CENTRAL DOPAMINERGIC NEURONS: NEUROPHYSIOLOGICAL IDENTIFICATION AND RESPONSES TO DRUGS*

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INTRODUCTION

FLUORESCENCE histochemical studies have shown that the soma of central dopamine (DA)-containing (dopaminergic) neurons are mainly located in the zona compacta (ZC) of the substantia nigra and adjacent ventral tegmental (VT) area (DAHLSTRÖM and FUXE, 1965; UNGERSTEDT, 1971a). Neurons in the zona reticulata (ZR) of the substantia nigra, on the other hand, do not contain DA and are thus distinguishable from ZC and VT cells by fluorescence histochemical methods. Despite these histochemical differences, in neurophysiological studies involving either stimulation of or recording from the substantia nigra it has not been customary to distinguish between dopaminergic and non-dopaminergic neurons. In view of the histochemical studies, however, it was of interest to determine if ZC and VT neurons could be distinguished from ZR and other cells according to their pattern of single unit activity and whether they would show selective responses to drugs and other substances known to affect dopaminergic mechanisms.

IDENTIFICATION OF DOPAMINERGIC NEURONS

To establish the dopaminergic identity of neurons observed in single unit recordings conducted in rat brain, three independent procedures were employed (BUNNEY *et al.*, 1973b). As a first step, on the basis of standard histological examination, cells were presumptively identified as dopaminergic if a dye spot ejected from a stereotaxically placed recording electrode was located within the ZC or VT. This criterion, however, is by itself insufficient, since some non-dopaminergic neurons (e.g., in the ZR) are found on the immediate border of the ZC and VT. Therefore, two other criteria, both involving combined neurophysiological and fluorescence histochemical methods were employed. It was found that when an electrode tip was located in the ZC or VT, microiontophoresis of L-dopa led to a markedly enhanced histochemical fluorescence of neurons surrounding the electrode tip; in other areas, only the neuropil or capillaries became fluorescent under these conditions. In a counterpart of the preceding experiment, small amounts of 6-hydroxydopamine were injected in the vicinity of the substantia nigra, a treatment which results in a selective destruction of dopaminergic neurons (cf. UNGERSTEDT, 1971b). 1–4 days following this procedure, there was a selective loss of single units which had the firing pattern and responses to drugs which, by the above histological and histochemical techniques, were determined to be characteristic of the neurons in the ZC and VT (see below).

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NEUROPHYSIOLOGICAL PROPERTIES OF DOPAMINERGIC NEURONS

Neurons in the rat brain identified as "dopaminergic" by the above criteria were found to be remarkably homogeneous with respect to their firing pattern and responses to drugs and other substances administered systemically (BUNNEY *et al.*, 1973a,b) or applied iontophoretically. In these same respects, dopaminergic neurons were clearly distinguishable from neurons in nearby areas such as the ZR of the substantia nigra, the red nucleus, or the reticular formation. Dopaminergic cells typically show extracellular action potentials with a positive-negative wave form of unusually long duration (~2msec). They tend to have a regular rhythm and a slow rate of firing (2–6 spikes/sec) in unanaesthetised, gallamine-paralysed preparations, but curiously exhibit an increase in firing rate and a bursting pattern when the animal is anaesthetised with chloral hydrate or halothane. In contrast, ZR cells tend to have a relatively rapid rate of firing and are either unaffected or depressed by anaesthesia.

Microiontophoretic studies show still further differences between the dopaminergic neurons of the ZC and VT and the non-dopaminergic neurons of the ZR. Dopaminergic cells showed little or no response to acetylcholine whereas neurons in the ZR were consistently excited by acetylcholine even at very low microiontophoretic currents (Fig. 1). On the other hand, with one exception, ZR neurons tested ($N = 24$) showed no response to DA applied microiontophoretically (Fig. 1, bottom trace), whereas *all* dopaminergic neurons tested showed at least an initial depressant response to DA at low ejection currents (Fig. 1, top trace). Interestingly, after repeated applications there tended to be an attenuation of the response to DA. As can be seen from Fig. 1 (top trace) the initial application of DA at an ejection current of 10 nA temporarily produced a total inhibition of firing but at later times as much as 60 nA was required to cause approximately this same degree of inhibition. At this later time, 10 nA of DA had no appreciable effect In postsynaptic areas (i.e., those receiving dopaminergic input, such as the caudate nucleus, accumbens nucleus and the olfactory tubercles (FUXE, 1965; ANDEN *et al.*, 1966; UNGERSTEDT, 1971a) no attenuation of response to DA was seen after repeated applications (AGHAJANIAN and BUNNEY, unpublished data).

ZC and VT cells as well as ZR cells were inhibited by γ-aminobutyric acid and excited by glutamate applied microiontophoretically; thus these substances did not serve to distinguish dopaminergic from non-dopaminergic neurons.

DOPAMINERGIC NEURONS: EFFECT OF DA-RECEPTOR BLOCKADE

Drugs believed to have actions upon the dopaminergic system in the CNS have been found to have dramatic effects upon the rate of firing of ZC and VT neurons (BUNNEY *et al.*, 1973a,b). Such drugs fall into two general classes: those which appear to block DA receptors and those which either increase DA availability or mimic the action of DA at receptors. The antipsychotic drugs of the phenothiazine and butyrophenone type have been shown to have a structural relationship to DA and thus might competitively block DA receptors (HORN and SNYDER, 1971). These drugs have been shown to markedly increase DA turnover in the neostriatum (CARLSSON and LINDQVIST, 1963; NYBÄCK *et al.*, 1968; CORRODI *et al.*, 1967; GEY and PLETSCHER, 1968), an effect requiring an anatomically intact nigrostriatal pathway (CHERAMY *et al.*, 1970; ANDÉN *et al.*, 1971; NYBÄCK and SEDVALL, 1971). It has been hypothesised that a blockade of DA receptors produced by these drugs might

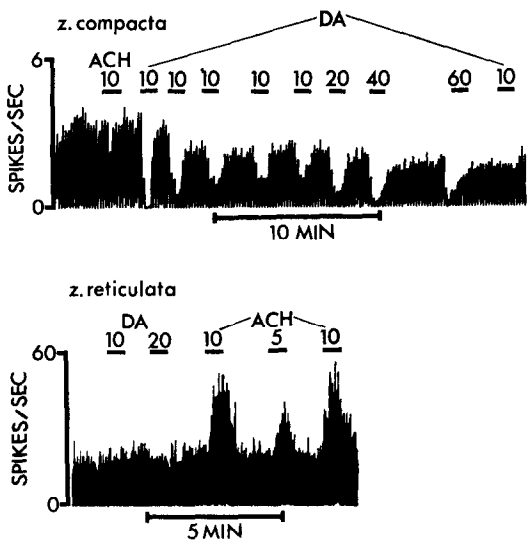

FIG. 1.—Comparison of responses of neurons in zona compacta (ZC) and zona reticulata (ZR) of the substantia nigra to the microintophoretic application of dopamine (DA) and acetylcholine (ACh).

In the *top trace*, ACh ejected at a current of 10 nA upon a ZC neuron is seen to produce no effect. There was no appreciable response in any of the ZC and VT cells tested with ACh ($N = 8$), even with ejection currents up to 40 nA. On the other hand, all ZC and VT cells tested with DA ($N = 16$) responded, at least initially, with a depression in firing rate. Typically as is shown in the *top trace*, there was an attenuation of discrete responses to DA with repeated applications, associated with a downward drift of baseline rate.

The *bottom trace* illustrates that ejection of DA usually causes no change in firing rate of ZR neurons. In only 1 of 24 ZR cells tested was there a response (depression) to microiontophoretic DA. In contrast, ACh was consistently excitatory on all ZR cells tested ($N = 24$) and was usually effective at low ejection currents (5–10 nA, as shown in *bottom trace*).

Methods for recording and microiontophoretic drug applications through 5-barreled micropipettes were as previously described (AGHAJANIAN *et al.*, 1972; HAIGLER and AGHAJANIAN, 1973). The duration of ejecting current is indicated by length of bar and intensity of ejection current by number (in nA) above bar. Rate of firing is displayed by on-line integrated rate record calibrated on ordinate in spikes/sec. Concentration of substances in micropipettes: ACh, 0·2 M (pH 6) ; DA. 0·2 M (pH 4).

lead to a compensatory increase in the activity of dopaminergic neurons via a neuronal-feedback mechanism (CARLSSON and LINDQVIST, 1963; ANDÉN *et al.*, 1964). In direct support for this hypothesis it has been found that the systemic administration of antipsychotic compounds including chlorpromazine (CPZ) and haloperidol (HAL) causes an increase in the rate of firing of ZC and VT neurons (BUNNEY *et al.*, 1973a,b). However, when CPZ is applied directly to dopaminergic neurons it has little or no effect of its own and does not block the inhibitory action of DA (Fig. 2A). In contrast small intravenous doses (0·5–1·0 mg/kg) of CPZ produced a doubling in the rate of firing of ZC and VT neurons (Fig. 2B). HAL has been found to accelerate the firing of dopaminergic cells to the same extent as does CPZ (BUNNEY *et al.*, 1973b). When HCL is given after CPZ it produces no further effect (Fig. 2B), suggesting that these drugs act upon a common set of receptors within the dopaminergic system.

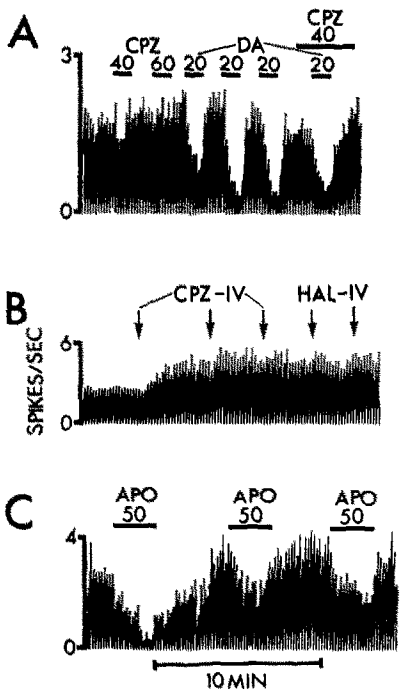

FIG. 2.—Effects of chlorpromazine (CPZ), dopamine (DA) and apomorphine (APO) on the rate of firing of dopaminergic neurons of the zona compacta (ZC) and ventral tegmental (VT) areas.

In "A", CPZ is seen to have no effect on firing of a dopaminergic cell nor does it block DA inhibition when applied concurrently by microiontophoresis. This was the case with all ZC and VT cells tested with CPZ ($N = 8$) despite the fact that the drug was ejected up to levels sufficient to produce some degree of local anaesthetic effect.

In "B", CPZ given intraveniously (IV) in a sequence of low doses (0·5, 0·5 and 1·0 mg/kg) is seen to maximally accelerate the firing of a dopaminergic neuron. This result is in accord with previous studies (BUNNEY *et al.*, 1973b). Once a maximal effect is achieved with CPZ no further effect is seen with haloperidol (HAL: 0·1 and 0·5 mg/kg).

In "C", APO given iontophoretically is seen to inhibit the firing of a dopaminergic neuron. This effect was found with all dopaminergic neurons tested with APO ($N = 10$). As can be seen in this example, there was some attenuation of response with repeated applications.

Methods as described in Fig. 1. Concentrations of drugs used in the 5-barreled pipettes: CPZ, 0·2 M (pH4); APO, 0·06 M (pH 3·5).

DOPAMINERGIC NEURONS: EFFECTS OF DA RECEPTOR STIMULATION

Drugs that are believed to directly or indirectly stimulate DA receptors have been found to cause a depression of the firing rate of dopaminergic neurons (BUNNEY *et al.*, 1973a,b). Amphetamine has been shown to increase the release and/or block the reuptake of central catecholamines (CA) (MCLEAN and MCCARTNEY, 1964; MOORE and LARIVIÈRE, 1963; STEIN, 1964; FARNEBO, 1971; GLOWINSKI and AXELROD, 1965; MCKENZIE and SZERB, 1968; COYLE and SNYDER, 1969; BESSON *et al.*, 1969, 1971a,b; TILSON and SPARBER, 1972; VOIGTLANDER and MOORE, 1973). It has been suggested that amphetamine, by increasing the concentration of CA post-synaptically might initiate a neuronal-feedback inhibition of CA-containing neurons (CORRODI *et al.*, 1967). Consistent with this hypothesis, systematically administered

d-amphetamine has been found to inhibit the firing of dopaminergic neurons (BUNNEY *et al.*, 1973b); this effect can be reversed by CPZ or HAL. A direct inhibitory mechanism seems unlikely since *d*-amphetamine has little effect on ZC and VT cells when applied by microiontophoresis and the effect of systemic *d*-amphetamine is lost when connections between the substantia nigra and neostriatum are interrupted (BUNNEY and AGHAJANIAN, this volume). Taken together, these results point to a neuronal-feedback circuit as the mechanism by which amphetamine inhibits dopaminergic neurons.

Apomorphine, a drug thought to stimulate DA receptors directly (ERNST, 1967; ANDÉN *et al.*, 1967; PERSSON, 1970) was also found to depress the firing of dopaminergic neurons when it is administered systematically (BUNNEY *et al.*, 1973a). However, unlike amphetamine, apomorphine has a powerful inhibitory effect when applied by microiontophoresis directly to dopaminergic neurons (Fig. 2C). As with DA, there appears to be an attenuation of this effect with repeated ejections. Thus, in addition to its postulated effects upon "postsynaptic" DA receptors, apomorphine appears to mimic the action of DA on "presynaptic" DA receptors (i.e., receptors upon the dopaminergic neuron itself). These results are consistent with the findings of KEHR *et al.*, (1972) which show that apomorphine can inhibit DA synthesis in the neostriatum even following interruption of the nigrostriatal pathway. It was suggested that there may be a receptor-mediated feedback inhibition of tyrosine hydroxylase by apomorphine. An alternative explanation for these findings would be that apomorphine can directly inhibit the enzyme tyrosine hydroxylase (GOLDSTEIN *et al.*, 1970). In any case, our electrophysiological results give support for the notion that DA and DA agonists can exercise a local feedback control upon synthesis or release of this putative transmitter by acting upon pre-synaptic DA receptors.

SUMMARY AND CONCLUSIONS

(1) By combined neurophysiological and histochemical methods, dopaminergic neurons of the ZC and VT areas were found to be clearly distinguishable from adjacent non-dopaminergic neurons (e.g., in ZR). Of particular interest was the finding that ZC and VT cells are inhibited by microiontophoretic DA but are insensitive to acetylcholine. On the other hand, ZR neurons are relatively insensitive to DA but are excited by acetylcholine at very low ejection currents. This suggests that if there are cholinergic afferents to the substantia nigra they would need to impinge upon ZR rather than upon ZC cells to produce a physiological effect.

(2) Drugs which either increase DA availability at DA receptors (e.g., amphetamine or have a direct DA-agonist action (e.g., apomorphine) inhibit the firing of dopaminergic neurons; these results are consistent with the operation of a compensatory negative feedback system. Conversely, presumed DA-receptor blockers (e.g., CPZ and HAL) increase the rate of firing of dopaminergic neurons; these results are consistent with the operation of a compensatory positive feedback system.

(3) The soma of dopaminergic neurons appear to have DA receptors since they are responsive to the direct, microiontophoretic application of either DA or the DA-agonist, apomorphine. If the terminals of dopaminergic neurons also have such DA-receptors (i.e., "presynaptic DA-receptors"), then this might explain the postulated receptor-mediated feedback control of striatal tyrosine hydroxylase activity at dopaminergic synapses (cf. KEHR *et al.*, 1972).

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