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Dopamine "Autoreceptors": Pharmacological Characterization by Microiontophoretic Single Cell Recording Studies

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Summary. The effects on the firing of single dopamine (DA) neurons in the substantia nigra (and adjacent ventral tegmental area) of a representative group of catecholamine agonists and antagonists were studied in rats using single cell recording and microiontophoretic techniques. Microiontophoretic application of DA or the DA agonist apomorphine depressed the firing of these cells; the DA antagonist trifluoperazine blocked this effect. However, the α-agonist clonidine had no depressant effect and the β-agonist isoproterenol had only a weak depressant action on DA neurons. Furthermore, the α-antagonist piperoxane and the β-antagonist sotolol were completely ineffective in blocking the depressant effects of DA. These results show that DA-sensitive receptors on the soma of DA neurons are pharmacologically distinct from α or β adrenoreceptors. Because of their location and selective responsiveness to DA agonists, the catecholamine receptors on the soma of DA neurons appear best classified as DA "autoreceptors".

Key words: Autoreceptor – Adrenoceptor – Dopaminergic – Microiontophoretic – Substantia nigra.

INTRODUCTION

Evidence for the existence of presynaptic or prejunctional dopamine (DA)-sensitive receptors located upon various components of central dopaminergic neurons has come from both biochemical and physiological studies. Some of these receptors, which have been termed dopamine "autoreceptors" by Carlsson (1975), appear to be located at DA nerve terminals. DA receptors in this location are believed to regulate the rate of DA synthesis and DA release by a local negative feedback mechanism. The presence of autoreceptors at DA nerve terminals was first revealed by certain surprising biochemical changes seen in an early time period following a blockade of impulse flow in the dopaminergic nigro-neostriatal pathway. If impulse flow in DA neurons is interrupted by an electrothermic lesion, mechanical transection, injection of local anesthetics or the administration of γ-hydroxybutyric acid, a marked increased in neostriatal DA content and synthesis is observed (Faull and Laverty, 1969; Andén et al., 1971; Nybäck, 1971; Carlsson et al., 1972; Walters et al., 1973). The increase in DA can be prevented by the administration of apomorphine (Kehr et al., 1972; Roth et al., 1973), a DA agonist, or pretreatment with d-amphetamine (Roth et al., 1973), a drug which releases DA. These effects of apomorphine and amphetamine can in turn be nullified effectively by haloperidol and to some extent by chlorpromazine (Kehr et al., 1972; Walters and Roth, 1976). Since the above increases in DA levels and synthesis occur under circumstances in which there can be no further modulation of impulse flow, a feedback mechanism based upon a neuronal circuit appears to be ruled out. Moreover, a biochemical feedback mechanism based on end-product inhibition, which is operative under certain circumstances (Costa and Neff, 1966; Javoy et al., 1972), could not explain the phenomenon since there is actually an increase in DA level. It has therefore been postulated that a diminished release of DA into the synaptic cleft resulting from an interruption of impulse flow leads to a depletion of DA acting upon a receptor site located on the presynaptic as well as the postsynaptic junctional membrane (Carlsson et al., 1972; Kehr et al., 1972). A lowering of DA receptor activation may then cause a local compensatory increase in DA synthesis.

The above biochemical phenomena are paralleled by the physiological responses of dopaminergic neu-

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ronal soma in the substantia nigra (zona compacta) and adjacent medial ventral tegmentum (respectively, A9 and A10 cells of Dahlström and Fuxe, 1965) to the systemic or microiontophoretic administration of DA agonists and antagonists. Earlier studies showed that the systemic injection of apomorphine or the microiontophoretic ejection of apomorphine or DA has a powerful inhibitory effect on the firing of identified A9 and A10 cells (Aghajanian and Bunney, 1973; Bunney et al., 1973a). The inhibition was associated with a marked increase in the amplitude of action potentials recorded extracellularly, indicating a hyperpolarization of the neuronal membrane potential. In a more recent study it was shown that the inhibitory effect of microiontophoretically applied DA can be blocked partially or completely by the systemic injection of the antipsychotic drugs haloperidol and chlorpromazine (Aghajanian and Bunney, 1974). The aim of the present study is to provide a more complete pharmacological characterization of the DA-sensitive receptors which occur in the region of the dopaminergic neuronal soma. The effects on the firing of single dopaminergic neurons of a representative group of catecholamine agonists and antagonists were studied using single cell recording and microiontophoretic methods.

METHODS

Male albino rats (Charles River Laboratories, Wilmington, MA) weighing approximately 250 g were used. The animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). The animals were then mounted in a stereotaxic instrument for unit recordings from the substantia nigra, zona compacta (A9) and ventral tegmental area of Tsai (A10). For A9 recordings, a burr hole was drilled with its center 2.8–3.2 mm anterior to lambda and 1.8–2.2 mm lateral to the midline. For A10 recordings, the same frontal plane was used but the laterality was only 0.5–0.8 nm.

Five barrel micropipettes were prepared using a Narishige pipette puller, as described previously (Haigler and Aghajanian, 1974). To improve recording quality the pipettes were pulled with relatively low heat and a delayed magnet pull to produce a wide tip angle rather than a filamentous tip. The tips were broken back to 4–5 μm under microscopic control and then directly filled by injection of solutions into the various barrels; the presence of several fiberglass filaments (placed in each barrel prior to pulling) allowed the tips to become filled rapidly by capillary action (Tasaki et al., 1968). The central (recording) barrel was filled with 2 M NaCl saturated with fast green dye. The impedences of recording barrels were typically between 3 and 5 meghoms. One side barrel was filled with 4 M NaCl and this channel was used for automatically balancing (neutralizing) tip current (Salmoiraghi and Weight, 1967). The remaining side barrels were filled with various sets of 3 of the following solutions: dopamine HCl (0.1 M, pH 4.0; Calbiochem); *l*-norepinephrine bitartrate (0.1 M, pH 4.0; Regis); apomorphine HCl (0.05 M, pH 5.0; Merck); isoproterenol HCl (0.1 M, pH 4.0; K & K Labs); *d*-amphetamine sulfate (0.1 M, pH 4.0; Aldrich); clonidine HCl (0.01 M in 0.1 M NaCl, pH 4.0; Boehringer Ingelheim Ltd); trifluoperazine HCl (0.05 M in 0.05 M Na tartrate, pH 3.6; SKF); piperoxane HCl (0.1 M, pH 4.0; Rhone-Poulenc); sotalol HCl (0.1 M, pH 4.0; Regis); γ-aminobutyric acid (0.1 M, pH 4.0; Calbiochem). The trifluoperazine was used as a partial salt of tartaric acid since the pure HCl salt tended to result in electrode noise ("blocking") whereas the addition of tartrate improved electrical properties. Trifluoperazine was chosen as the antipsychotic drug for use in these microiontophoretic studies in preference to haloperidol or chlorpromazine, which were used in previous systemic studies (Aghajanian and Bunney, 1974), because of certain advantages as follows: trifluoperazine is readily soluble in water (unlike haloperidol) and it is highly potent at ejection currents which do not produce local anesthetic effects (unlike chlorpromazine; Aghajanian and Bunney, 1973). The clonidine was ionically diluted 10 fold by NaCl because previous studies had shown that noradrenergic neurons of the locus coeruleus are extremely sensitive to this compound and it was necessary for the drug to be used in a diluted form (Svensson et al., 1975).

During periods of baseline unit recording, a negative retaining current of 10 nA was applied to each drug barrel. Drugs, which were all in cationic form, were ejected by passing a positive current of 5–20 nA through the appropriate barrel or barrels. Electrical signals of spike activity were followed by a high input-impedence amplifier, displayed on an oscilloscope, and led into an electronic counter whose threshold was set so that it was triggered by the individual spikes of the single neuron under study. Counts of unit activity were then transformed into integrated rate histograms with an interval time of 10 s. A mercury vapour lamp oscillograph was used to record spike activity directly.

The anatomical location of the units tested was determined by passing a 20 nA negative current through the recording barrel for 10 min. This resulted in the deposition of fast green in a discrete spot (Thomas and Wilson, 1965). Animals were anesthetized and perfused with 10% formalin (phosphate buffered, pH 7.0). Serial sections were then cut, mounted and counterstained with neutral red. Although the identity of a cell was retrospectively determined by histological examination after each experiment there were certain practical guidelines which were useful for locating units in the A9 and A10 areas during the actual recording procedure. In both areas the DA cells were usually found 6.8–7.5 mm ventral to skull surface at the coordinates given above for the burr holes. Both A9 and A10 cells typically displayed a positive-negative spike of long duration (≈ 2–3 ms) giving rise to a low-pitched sound as heard on the audiomonitor. Also characteristic was the slow (2–8/s) bursting, complex spike pattern in which there is a progressive decrease of spike amplitude within each cluster of action potentials (Bunney et al., 1973b). Great care was taken to distinguish the DA neurons from nearby neurons (e.g. in zona reticulata, red nucleus, interstitial nucleus of ventral tegmental decussation, and reticular formation) since these other cells have quite different histochemical, physiological and pharmacological properties (Bunney et al., 1973b; Aghajanian and Bunney, 1973, 1974).

RESULTS

A. Agonists

In comparing DA cell response to the various catecholamine agonists, the response to DA from the same 5-barrel pipette was always determined at a duration and current of application equal to that used for the other agonists. When applied in this manner from different barrels of the same electrodes no difference was found between the degree of DA cell inhibition produced by DA and norepinephrine

Table 1. Effects of catecholamine agonists and antagonists upon dopaminergic neurons[a]

Agonists	Number of cells tested	Inhibitory Effect on dopaminergic neurons (A9 and A10)[b]
dopamine	49	+−+++
norepinephrine	7	+−+++
apomorphine	6	+−++
isoproteronol	6	+
d-amphetamine	8	0− +
clonidine	7	0
Antagonists	**Number of cells tested**	**Blockade of inhibition**
trifluoperazine	16	+++
piperoxane	7	0
sotalol	6	0

[a] Results based on use of iontophoretic currents of up to 20 nA applied for 1 min, except for amphetamine where a duration of 2 min was used. Ratings given as follows: +++, 75−100% inhibition (or antagonism); ++, 50−75% inhibition; +, 25−50% inhibition.

[b] Data from A9 and A10 cells was combined because no differences in responses of cells in these areas was detected

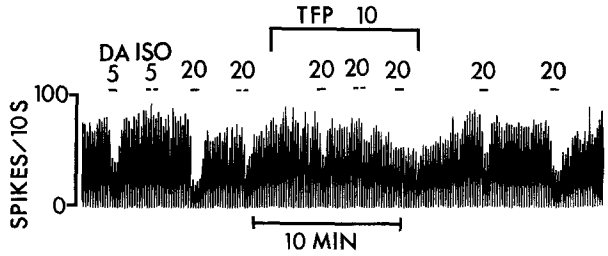

Fig. 1. Average rate record showing the inhibitory effects of dopamine (*DA*) and isoproterenol (*ISO*) applied microiontophoretically to a single neuron in the substantia nigra, zona compacta (A9). Note that an application of 20 nA of *ISO* produces somewhat less of an inhibitory response than 5 nA of *DA*. The concurrent ejection of trifluoperazine (*TFP*) blocks the effects of both *DA* and *ISO*. The *TFP* produces some direct depression toward the end of the ejection period. After the ejection of *TFP* is terminated, recovery of inhibitory responses to *DA* is very gradual. Solid lines above trace indicate durations of *DA* ejections, dashed lines are periods of *ISO* ejections; numbers above lines are ejection currents in nA

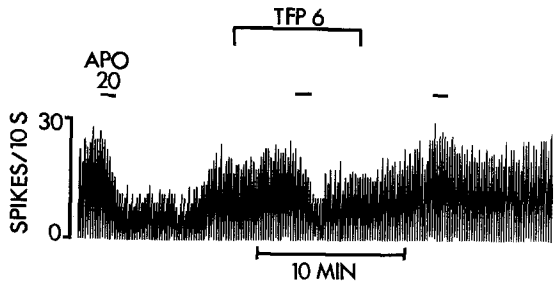

Fig. 2. Inhibitory response of an A9 dopaminergic neuron to the microiontophoretic application of apomorphine (*APO*). Note the delayed onset and prolonged duration of inhibition. Trifluoperazine attenuates the inhibition on succeeding ejections of *APO*

(Table 1). In both cases, a 25−100% depression of DA cell firing was achieved by 1 min of iontophoretic ejection at currents up to 20 nA. There was variation from cell to cell in the degree of inhibition at these parameters, but the effects of DA and norepinephrine were the same for each cell tested. On the other hand, in similar paired comparisons the β-agonist isoproterenol had only about one-fifth the inhibitory potency of DA (Table 1, Fig. 1). Furthermore, the α-agonist clonidine was entirely lacking in inhibitory activity (Table 1). Apomorphine had an intermediate degree of inhibitory activity in terms of % inhibition from baseline rate. However, in contrast to DA, its effects were delayed in onset and prolonged in duration (Fig. 2). Amphetamine had relatively little inhibitory activity when applied at equal or greater currents and periods as DA (Table 1, Fig. 3). Even when it was given for prolonged periods, amphetamine had a relatively weak inhibitory effect.

B. Antagonists

The antipsychotic drug trifluoperazine, the α-antagonist piperoxane, and the β-antagonist sotalol were tested for their DA-blocking activity. Of these compounds only trifluoperazine showed blocking activity. To avoid local anesthetic effects trifluoperazine was applied at low ejection currents over long periods. Under these conditions trifluoperazine was capable of blocking totally the effects of DA. This is illustrated in Figure 4 which shows a direct oscillographic tracing from an A9 cell. In the top trace, the predrug inhibitory response to iontophoretic DA can be seen; note the marked increase in spike amplitude during the inhibitory period. The lower trace, taken after trifluoperazine was applied for 8 min (at 6 nA), shows a total block of the DA. The ability of trifluoperazine to block DA or apomorphine is also illustrated in some of the integrated rate records shown above (see Figs. 1−3). Note that the antagonism is gradual in onset and that the effect persists beyond the cessation of trifluoperazine ejection. The specificity of the trifluoperazine antagonism of DA is shown by the fact that the inhibitory response to alternate pulses of GABA are not blocked at a time when there is total antagonism of DA (Fig. 5). In contrast to the potent effect of trifluoperazine, piperoxane and sotalol were entirely without blocking activity (Fig. 6, Table 1).

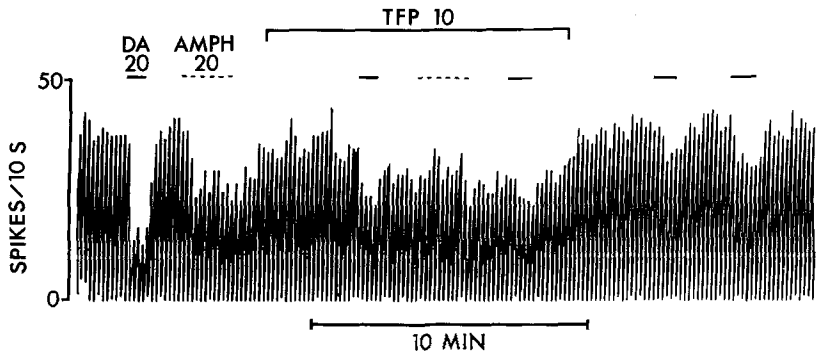

Fig. 3. Comparison of the inhibitory effects of dopamine (*DA*) and amphetamine (*AMPH*) on a dopaminergic (A9) neuron. At equal currents, *AMPH* has only about half the inhibitory effect of DA despite the fact that it was applied for twice the duration. *TFP* attenuates the response to both *DA* and *AMPH*

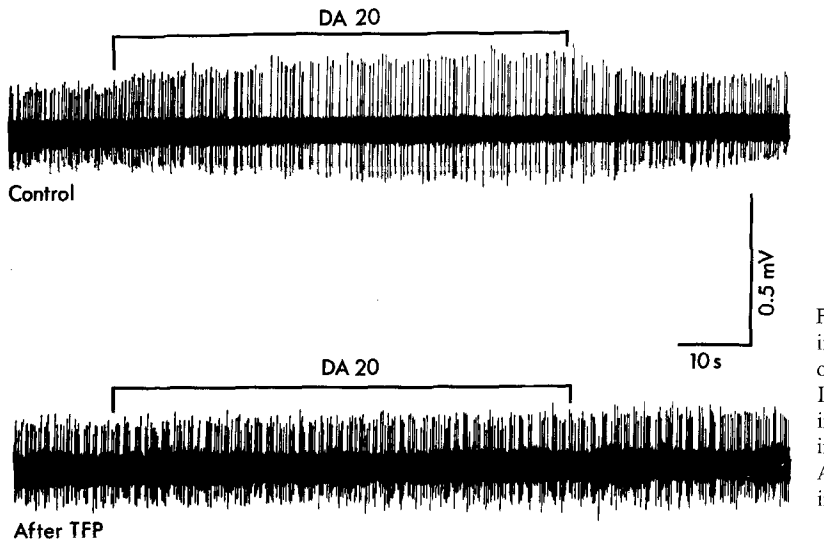

Fig. 4. Direct oscillographic tracing showing the inhibitory effect of *DA* on an A9 neuron and a blockade of this effect after *TFP* (applied for 8 min at 6 nA). In the top trace *DA* produces about a 50% decrease in firing rate which is associated with a marked increase in the amplitude of the action potentials. After *TFP* (lower trace) both the decrease in rate and increase in action potential amplitude is blocked

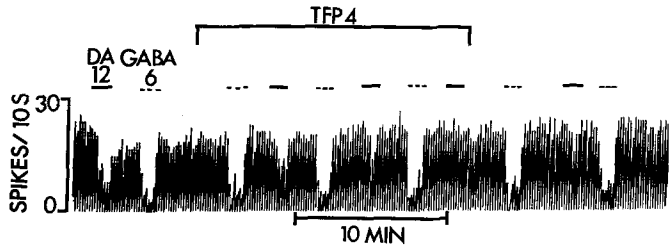

Fig. 5. Block of inhibitory responses of an A9 cell to *DA* but not *GABA* by the concurrent ejection of *TFP*. Note that recovery of responses to *DA* is nil in the time period shown after the *TFP* is terminated

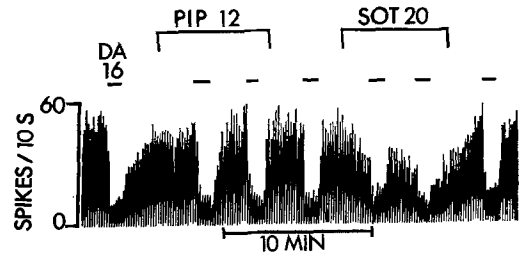

Fig. 6. Failure of piperoxane and sotalol to block the inhibitory responses of an A9 cell to *DA*. Note that sotalol itself produces some degree of inhibition at the ejection current employed

DISCUSSION

The results of this microiontophoretic study indicate that pharmacologically distinctive DA-sensitive receptors are present upon the soma of DA neurons located in the zona compacta of the substantia nigra and the adjacent ventral tegmental area (A9 and A10 neurons, respectively). Microiontophoretic application of DA or the DA agonist apomorphine was able to depress the firing of these cells. On the other hand, the α-agonist clonidine failed to inhibit the firing of

DA neurons. Under the same experimental conditions, clonidine has been shown to have a powerful inhibitory effect on noradrenergic neurons of the locus coeruleus (Svensson et al., 1974). Similarly, the α-antagonist piperoxane, which is highly potent in blocking the inhibitory responses to catecholamines in the locus coeruleus (Cedarbaum and Aghajanian, 1976) was completely ineffective in blocking DA inhibition of A9 and A10 cells. Furthermore, the β-agonist isoproterenol only had a weak depressant effect and the β-antagonist sotalol had no blocking activity when applied to DA neurons. These results demonstrate that DA-sensitive receptors on the soma of DA neurons are pharmacologically distinct from α or β-adrenoceptors. In marked contrast to the α and β-antagonists tested, the antipsychotic drug trifluoperazine applied by iontophoresis was able to block totally the inhibition of DA neurons by DA or apomorphine. This is the first demonstration that the local (microiontophoretic) application of an antipsychotic drug can block the inhibition of DA neurons by DA; in previous studies the antipsychotic drugs (i.e., haloperidol and chlorpromazine) which were used to block microiontophoretic DA were given systemically (Aghajanian and Bunney, 1974). Trifluoperazine resembles other classical antipsychotic drugs in its ability to accelerate the firing of DA neurons when it is given systemically (Bunney et al., 1973b). In view of their selective responsiveness to DA agonists and antagonists the catecholamine receptors on the soma of DA neurons would appear best classified as DA receptors. Moreover, these DA receptors in the region of the soma bear a strong resemblance to DA "autoreceptors" (Carlsson, 1975) found in the terminal region of DA neurons.

Results from our in vivo pharmacological studies of autoreceptors on the DA cell soma are in accord with a recent detailed in vitro biochemical study on the pharmacological properties of "presynaptic" DA receptors (Iversen et al., 1976). In the latter study, the inhibitory effects of apomorphine on catechol formation in intact striatal synaptosomes was used in an index of presynaptic DA receptor activity. In parallel with our physiological results trifluoperazine was found to block the inhibitory effect of apomorphine on catechol formation. In this same biochemical system, dopamine and *l*-norepinephrine were approximately equipotent in depressing catechol formation in striatal synaptosomes. On the other hand, isoproterenol and amphetamine had low activity in this system. This parallels the finding in the present study that dopamine and *l*-norepinephrine were approximately equal in their ability to depress the firing of DA neurons but isoproterenol and amphetamine were much less active.

What is (are) the function(s) of dopamine "autoreceptors"? It is possible that such autoreceptors are incorporated into the entire dopaminergic neuronal membrane but have a function only at terminal regions as part of a postulated local negative feedback mechanism for regulating the synthesis and release of dopamine (Carlsson et al., 1972). Our microiontophoretic studies at the soma of dopaminergic neurons suggest one possible mechanism by which autoreceptors could regulate dopamine synthesis at dopaminergic terminals. If stimulation of these receptors located within the synaptic cleft produces a hyperpolarization of the membrane of dopaminergic nerve terminals (analogous to that seen when dopamine is applied to the soma) then axonal impulses may fail to invade the terminal region. Such a mechanism could account for the following effects of various DA agonists: 1) depression of the impulse-flow dependant activation of tyrosine hydroxylase produced by electrical stimulation of the nigrostriatal pathway in vivo (Murrin and Rath, 1976) and 2) depression of stimulus-dependant release of DA from DA terminals in striatal slices in vitro (Farnebo and Hamberger, 1971). Similarly, antipsychotic drugs that block the depressant effect of DA agonists on DA synthesis (Kehr et al., (972; Roth et al., 1973), enhance the release of DA in vivo (Cheramy et al., 1970), and markedly enhance the stimulation-induced activation of tyrosine hydroxylase (Roth et al., 1975) may do so preventing a hyperpolarization of the terminal membrane produced by an accumulation of dopamine released into the synaptic cleft. It should be noted, however, that there is as yet no direct evidence that an activation of DA autoreceptors produces an hyperpolarization of DA nerve terminals or that such an hyperpolarization could suppress invasion of the terminals by axonal impulses. The latter point is of crucial importance for the validity of the above suggestions because, in general, when impulses *do* invade the terminal there is a direct correlation between the resting potential and the amount of transmitter released by a nerve impulse (cf. Eccles, 1964, for review).

It is important, therefore, to consider the possibility that dopamine "autoreceptors" in the terminal region may modulate tyrosine hydroxylase through some mechanism independant of terminal membrane hyperpolarization. For example, the dopamine autoreceptors might gate the fluxes of a specific species of ion, such as Ca^{2+}, which may be critical in the activation or suppression of tyrosine hydroxylase. These speculations are complicated by the fact that the activation of tyrosine hydroxylase observed after nigral stimulation and the activation observed after abolition of impulse flow in the nigrostriatal pathway appear to be produced by different mechanisms: the activation

after cessation of impulse flow can be reversed in vitro by the addition of Ca^{2+} ions whereas the activation after increased impulse flow cannot (Roth et al., 1975). Nevertheless, in both cases stimulation of dopamine "autoreceptors" in the terminal region suppresses the enzyme activation.

The present results are also compatible with the possibility that dopamine "autoreceptors" in the region of the soma have a function separate from that at DA terminals. For example, they could participate in mediating transmission at dendro-dendritic junctions which are believed to exist between dopaminergic neurons (Björklund and Lindvall, 1975). Support for this latter concept has some from recent biochemical work which indicates that dopamine can be released from dendrites in the rat substantia nigra by antidromic stimulation in vivo (Korf et al., 1976) or K^+ stimulation in vitro (Geffen et al., 1976). In addition, DA-sensitive adenylate cyclase activity, which has been linked to the physiological action of DA at postsynaptic structures, has also been found in the substantia nigra (Kebabian and Saavedra, 1976).

It is evident, both from the biochemical and physiological studies, that feedback mechanisms involved in the regulation of dopaminergic neurons are far more complex than originally supposed (Carlsson et al., 1972). The apparent existence of presynaptic DA receptors and their possible role in local feedback regulation must now be considered along with feedback mechanisms involving neuronal circuits and modulation of impulse flow. Originally, it was suggested from biochemical data that there is an increase in activity of dopaminergic neurons induced by some antipsychotic drugs mediated through a neuronal feedback circuit (Carlsson and Lindqvist, 1963). Direct support for the concept that a block of postsynaptic DA receptors by antipsychotic drugs is responsible for modulating the rate of impulse flow within the dopaminergic pathway has come from physiological studies (Bunney et al., 1973b). The *d*-amphetamine-induced depression of firing of dopaminergic neurons also appears to be in part mediated by such a postsynaptic feedback loop (Bunney and Aghajanian, 1976). However, based on our microiontophoretic studies as well as the microinjection results of Groves et al. (1975), it is also possible that amphetamine can depress the firing of DA neurons in part through a local effect on these cells – perhaps by releasing DA from dendrodendritic junctions (see above).

If it is accepted that there are both local and postsynaptic feedback mechanisms modulating dopaminergic function, the question arises as to the relative importance of these two mechanisms under various physiological conditions and drug treatments. It has been shown that in low doses apomorphine may directly inhibit the firing of dopaminergic neurons. Conceivably in a low dose range the presynaptic action of the drug may predominante, resulting in diminished dopaminergic function. Such a mechanism may explain the biphasic behavioral effects of apomorphine in rats: low doses reduce motor activity while high doses increase motor activities such as stereotyped behaviors (Carlsson, 1975). The question as to the relative importance of pre- and postsynaptic mechanisms also can be raised in the case of amphetamine. It has been reported that amphetamine-induced release of DA from the neostriatum is in large dependant upon a maintenance of impulse flow within the nigrostriatal pathway (Von Voigtlander and Moore, 1973). Thus, either a neuronal feedback inhibition of dopaminergic neurons secondary to a stimulation of postsynaptic DA receptors or a stimulation of DA autoreceptors following amphetamine-induced release of DA into the synaptic cleft would serve to dampen the extent of DA release from DA terminals, thereby partially compensating for the direct releasing action of the drug. In the case of the antipsychotic drugs, an increase in DA synthesis and release may result from an increase in impulse flow secondary to a blockade of postsynaptic receptors, from a blockade of presynaptic DA receptors, or a combination of the two factors. An increase in DA release mediated by a block of presynaptic DA receptors and a block of postsynaptic DA receptors by antipsychotic drugs represent opposing functional forces, the net effects of which are not presently well understood in terms of either therapeutic actions or adverse side effects. In general, it now appears necessary to assess the relative potency of drugs on both pre- and postsynaptic DA receptors in order to fully understand the basis for their physiological and behavioral actions.

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