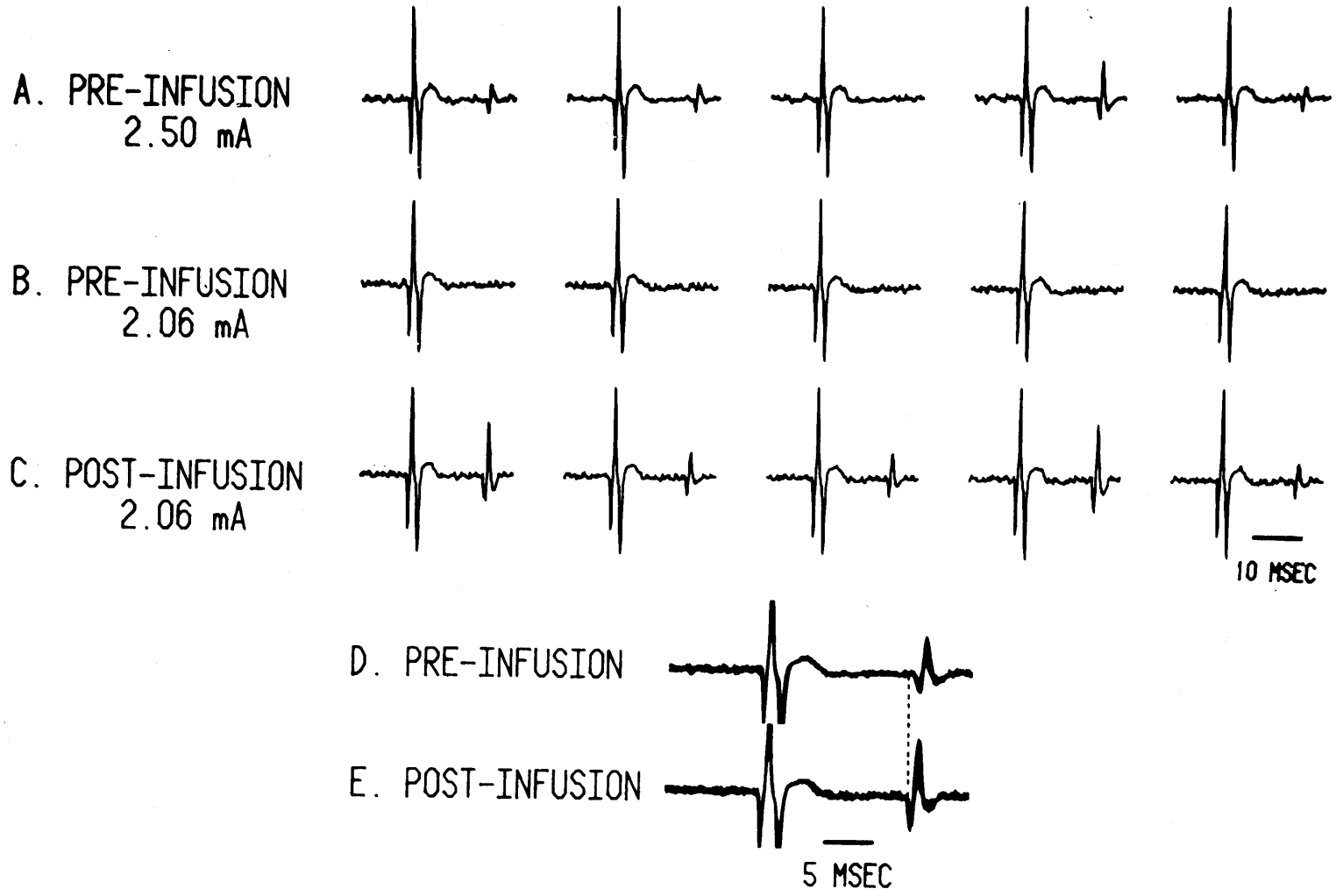

Fig. 5. Effects of neostriatal infusion of haloperidol on dopaminergic terminal excitability. A: prior to infusion, a neostriatal stimulating current of 2.50 mA is just subthreshold. B: prior to infusion, 2.06 mA is far below threshold, and does not evoke any antidromic responses. C: following local infusion of haloperidol, terminal excitability is markedly increased, and 2.06 mA is at threshold, and elicits antidromic responses on every trial. D and E: haloperidol infusions lead to a decrease in antidromic latency and latency variability.

ponse curve was displaced to the left, reflecting the increased excitability of the terminal field. A second infusion of fluphenazine caused an additional small increase in terminal excitability, indicated by a further shift to the left in the excitability curve.

Similar effects were obtained following the local infusion of the benzamide dopamine antagonist, sulpiride. With both sulpiride and fluphenazine, as with the low concentrations of haloperidol, increases in terminal excitability were often accompanied by reductions in the antidromic conduction time, and by reductions in the variability of the antidromic latency.

Relationship of changes in terminal excitability to baseline firing rate

The magnitudes of the changes in terminal excitability following local infusions of dopamine agonists and antagonists were dependent on the pre-infusion baseline firing rate for each neuron, as shown in Fig. 7. With both amphetamine and apomorphine, increases in threshold were greatest at low firing rates and declined as firing rate increased ($r = -0.61$, df = 13, $P < 0.05$ and $r = -0.73$, df = 9, $P < 0.05$, respectively). In contrast, following local infusion of the dopamine antagonists haloperidol and fluphenazine, decreases in threshold current were relatively small in slowly firing neurons, and greatest in the most rapidly firing cells ($r = 0.65$, df = 11, $P < 0.05$ and $r = 0.79$, df = 7, $P < 0.05$, respectively).

Vehicle infusions

In 11 cases the effects of vehicle infusion on terminal excitability were ascertained. These results are summarized in Table I. Eight infusions were made with 0.9% saline, the vehicle for all infusions except for apomorphine. The remaining 3 control infusions consisted of 0.9% saline in 0.1% ascorbate, the vehicle for apomorphine infusions. In 6 of the 8 cases, up to 3 successive infusions of vehicle failed to alter threshold in the cells tested. In the 2 remaining neurons, infusion of 0.9% saline led to modest increases in threshold. In the 3 cases in which it was attempted, infusions of the ascorbate-saline vehicle did not alter terminal excitability. In 3 cases, following a single in-

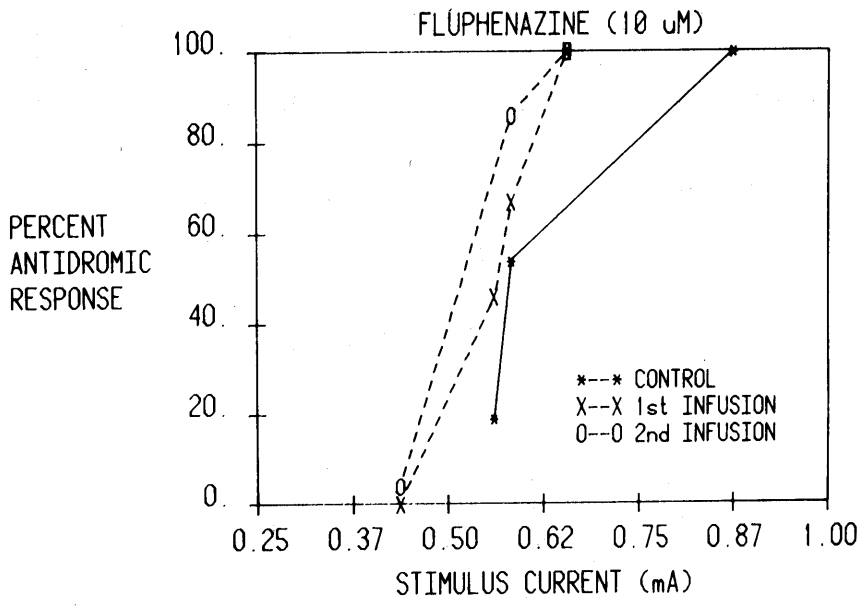

Fig. 6. Neostriatal infusions of fluphenazine (10 μM) increase dopaminergic terminal excitability. Pre-drug control (*— —*). Following infusion of fluphenazine, excitability is increased (×— —×). A second infusion results in an additional modest increase in excitability (○— — ○), attesting to the dose-dependent nature of the effect.

fusion of vehicle which was without effect, subsequent infusions of apomorphine (n = 2) or haloperidol (n = 1) were found to increase and decrease threshold, respectively.

Potassium

Potassium chloride, infused at concentrations of 50 and 100 mM, generally led to an increase in terminal excitability, as summarized in Table I. As was the case with other agents that led to a decrease in the threshold current, antidromic latency and latency variability were often decreased following potassium infusion.

One of the neurons tested with 100 mM KCl responded during the first 2 min of the infusion with an initial small decrease in threshold, which suddenly reversed to a very large (over 400%) increase in threshold, presumably the result of a depolarization block. Over the course of the following 30 min, the threshold gradually decreased and at the time that the cell was lost had decreased to slightly below the starting value.

Clonidine

The effects of the alpha-2-adrenergic agonist clonidine on terminal excitability were tested in 7 cases. As indicated in Table I, infusions of 10 μM clonidine resulted in very small increases in excitability in 2 cases, and no change in the remaining 5 cases.

MFB infusions

The effects of local apomorphine infusion (10 μM) into the MFB on antidromic excitability from this site were examined in 5 cases (Table I). In marked contrast to the changes in terminal excitability seen in striatum following local infusions of apomorphine, similar infusions into the MFB were without effect on antidromic excitability tested from this site.

Effects of kainic acid treatment

Antidromic responses obtained from rats 3–6 days following bilateral injection of 1.25 mg/ml kainic acid did not differ from responses obtained in control rats. The mean latency in 8 cells activated antidromically from neostriatum was 16.18 ± 1.62 ms, compared to 15.06 ± 0.25 ms in untreated controls. Multiple antidromic latencies from neostriatal stimulation were observed in 4 of these neurons, similar to the proportion observed in the control population.

In kainate-treated rats, the local infusion of 10 μM apomorphine led to decreased terminal excitability in all 4 attempts. The mean percent increase in threshold (27 ± 6.1%) was not significantly different from that seen in intact controls ($t = 0.616$, df = 14, $P > 0.05$). Haloperidol was infused twice at a concentration of 1 μM and led to an increase in excitability in both cases. In the single attempt, 1 μM haloperidol was able to partially reverse the decreased terminal excitability induced by a prior infusion of 10 μM apomorphine.

Histology

The infusion of kainic acid into the neostriatum led to a widespread destruction of neostriatal tissue as evidenced by the disappearance of neuronal cell bodies and a marked gliosis for several cubic millimeters around the injection site as revealed by the Nissl and fiber stains.

The neostriatal damage was constrained to a region approximately 1.5–2.5 mm in diameter surrounding the tip of the infusion cannulae, and the neurotoxic effects were largely contained within the confines of the dorsolateral neostriatum. Fiber bundles running through the neostriatum appeared normal, although the lateral ventricles were moderately dilated.

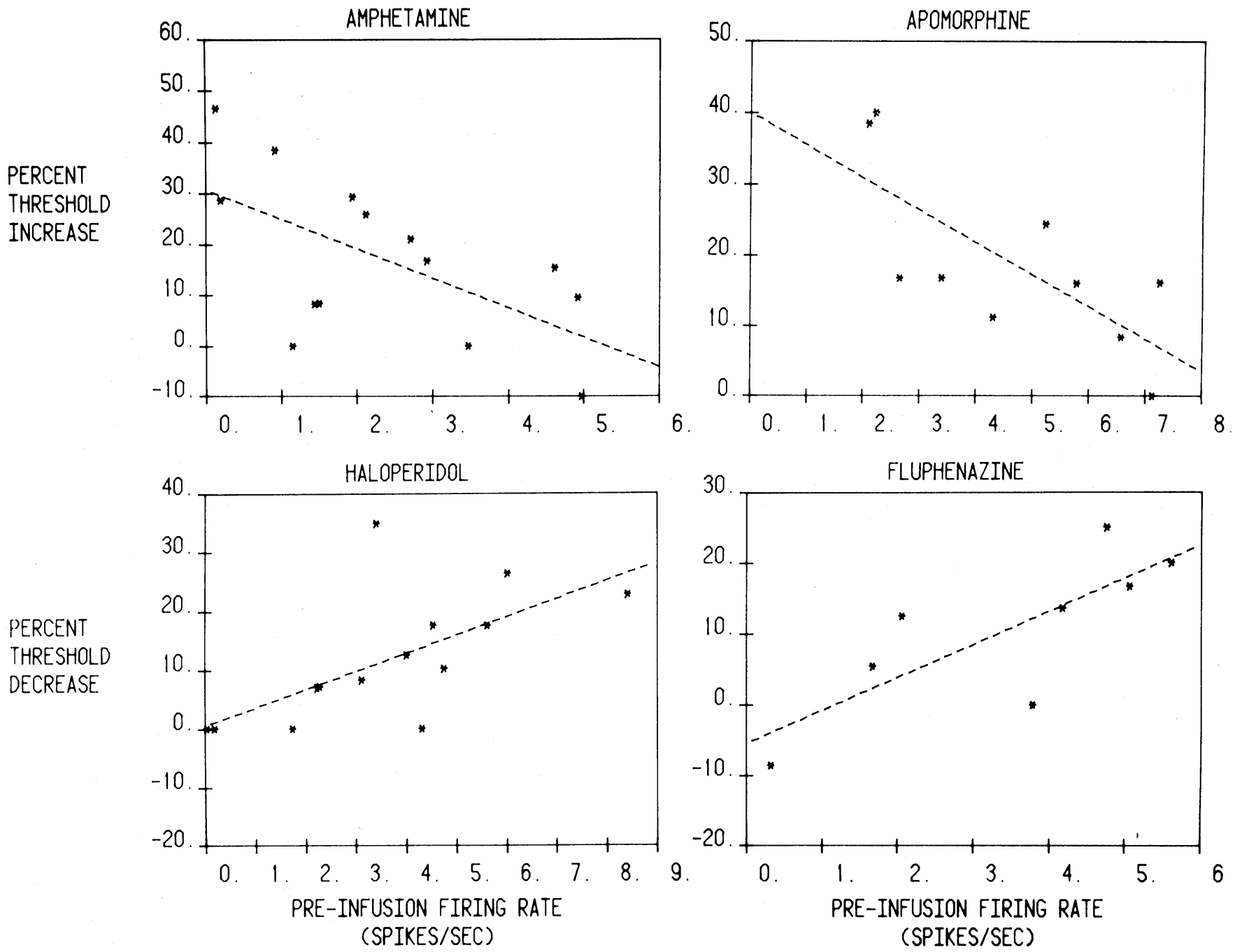

Fig. 7. Changes in threshold following striatal infusions of dopamine agonists and antagonists are correlated with pre-drug firing rates. Following infusions of 10 μM amphetamine or 10 μM apomorphine, threshold increases are larger for slowly firing cells and smaller for more rapidly firing neurons, (AMP: $r = -0.61$, df = 13, $P < 0.05$), (APO: $= r -0.73$, df = 9, $P < 0.05$). The opposite relationship obtains following infusions of 1 μM haloperidol or 10 μM fluphenazine, where decreases in threshold are small in slowly firing neurons but are more pronounced in faster firing cells (HAL: $r = 0.65$, df = 13, $P < 0.05$), (FLU: $r = 0.79$, df = 7, $P < 0.05$). The percent change in threshold stimulating current is shown on the ordinates and the pre-infusion firing rate is plotted on the abscissae.

DISCUSSION

Origin of the changes in antidromic responsivity

Changes in the proportion of antidromic responses recorded in substantia nigra following infusions of drugs into the neostriatum could originate from orthodromically transmitted effects on dopaminergic neurons in substantia nigra, or from alterations in excitability at the site of antidromic impulse initiation in the neostriatum. Since the propagation of action potentials along the axon can be envisioned as an 'all-or-none' event, our observation that the proportion of antidromic responses detected in substantia nigra is a function of the neostriatal stimulating current suggests that this parameter reflects conditions obtaining at the site of initiation of the antidromic potential. Consistent with this interpretation, although the proportion of antidromic responses to neostriatal stimulation can be altered by local infusions of dopaminergic agonists or antagonists, concurrent measurements of antidromic excitability at intermediate sites along the nigrostriatal axon in the MFB reveal no change in the frequency of antidromic responses elicited from this site. In addition, when antidromic responding is decreased by the local infusion of apomorphine or amphetamine, increases in neostriatal stimulus strength can reinstate 100% antidromic responding, indicating that the decreases in excitability are not due to orthodromically mediated alterations in excitability occurring between the sites of stimula-

tion and recording. Thus, alterations in the probability of obtaining antidromic responses resulting from the local infusion of various substances into the region of the axon terminal reflect changes in terminal excitability, and are due to phenomena occurring locally, and not at some distal locus along the axon or cell body.

Multiple antidromic latencies

Although verified as antidromic by several criteria including collision testing and high frequency following, antidromic responses evoked from neostriatum occurred at discrete multiple latencies in almost one-third of the neurons in this study. These multiple latencies never occurred simultaneously; there was always only one response per stimulus but its latency 'jumped' in discrete steps, typically from 0.5 to 2.0 ms. Each individual latency was very constant, as illustrated in Fig. 1. This phenomenon has been reported previously[9,24], and has been interpreted as arising from variable sites of antidromic impulse initiation due to the highly branched nature of the terminal arborizations of nigrostriatal neurons.

When antidromic responses were elicited from the MFB, the incidence of multiple latencies was much reduced (11.4%), but the phenomenon was still present. The multiple latency antidromic responses arising from stimulation of the MFB probably originate from stimulation of different collateral branches of the ascending projections; i.e. the nigrostriatal axons have issued collaterals by the time they reach the site of the MFB electrode, 2.1 mm anterior to the substantia nigra. Although Golgi studies failed to reveal evidence of local axonal collateralization in rat[32], recent retrograde double-labelling studies have indicated that many mesotelencephalic dopamine neurons send divergent projections to striatum, septum and cerebral cortex[18].

Terminal excitability changes: receptor characterization

The decreases in terminal excitability following neostriatal infusions of amphetamine were almost totally prevented by pretreating the rats with α-methyl-*p*-tyrosine, an inhibitor of catecholamine synthesis, which suggests that the effects of amphetamine were mediated indirectly, through an endogenous catecholamine. The amphetamine effects could be reversed by subsequent local infusions of haloperidol, which implicated dopamine as the endogenous amine mediating the effect. This hypothesis was supported by the finding that local infusions of apomorphine, a relatively specific agonist for dopamine receptors, led to decreases in terminal excitability similar to those following amphetamine administration.

In marked contrast to the effects of apomorphine and amphetamine, local infusions of the alpha-2 agonist, clonidine, did not result in a decrease in dopaminergic terminal excitability. In previous studies it has been shown that clonidine is extremely potent at stimulating the noradrenergic autoreceptor as inferred from evoked release studies in cortex and midbrain[19,20,42,57], inhibition of firing rate in noradrenergic locus coeruleus neurons[7] and decreases in noradrenergic terminal excitability[40,41]. The inability of clonidine to alter dopaminergic terminal excitability is consistent with reports which indicate that this alpha-2 agonist does not significantly inhibit the firing rate of nigral dopaminergic neurons[1]. Furthermore, sulpiride was effective at increasing terminal excitability, and could antagonize the decrease in excitability arising from a prior local infusion of apomorphine. Since sulpiride is devoid of alpha-adrenergic antagonist properties[16], it follows that the agonist-induced decreases in excitability and their blockade by sulpiride and the other neuroleptics are mediated by a receptor possessing dopaminergic but not alpha-2 adrenergic character.

Although isoproterenol led to decreases in excitability in 2 of the 3 cases in which it was tested, this may have been secondary to increased dopamine release from striatal terminals. It has been inferred that, in addition to the autoreceptor, other presynaptic receptors exist on the terminals of nigrostriatal neurons which are capable of regulating the evoked release of dopamine[49]. Recently, it has been demonstrated that isoproterenol increases the spontaneous release of dopamine in vivo and in vitro from striata of cat and rat[43,44]. An indirect effect of the beta-agonist on terminal excitability is supported by electrophysiological data which show that iontophoresis of isoproterenol onto dopamine neurons causes a decline in firing rate which can be blocked by dopamine antagonists but not by alpha- or beta-adrenergic antagonists[1]. These data thus suggest that the local infusion of isoproterenol induced release of dopamine,

which led to a decrease in terminal excitability by interacting with the terminal dopamine autoreceptor.

The present results are consistent with the proposal that autoinhibition of dopamine release and changes in terminal excitability, as well as inhibition of neuronal firing rate, are mediated by the same type of receptor, which is distinct from the alpha-2 adrenergic receptor that mediates noradrenergic autoinhibition[7,12,13,35,40,41].

Site of the receptor mediating changes in antidromic excitability

In an attempt to localize the site of the striatal dopamine receptor mediating the drug-induced changes in terminal excitability in order to rule out the possibility of a local, transsynaptic effect, striatal neurons were destroyed by intrastriatal injections of the neurotoxin, kainic acid[10,39]. In kainate-treated animals, the neurophysiological responses to neostriatal stimulation were indistinguishable from control animals and infusions of dopamine agonists and antagonists exerted effects identical to those seen in intact rats. Histological analysis of the striata in these animals revealed a relatively circumscribed locus of damage around the injection site with a complete absence of neuronal cell bodies.

This finding, when coupled with a lack of ultrastructural evidence for the existence of synapses between axon terminals in striatum, argues that the dopamine receptors mediating the drug-induced changes in terminal excitability are located on the dopaminergic axon terminals themselves, consistent with results from biochemical release studies[28,31]. Furthermore, since local infusions of apomorphine into stimulating sites along the dopamine axons in the medial forebrain bundle were unable to change the excitability from this region, the axonal dopamine autoreceptors responsible for altering excitability are apparently constrained to the axon terminal region.

Membrane effects of autoreceptor stimulation

The agonist-induced decrease in terminal excitability could result from a receptor-mediated hyperpolarization of the terminal regions of the axon[24,54]. This would have the effect of moving the site of antidromic impulse initiation further from the firing threshold, resulting in increased currents necessary to initiate antidromic responses. However, it is also possible that a change in the conductance of the terminal membrane at the site of impulse initiation could exert similar effects on excitability in the absence of any polarization change. If autoreceptor stimulation resulted in an overall decrease in the membrane resistance, then the potential difference created across the terminal membrane by a given stimulus current would be decreased, and the membrane thus rendered less excitable to direct electrical stimulation. It is also conceivable that a decrease in conductance, specific to calcium, could lead to a threshold increase in the absence of a change in the overall input impedance of the terminal, if a significant depolarizing current at the terminal were carried by calcium ions, as has been shown to be the case in squid axon terminal by Llinas and colleagues[37,38].

Although intracellular recording from the axon terminals under conditions of autoreceptor stimulation and blockade is technically impossible at the present time due to the small size of the terminals, intracellular recordings from cell bodies might reflect processes common to autoreceptor stimulation at both sites. In one study, intravenous administration of apomorphine led to a hyperpolarization of dopaminergic somata, associated with a decrease in overall conductance[23]. On the other hand, a recent intracellular study in noradrenergic locus coeruleus neurons indicates that stimulation of the soma-dendritic autoreceptor in these cells also resulted in a membrane hyperpolarization, although this was associated with a more typical increase in conductance to potassium[2]. Thus, there may not be a single ionic mechanism underlying the autoreceptor-mediated hyperpolarizations. The precise changes in ionic conductance at the sites of transmitter release remain to be determined.

Effects of dopamine antagonists

When infused at concentrations of 0.1 μM or 1.0 μM, haloperidol led to a modest but consistent increase in terminal excitability. However, striatal infusions at concentrations of 10 μM or 50 μM led to equivocal alterations in terminal excitability that were occasionally biphasic. These anomalous effects were not observed with sulpiride or fluphenazine.

The most likely explanation for this phenomenon results from the local anesthetic action of haloperidol at higher concentrations[26,45,46]. Fluphenazine was

found not to exhibit this property at the concentration used, consistent with the observation that the ED_{50} for inhibition of dopamine release by a similar phenothiazine was between 1 and 2 orders of magnitude greater than that for haloperidol[46]. This interpretation is further supported by the lack of threshold increases following sulpiride, a drug devoid of membrane stabilizing properties[33]. These data are consistent with the interpretation that the threshold increases and biphasic effects seen following striatal infusions of the higher concentration range of haloperidol were due to a local anesthetic effect at the terminal region. At the lower range of concentrations, haloperidol, as well as fluphenazine and sulpiride, consistently led to decreases in threshold indicative of increases in terminal excitability, consistent with a blockade of an inhibitory dopamine autoreceptor.

The present results indicate at least the possibility that some of the discrepant results in the literature with respect to neuroleptic-induced facilitation of dopamine release may be due to a combination of a local anesthetic action of the drug at the terminal which would tend to reduce excitability and transmitter release[57], with autoreceptor blockade, which would facilitate terminal excitability and transmitter release[15,30,45,47].

Terminal excitability changes: physiological relevance

Changes in terminal excitability in response to striatal infusions of amphetamine or apomorphine were found to be inversely correlated with the predrug rate of firing of the cell. This suggests that terminal excitability is a function of the total amount of autoreceptor stimulation, resulting from endogenous and exogenous sources. Thus, if a cell is firing rapidly, there is a relatively large amount of neurotransmitter liberated and available for binding to the presynaptic receptor. The state of occupancy of the autoreceptor is high under these conditions, and exogenously elicited stimulation, by amphetamine-induced dopamine release or by the direct-acting agonist apomorphine, adds a relatively smaller increment to total autoreceptor occupancy than when the cell is not firing rapidly.

Consistent with this interpretation, the effects of the dopamine antagonists, haloperidol and fluphenazine, were correlated directly with pre-infusion firing rate. This latter observation suggests that under the conditions of this experiment the terminal autoreceptors were being stimulated tonically, most likely by dopamine released from the terminal from which the antidromic response was being initiated or from nearby dopamine release sites. This interpretation is in good agreement with previous results showing a similar relationship between stimulation frequency and neuroleptic-induced facilitation of dopamine release in vitro[29,36]. These results indicate that the effects of the dopamine antagonists are likely due to a blockade of autoreceptor stimulation that results from the rate-dependent release of endogenous dopamine.

Thus, autoreceptor-mediated control of terminal excitability is a phenomenon that occurs in situ, similar to the tonic inhibition of dopamine neuronal firing by dendritic release of dopamine[25,26]. The results obtained in these experiments indicate that the excitability of the nerve terminal, and thus presumably the level of autoinhibition, varies as a function of the frequency of impulses reaching the terminal. Additional findings on the relationship of impulse traffic to terminal excitability are reported in the following paper[55].

Mechanisms of autoinhibition

There is as yet a paucity of information concerning the mechanism(s) whereby stimulation of the dopamine autoreceptor leads to a reduction in the amount of stimulus-evoked transmitter release. However, recent findings on the biophysical bases of alpha-adrenergic autoinhibition in peripheral noradrenergic systems may be pertinent to the question.

Stjarne and associates have proposed a dual process hypothesis of noradrenergic autoinhibition[3,50,51,52]. From kinetic analyses of the relationship between extracellular calcium levels and the frequency dependence of autoinhibition, these researchers suggest that 'alpha-autoinhibition acts in an all-or-none manner, turning off rather than gradedly depressing the sensitivity of units'[52]. Furthermore, comparisons of the ability of phentolamine to increase norepinephrine release induced by potassium versus electrical stimulation revealed that, although the antagonist was effective at augmenting release evoked by both types of stimuli, release was enhanced considerably more in electrically stimulated tissue[3,51,52].

These two lines of evidence indicate that the alpha-adrenergic autoreceptor-mediated inhibition of norepinephrine release occurs primarily by way of suppressing the 'recruitment', or active invasion of potential release sites. However, the fact that the alpha blockers were still able to increase the release of norepinephrine evoked by the direct potassium depolarization suggests that there may be some modification of the depolarization-release coupling induced by the autoreceptor activation, perhaps arising from a decrease in calcium entry at the nerve terminal[13,50].

Although decreases in dopamine terminal excitability following neostriatal infusions of dopaminergic agonists are consistent with a reduced safety factor for impulse conduction, the fact that increases in the strength of stimulation can re-establish antidromic responding on 100% of the stimulus deliveries argues that there cannot be complete blockade of the antidromic impulse at any point between the sites of stimulation in the terminal fields and recordings in the substantia nigra. Since most of the synapses along central dopaminergic and noradrenergic fibers are en passant, it follows that if impulse failure occurs under conditions of autoinhibition, it must take place only at terminal boutons or axonal branch points, and not along the main fiber.

However, it is possible that autoinhibition functions at en passant varicosities as well. Conduction along a varicose axon would be most sensitive to polarization or conductance changes at the proximal side of the varicosity due to the rapid increase in fiber diameter[53]. By causing a local conductance increase, autoreceptor stimulation could act to shunt action currents before invading such release sites, causing a failure of active invasion. Failure of the action potential at this site could greatly reduce or abolish the voltage-dependent calcium current, and lead to the failure of transmitter release. However, a decremented graded potential could still propagate into the varicosity, and due to a reduction in the diameter of the fiber at the intervaricose segment, might produce a current source large enough to re-initiate the action potential on the distal side of the inhibited region. In this way, autoinhibition could still act to reduce the number of sites releasing transmitter in response to impulse flow along a main fiber, as suggested by Stjarne and associates[3,50–52], without blocking propagation of the inpulse to more distal regions. A mechanism of this type would be consistent with recent data which suggests that transmitter release from certain synapses is 'all-or-none', with variations in the magnitude of the postsynaptic potential arising from changes in the number of sites releasing transmitter, rather than from the amount of transmitter released from each site[11,17].

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