J. Physiol. (1987), **392**, *pp.* 397–416
With 10 text-figures
Printed in Great Britain

DOPAMINE ACTS ON D_2 RECEPTORS TO INCREASE POTASSIUM CONDUCTANCE IN NEURONES OF THE RAT SUBSTANTIA NIGRA ZONA COMPACTA

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(Received 23 December, 1986)

SUMMARY

1. Intracellular recordings were made from neurones in the substantia nigra zona compacta in slices of rat mesencephalon *in vitro*. The majority of neurones fired action potentials spontaneously at 0·2–5·6 Hz. Dopamine, applied either by superfusion or from the tip of a pressurized pipette, prevented spontaneous action potential firing and hyperpolarized the membrane.

2. When the membrane potential was held negative to the threshold for action potential firing, the hyperpolarization evoked by dopamine was accompanied by a fall in input resistance. Under voltage clamp, dopamine produced an outward membrane current associated with an increase in membrane conductance. The effects of superfused dopamine on firing rate, membrane potential and membrane current were concentration dependent in the range 1–100 μM.

3. The reversal potential for the hyperpolarizations and the outward currents produced by dopamine was $-109{\cdot}7 \pm 1{\cdot}7$ mV ($n = 12$) when the potassium concentration was 2·5 mM and $-74{\cdot}0 \pm 5{\cdot}0$ mV ($n = 4$) when the potassium concentration was 10·5 mM. The change in reversal potentials in these and intermediate potassium concentrations was described by the Nernst equation.

4. The outward current induced by dopamine was reversibly reduced by barium (100–300 μM) and by high concentrations of tetraethylammonium ($\geqslant$10 mM). Calcium-free solutions with cobalt (0·5–2 mM) did not reduce the current in response to dopamine during the first 5 min of their application. Currents and hyperpolarizations caused by dopamine were unaffected by tetrodotoxin (1 μM).

5. The hyperpolarization produced by dopamine was mimicked by the D_2 receptor agonist quinpirole (LY171555, 0·1–3 μM) and was blocked by the D_2 receptor agonists domperidone and (−)-sulpiride. Agonists and antagonists at D_1 receptors had no effect.

6. (−)-Sulpiride (30 nM–30 μM) produced a progressive shift to the right in the concentration–response curve to either dopamine or quinpirole. Schild analysis of the

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antagonism between (−)-sulpiride and quinpirole suggested competitive antagonism with a dissociation equilibrium constant for (−)-sulpiride of about 13 nM.

7. It is concluded that dopamine acts on D_2 receptors on neurones of the rat substantia nigra pars compacta to increase the membrane potassium conductance.

INTRODUCTION

More than 90% of the dopamine-containing neurones in the rat brain are found in two cell groups of the mesencephalon, the ventral tegmental area (A10 region) and the zona compacta of the substantia nigra (A9 region; Björklund & Lindvall, 1984). The dopamine-containing neurones of the substantia nigra compacta project predominantly to the corpus striatum and the integrity of this pathway is of considerable importance for the control of voluntary movement. The release of dopamine by these neurones occurs not only in their striatal terminal fields, but also in the substantia nigra itself, probably from dendrites; this release appears to be self-regulating in that it can be inhibited by the evoked release of dopamine (Cheramy, Leviel & Glowinski, 1981).

Electrophysiological studies of substantia nigra compacta neurones *in vivo* have demonstrated that ionophoretic application of dopamine reduces the rate of action potential firing (Aghajanian & Bunney, 1973; Guyenet & Aghajanian, 1978). This effect is mimicked by systemic administration of dopamine receptor agonists, notably apomorphine, and by amphetamine (which releases dopamine), and is blocked by neuroleptic drugs (Bunney, Aghajanian & Roth, 1973*a*; Bunney, Walters, Roth & Aghajanian, 1973*b*; Groves, Wilson, Young & Rebec, 1975; Aghajanian & Bunney, 1977). More recently this has been demonstrated *in vitro* (Pinnock, 1983) with the additional evidence provided that this was probably mediated by a receptor of the D_2 type (Pinnock, 1984).

Intracellular recordings from substantia nigra zona compacta neurones and some characterization of the membrane properties of the cells has been previously reported by Grace & Bunney (1980, 1983*a*, *b*) (*in vivo*, rat), by Kita, Kita & Kitai (1986) (*in vitro*, rat) and by Llinás, Greenfield & Jahnsen (1984) (*in vitro*, guinea-pig). In the present paper we report results of intracellular electrophysiological studies of substantia nigra zona compacta neurones, recorded in a rat brain slice preparation, in which the ionic basis of the action of dopamine on these neurones is established and the dopamine receptor type pharmacologically characterized. A preliminary report of some of these results has been presented to the Physiological Society (Lacey, Mercuri & North, 1986).

METHODS

The experimental preparation

The preparation and *in vitro* maintenance of slices of rat brain containing the substantia nigra was similar to that described by Williams, Henderson & North (1984) with reference to pontine slices containing the nucleus locus coeruleus. Male Sprague–Dawley rats weighing between 180 and 300 g were anaesthetized with ether and killed by a blow to the chest. The scalp and skull were removed and the brain was excised from the cranial cavity. A block of tissue containing the mesencephalon was prepared, and serial coronal slices of mesencephalon (thickness 300 μM) were cut on a vibratome (Oxford). A single brain slice, taken caudal to the third ventricle at the level

of the mammillary bodies, was transferred to a recording chamber, in which it rested on a nylon mesh. The tissue was held in position with two electron microscopy grids placed bilaterally over each of the two regions containing the substantia nigra; these were weighted down by short (2–3 mm) pieces of 500 μm diameter platinum wire. The slice was totally immersed in a continuously flowing solution at a rate of 1·5 ml/min at 36 °C. The solution contained (mM): NaCl, 126; KCl, 2·5; NaH_2PO_4, 1·2; $MgCl_2$, 1·3; $CaCl_2$, 2·4; glucose, 10; $NaHCO_3$, 26; and was saturated with 95% O_2 and 5% CO_2 at 36 °C. When cobalt chloride was added to the solution, NaH_2PO_4 was omitted.

The substantia nigra was identified in the slice using a dissection microscope and illumination from above. The zona compacta, from which all recordings were made, was taken to be the dorsal rim of the region of grey matter lying adjacent to the ventral surface of the mesencephalon, at least 1·5 mm lateral to the mid-line. This area was clearly separated from the more medial area of grey matter. These slices were then fixed in 4% formaldehyde and 0·5% glutaraldehyde in order to stain accessory optic tract. At the end of some experiments the position of the recording electrode was noted with reference to the position of the electron microscopy grid overlying the region of grey matter. These slices were then fixed in 4% formaldehyde and 0·5% glutaradehyde in order to stain for catecholamines (Furness, Costa & Wilson, 1977). When the slice was subsequently viewed with a fluorescence microscope, the region where recordings had been made was identifiable from the impression of the electron microscopy grid left on the surface of the fixed brain slice; recordings were made from an area of intense catecholamine fluorescence corresponding to the A9 region of dopamine-containing neurones described by Björklund & Lindvall (1984) using tyrosine hydroxylase antibody staining.

Electrical recording techniques

Intracellular recordings were made using glass microelectrodes filled with 3 M-KCl with resistance of 30–80 MΩ. Membrane potential was amplified with a WPI M707 amplifier; either a Dagan 8100 or Axoclamp 2 single-electrode voltage-clamp amplifier was used for both current- and voltage-clamp recordings. For current-clamp recordings, membrane potential was altered by passing current through the recording electrode using the active bridge circuit, with capacitance fully compensated and bridge balance monitored on a storage oscilloscope. Voltage-clamp recordings of membrane currents were made using a 2–3 kHz switching frequency and either a 25 or 33% duty cycle. Headstage current was continuously monitored on a separate oscilloscope. Membrane potentials and currents were recorded on Gould 2400 chart recorders. Steady-state current–voltage plots were obtained by hyperpolarizing neurones to between −110 and −130 mV and then depolarizing at a rate of 1 mV/s; membrane current was continuously plotted as a function of membrane potential using an X–Y plotter (Houston Instruments 200).

Drugs

Stock solutions of dopamine hydrochloride (Sigma) dissolved in 0·9% NaCl were prepared freshly before each experiment and kept on ice and gassed with nitrogen to prevent oxidation. Dopamine was also applied by pressure ejection from micropipettes containing dopamine (10 mM) with their tips (5–20 μm diameter) positioned in the superfusing solution above the slice surface. Reproducible responses to ejection of dopamine were achieved using pressure pulses of 10 lb/in^2 (1 lb/in^2 = 6·8 kPa) and 50–1000 ms duration (Picospritzer II, General Valve Corporation). Dopamine was also applied in known concentrations by changing the superfusion solution to one differing only in its content of dopamine. To minimize oxidative degradation, dilutions of the dopamine stock solutions in the oxygenated superfusion solution were not used more than 10 min after preparation. Solutions containing dopamine or other drugs entered the recording chamber within 30 s of turning a tap, the delay being necessary for passage of the solution through a heat exchanger; complete exchange of the bath solution occurred within 2·5 min. This method of application was also used for the following drugs: cocaine hydrochloride (Sigma), 4,4a,5,6,7,8,8a,9-octahydro-5-*n*-propyl-2H-pyrazolo-3,4-g quinoline (quinpirole; LY171555; Lilly), domperidone (Janssen), 2-[2-(1,4 benzodioxanyl)]-2-imidazoline HCl (RX781094; idazoxan; Reckitt & Colman), 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-3-benzazepine hydrocholoride (SKF38393; Smith, Kline and French), 7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol maleate (SCH 23390; Schering) and (−)-sulpiride (gift of Dr Forgione, Ravizza, Milan).

Numerical data are expressed as mean ± standard error of the mean.

RESULTS

Cell properties

Studies of the action of dopamine were based upon its application to 187 neurones within the substantia nigra zona compacta from which intracellular recordings were made, each recording being 0·3–6 h in duration. 142 of these neurones fired action potentials spontaneously at mostly regular rates which ranged from 0·2 to 5·6 Hz (mean $1·8 \pm 0·1$ Hz, 123 neurones). Several of these neurones were initially either quiescent or fired action potentials infrequently and irregularly, but following a short period (up to 15 min) of voltage or current clamping at -70 to -90 mV, they subsequently maintained regular spontaneous firing of action potentials when all applied current was removed. The threshold membrane potential for the generation of the spontaneous action potentials was $-56·2 \pm 0·67$ mV (sixty neurones); action potential discharge was readily prevented by 50–100 pA of applied hyperpolarizing current. The remaining forty-five neurones did not fire action potentials spontaneously and had resting membrane potentials of $-56·0 \pm 1·44$ mV (thirty-four neurones).

Input resistance was estimated in neurones in which spontaneous firing was prevented by passage of hyperpolarizing current. Superimposed hyperpolarizing pulses of currents of up to 100 pA and duration greater than 500 ms revealed a time-dependent inward membrane rectification. Input resistance measured at the peak of the electronic potential (usually within 200 ms of onset of the current pulse) was $186 \pm 7·5$ MΩ; at a steady-state level of hyperpolarization (at least 500 ms after the onset of the current pulse) the input resistance was $133 \pm 6·4$ MΩ ($n = 50$).

The action of dopamine

Dopamine, applied either by pressure ejection or in the superfusate, reduced the firing rate of those cells which were firing spontaneously (Fig. 1*A*). When cells were hyperpolarized by current injection so as to prevent firing, dopamine produced a membrane hyperpolarization accompanied by a fall in input resistance (Fig. 1*B*). Neurones which were not spontaneously active also exhibited a hyperpolarization accompanied by a fall in input resistance in response to dopamine. When applied to neurones that were voltage clamped at potentials between -58 and -78 mV, dopamine produced an outward membrane current (Fig. 1*C*). The hyperpolarizations and outward currents produced by dopamine reversed rapidly when the application was discontinued and could be elicited repeatedly in the cell throughout the period of impalement. However, in several instances repeated application of dopamine resulted in a cessation of cell firing which was not fully reversed either by superfusion with drug-free solutions for up to 20 min or by application of the D_2 receptor antagonist (−)-sulpiride (Kebabian & Calne, 1979).

When dopamine was applied by superfusion, the amplitude of the response was dependent on the concentration applied, whether reduction in firing rate, membrane hyperpolarization or outward current was used as the response (Figs 2 and 3). In experiments where high concentrations (100 μM or greater) of dopamine were applied, the response frequently exhibited desensitization. This was seen as a reduction in the amplitude of the hyperpolarization or outward current produced by dopamine while

it was still present in the superfusate (see Fig. 2*A*). The sensitivity to dopamine varied considerably from cell to cell (see Fig. 3). The largest hyperpolarization seen in this study was 18 mV and the largest outward current 260 pA. Cocaine hydrochloride (10 μM, a known blocker of neuronal reuptake of catecholamines (Axelrod, 1971) was frequently used to potentiate the response to dopamine although its actions

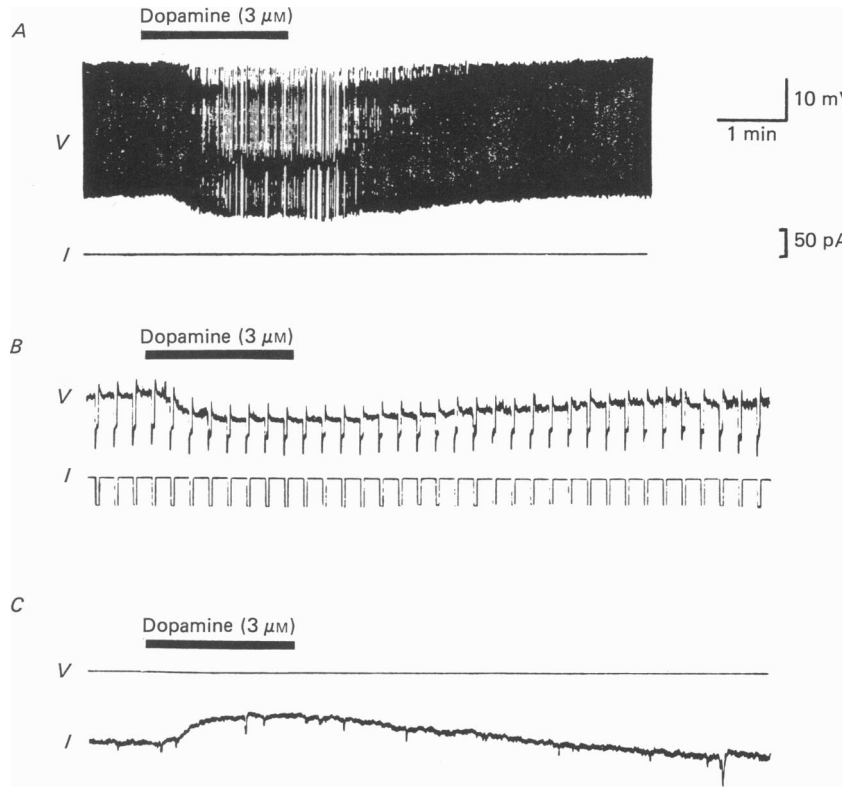

Fig. 1. Dopamine reduces firing rate (*A*), hyperpolarizes the membrane (*B*) and causes an outward current (*C*). Three pairs of records from the same neurone show membrane potential (*V*) and current (*I*). Dopamine (3 μM) was applied in the superfusate for the period indicated by the bars. *A*, at resting potential the cell is firing spontaneous action potentials (full amplitude not reproduced). Dopamine reduces the firing rate. *B*, membrane potential held at −63 mV by passing −120 pA direct current. Current pulses are 50 pA for 2 s. Dopamine hyperpolarizes the membrane by 6 mV, accompanied by a fall in input resistance. *C*, under voltage clamp at −63 mV, dopamine causes an outward current of 60 pA.

were not studied quantitatively. The desensitization phenomenon was also observed in the presence of cocaine, suggesting that it was not due to dopamine uptake. This concentration of cocaine had no obvious local anaesthetic action.

The ionic mechanism of the action of dopamine

The three experiments, hyperpolarizations were produced by repeated dopamine superfusions during prolonged conditioning potential changes: these reversed to

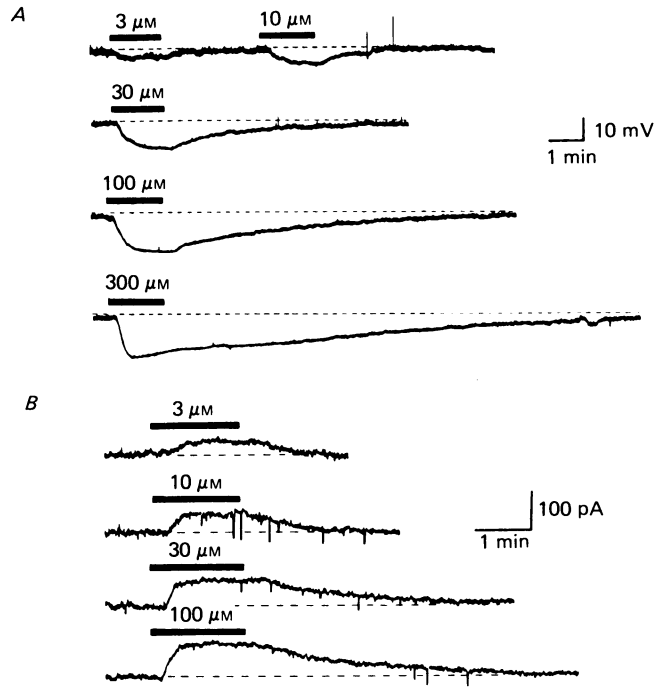

Fig. 2. Hyperpolarizations and outward currents caused by various concentrations of dopamine, in two different neurones. *A*, membrane potential. The superfusing solution contained dopamine during the periods indicated by the bars. Constant current was passed through the recording electrode of sufficient amplitude to prevent spontaneous firing and hold the membrane potential at −65 mV (dashed line) in the absence of dopamine. The four traces are continuous. *B*, membrane current. Holding potential was −67 mV. Dashed line indicates −120 pA.

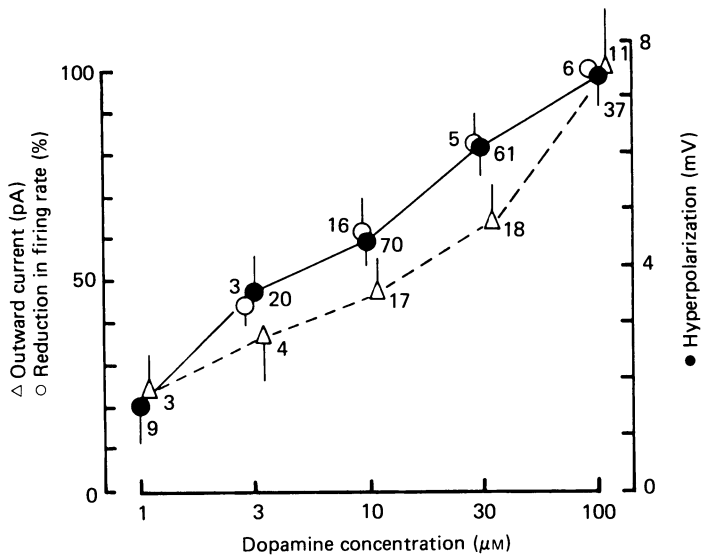

Fig. 3. Effects of dopamine as a function of the concentration applied. ○, inhibition of firing rate; ●, membrane hyperpolarization; △, outward current (holding at −58 to −70 mV). Points show mean effects and vertical bars are S.E. of mean, for the numbers of neurones indicated.

depolarizations at $-109{\cdot}2 \pm 4{\cdot}7$ mV. Steady-state current–voltage plots in the presence and absence of dopamine in the superfusate were made in three further experiments. The outward current produced by dopamine was accompanied by an increase in membrane slope conductance (Fig. 4), indicating that it was not likely to result from the reduction of a tonic inward current. These current–voltage plots also demonstrated that the outward current produced by dopamine became smaller as the membrane was hyperpolarized from -60 mV, reversing in polarity to become inward at $-109{\cdot}0 \pm 4{\cdot}4$ mV (three cells). A similar dependence of the amplitude of the dopamine response upon membrane potential was observed in experiments where membrane hyperpolarizations or outward currents were evoked by pressure ejection of dopamine (Fig. 5*A*). For voltage recordings, the dopamine potential change reversed at -103 and -105 mV in two cells; for current recordings the dopamine-induced current turned from outward to inward at $-113{\cdot}4 \pm 1{\cdot}5$ (four cells). The reversal potential of the responses to dopamine, studied in these four different ways, was $-109{\cdot}7 \pm 1{\cdot}7$ mV (twelve cells).

The above results were obtained with a potassium concentration of 2·5 mM. In four of these twelve cells the dependence of the dopamine reversal potential upon the concentration of potassium ions was examined; the dopamine reversal potential in 10·5 mM-potassium was $-74 \pm 5{\cdot}0$ mV. The results of the experiment illustrated in Fig. 5 demonstrated that for intermediate potassium concentrations, the reversal potential of the dopamine current shifted to less negative values as the concentration of potassium ions in the superfusate was increased. In this experiment, the plot of the reversal potential against the logarithm of the potassium ion concentration gave a straight line with a slope of 55·8 mV per log unit of potassium ion concentration (Fig. 5*B*). In three other cells, dopamine reversal potentials in two or more different concentrations of potassium gave slopes of 61·0, 63·5 and 59·2 mV per log unit of potassium ion concentration, respectively. These values are close to that predicted by the Nernst equation for a selective increase in potassium conductance (60 at 36 °C).

Tetraethylammonium (TEA) at concentrations of 1 and 3 mM produced increases of up to 40% in the outward current caused by dopamine (three cells). TEA also caused an increase in membrane resistance accompanied by a small depolarization, an increase in the frequency of spontaneous firing and, under voltage clamp, an inward current. At concentrations of 10–30 mM, TEA produced decreases in the amplitude of the action potential after-hyperpolarization and increases in action potential duration; the net effect was a reduction of firing rate. These higher concentrations reversibly depressed the amplitude of the outward current evoked by dopamine (Fig. 6); (in 10 mM-TEA, the reduction was to $40 \pm 5{\cdot}1$% (eight cells) of control amplitude). Barium (100–300 μM) also reversibly reduced the dopamine outward current by as much as 80% in two cells. These concentrations of barium additionally produced an inward membrane current, and, in current clamp, a depolarization occurred accompanied by an increase in input resistance, action potential duration and firing frequency.

Tetrodotoxin (TTX, 1 μM) rapidly blocked the spontaneous firing of action potentials, revealing oscillations of membrane potential of 3–20 mV peak-to-peak amplitude in eight out of fourteen cells (Fig. 7). These TTX-insensitive oscillations ranged in frequency between 0·3 and 3 Hz (mean $1{\cdot}4 \pm 0{\cdot}5$ Hz, $n = 5$), and were

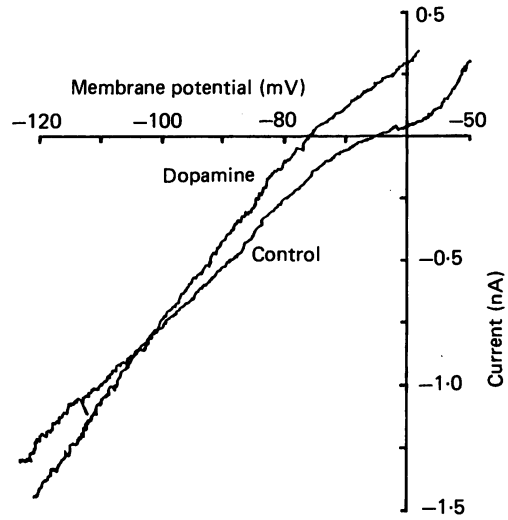

Fig. 4. Current–voltage relation before and during application of dopamine. The two current–voltage plots were constructed by depolarizing the same neurone from −120 to −50 mV at a rate of 1 mV/s while measuring membrane current. Dopamine concentration was 30 μM (cocaine (10 μM) also present throughout). Dopamine current reverses from outward to inward at about −100 mV (potassium concentration was 2·5 mM); note increase in conductance through the voltage range.

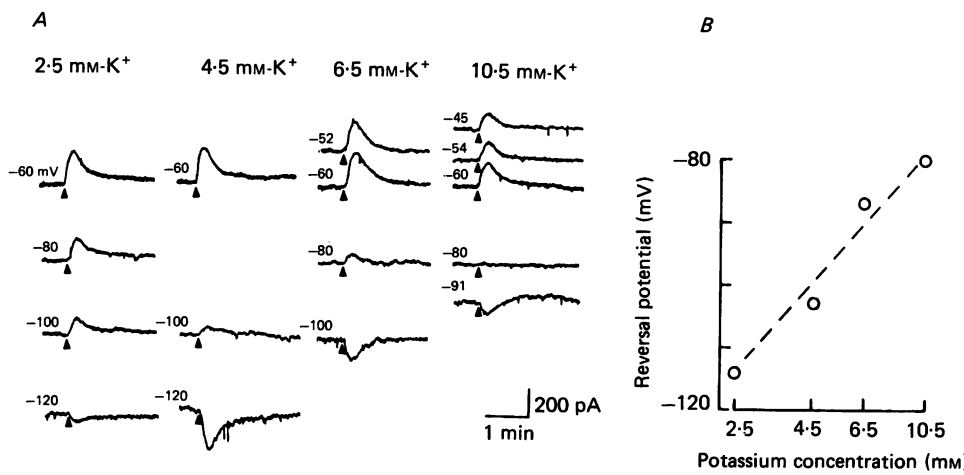

Fig. 5. Reversal of dopamine current in various potassium concentrations. *A*, records of membrane current from a single cell during pressure application of dopamine (triangles, 68 kPa for 100 ms). Holding potential is indicated beside each trace. In normal potassium concentration, the dopamine-induced current reversed from outward to inward at about −115 mV. When the potassium concentration was raised, the reversal potential became less negative. *B*, reversal potential of dopamine current (from *A*) plotted as a function of the logarithm of the potassium concentration. Line, fitted by least-squares method, has slope of 56 mV per tenfold change in potassium concentration.

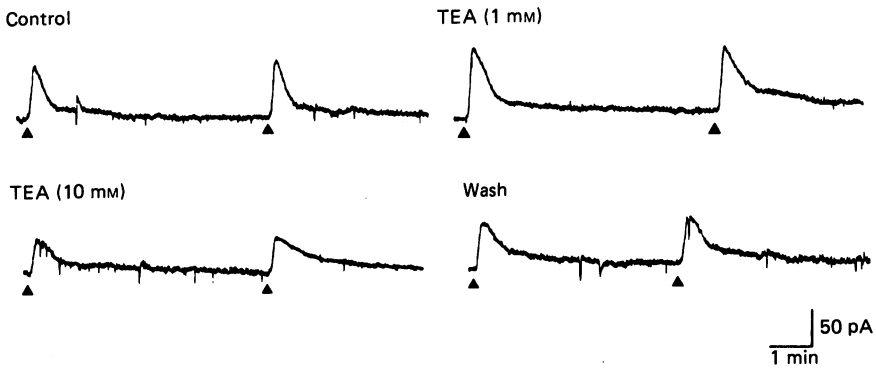

Fig. 6. Dopamine-induced current is insensitive to tetraethylammonium (TEA). Four records of membrane current are shown from a single cell. In each case, dopamine was applied twice (triangles, 68 kPa for 500 ms). TEA (1 mM) increased the amplitude of the current; this concentration of TEA caused a shift in holding current of 15 pA. At 10 mM, TEA reduced the amplitude of the dopamine current, but caused no further shift in holding current. The effects of TEA reversed partially after 30 min washing. Holding potential was −62 mV throughout.

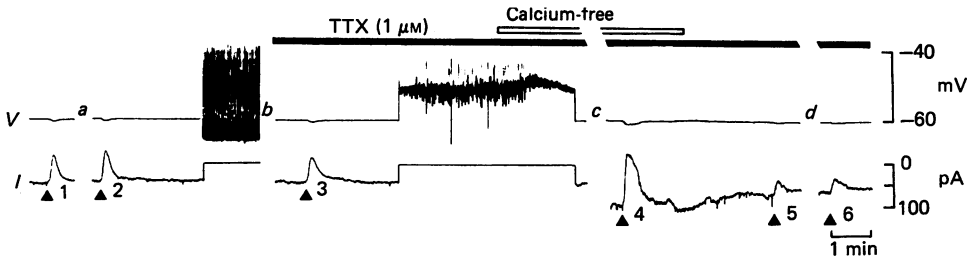

Fig. 7. Dopamine-induced current persists in tetrodotoxin (TTX) and 'calcium-free' solutions. Records are membrane potential (V) and membrane current (I) from a single cell; five segments of record are shown (breaks indicated *a–d*). Dopamine was applied by pressure ejection (numbered triangles, 68 kPa for 300 ms). 1 and 2, control applications under voltage clamp at −60 mV. Break at *a* is 3·5 min. After application 2, the voltage clamp was removed and the cell was allowed to fire spontaneously (full height of action potentials are not reproduced). Break at *b* is 7 min, and during this period voltage clamp was restored, and TTX (1 μM) superfusion was begun (filled bar). 3, after 4 min in TTX the response to dopamine was almost unchanged. Voltage clamp was released again, and spontaneous 'oscillations' of membrane potential occurred. The superfusing solution was next changed to one containing TTX which was also 'calcium-free' (open bar; actual composition was no added calcium, 0·5 mM-EGTA, and 2 mM-cobalt). This quickly blocked the spontaneous oscillations of potential. Break at *c* is 2 min, during which voltage clamp was restored. 4, dopamine-induced current was initially enhanced in 'calcium-free' solution, but later depressed (application 5), despite wash-out of 'calcium-free' solution. Break at *d* is 6 min. The control response to dopamine was not recovered after 8 min washing (application 6), although the spontaneous oscillations did recover (not shown).

abolished within 1–2 min by cobalt (0·5–2 mM, four cells) or calcium-free solution (Fig. 7). Furthermore, spontaneous action potentials were also completely abolished by cobalt (0·5–2 mM, six cells) in the absence of TTX. It therefore appeared that there were independent TTX- and cobalt-sensitive mechanisms underlying cell firing, but that calcium currents were an essential prerequisite for spontaneous regenerative depolarizations whether or not TTX was present.

Hyperpolarizations and outward currents caused by dopamine were unaffected by TTX (1 μM, thirteen neurones; Fig. 7). Applications of cobalt (0·5–2 mM) for 4–10 min reduced dopamine hyperpolarization by 24 ± 28 % (six cells), with the effect on the dopamine response ranging from a 100 % inhibition to a 70 % increase. When spontaneously occurring depolarizations were blocked with a solution containing zero calcium, ethylene-bis-(oxyethylenenitrilo) tetraacetic acid (EGTA, 0·5 mM) and cobalt (0·5–2 mM), the outward current produced by dopamine was initially increased, then decreased, but not abolished during 5 min of such treatment (Fig. 7). The reduction of dopamine hyperpolarizations or outward currents did not reverse following wash-out of the cobalt-containing solutions, although spontaneous regenerative depolarizations did exhibit partial recovery in some cells. The failure of these cobalt and 'calcium-free' solutions to abolish the action of dopamine implies that calcium entry, either into the neurone under study, or into presynaptic terminals releasing transmitter onto that neurone, is not necessary for dopamine to increase the membrane potassium conductance.

The type of dopamine receptor

Quinpirole (LY171555, 100 nM–3 μM), the selective D_2 receptor agonist (Tsuruta, Frey, Grewe, Cote, Eskay & Kebabian, 1981), was applied by superfusion to thirteen neurones. It caused concentration-dependent hyperpolarizations accompanied by falls in input resistance, effects which were sustained during applications of up to 10 min. The mean amplitude of the hyperpolarization caused by 1 μM-quinpirole was 10 ± 2·0 mV (five cells), the largest being 16 mV. The effects of quinpirole reversed in 15–30 min when superfusion was discontinued, which was considerably longer than the time required for reversal of the effects of dopamine (Fig. 8).

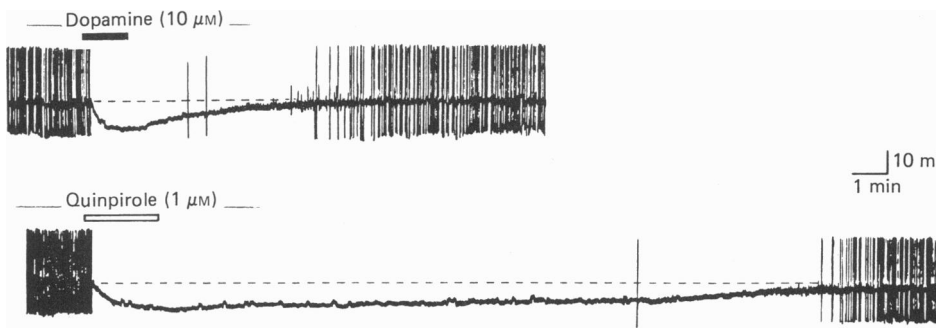

Fig. 8. Selective D_2 receptor agonist quinpirole mimicks the action of dopamine. Both records are from the same cell. Note the long duration of the hyperpolarization caused by a 2 min application of quinpirole. Dashed line indicates 56 mV.

Dopamine hyperpolarizations were antagonized by superfusion of domperidone (100 nM–1 μM, four cells). However, in no case was any response to dopamine observed following wash-out of domperidone for 45 min or more. Dopamine hyperpolarizations and outward currents recorded from twenty neurones were studied during superfusion of (−)-sulpiride at concentrations of 10 nM–1 μM. (−)-Sulpiride reduced the dopamine response, and this was surmountable in that the response amplitude

could be restored by increasing the dopamine concentration. In two cells, it was possible to maintain the recording for 60–80 min following wash-out of (−)-sulpiride; in these cases the initial sensitivity to dopamine recovered. Concentration–response curves for either dopamine currents or hyperpolarizations were determined in seven neurones before and after addition of at least one concentration of sulpiride. The experiments were carried out in the presence of cocaine (10 μM). If a competitive interaction is assumed to take place between dopamine and (−)-sulpiride at receptors on these neurones, then the dissociation equilibrium constant (K_D) can be calculated from $(DR-1) = [B]/K_D$ where [B] is the sulpiride concentration and DR is the ratio of concentration of dopamine required to produce a given response in the presence and in the absence of sulpiride. The K_D for sulpiride (at 30 nM) was 11·0 ± 4·1 nM (five cells), and at concentrations of 100 nM or greater the K_D was 47·3 ± 14·7 nM (four cells).

In a further experiment on a single cell, dopamine dose–response curves were constructed in the absence and then in the presence of four different concentrations of (−)-sulpiride; cocaine (10 μM) was present throughout (Fig. 9). Surmountable antagonism of the dopamine outward curve current by (−)-sulpiride was evident, but the Schild transformation of these results (Fig. 9*C*) suggests that the antagonism was competitive only when the sulpiride concentration was 100 nM or greater. The most likely explanation for the non-linear Schild plot was considered to be related to uptake of the applied agonist (see Furchgott, 1972; Kenakin, 1984; Pinnock, 1984). Cocaine produced an outward current (45 pA) which was reversed by (−)-sulpiride, presumably because it blocked the uptake of an unknown quantity of endogenous dopamine; the effect of this unknown amount of endogenous dopamine interferes with quantitative analysis of the responses to known added concentrations (see Discussion). An estimate of the sulpiride K_D from the more linear part of the Schild plot (which had a slope of 0·95) gave a value of approximately 52 nM.

In a further experiment, increasing concentrations of quinpirole were applied cumulatively to generate a concentration–response curve (Fig. 9*D*). This was done in the absence of cocaine (cocaine did not change the effect of quinpirole). After 40 min of washing, to reverse the effect of quinpirole, (−)-sulpiride was added to the superfusion solution and two further concentrations of quinpirole were applied; this procedure was repeated for two further concentrations of (−)-sulpiride. Figure 9*D* shows the result of this experiment, in which the shift to the right of the quinpirole dose–response curve appeared to be parallel. The Schild plot had a slope of 1·0, consistent with competitive antagonism, and the K_D for (−)-sulpiride was 13 nM (Fig. 9*E*).

SKF38393, a selective D_1 receptor agonist (Stoof & Kebabian, 1981), was superfused at concentrations of 10 or 30 μM ($n = 6$). No effect on membrane potential or firing rate was seen in four cells, but in two cells a small reduction in firing rate was seen; in one of these the hyperpolarization was reversed by (−)-sulpiride (100 nM). Two cells were also tested with a very high concentration (100 μM) of SKF38393. This caused a small reduction in firing rate of one cell and, in the presence of domperidone (1 μM), had no effect on the second cell. It would therefore appear that SKF38393 is without effect except at very high concentrations, at which it rather weakly mimics the effect of dopamine.

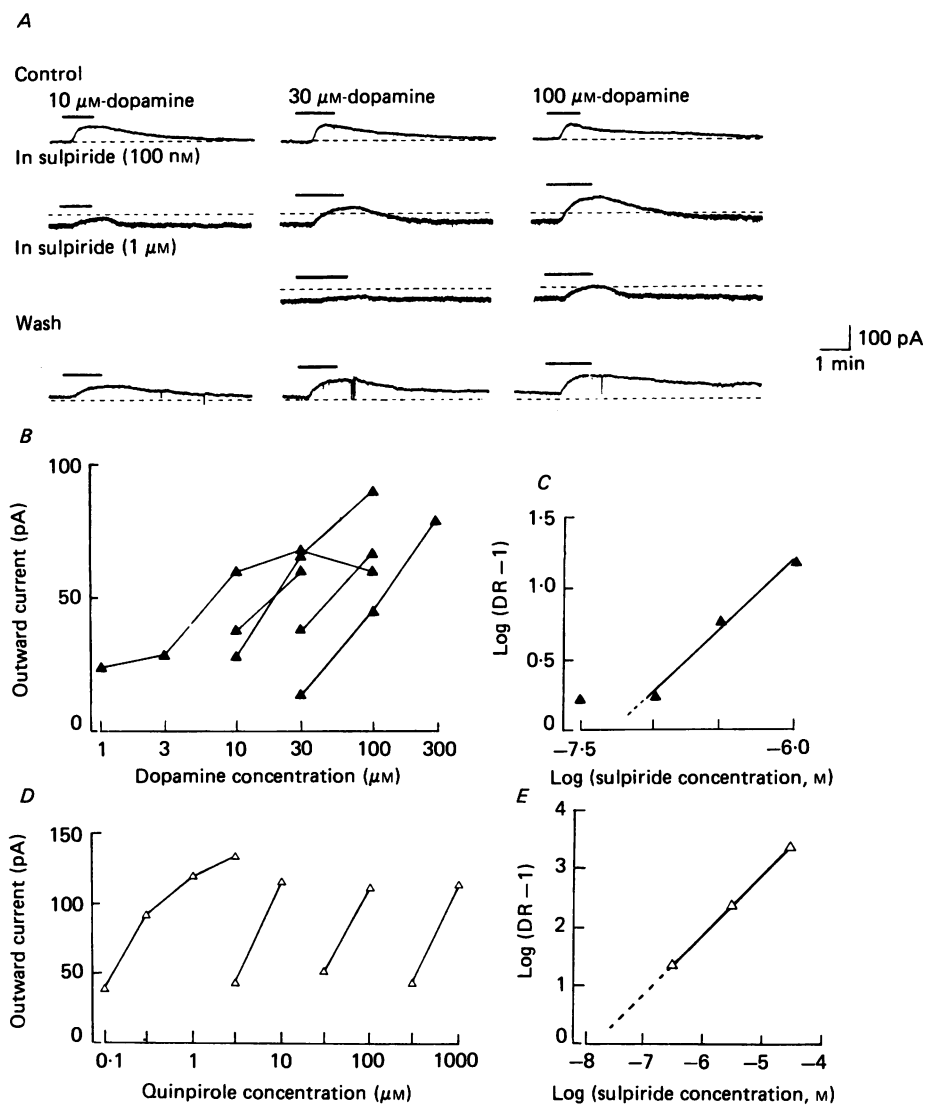

Fig. 9. (−)-Sulpiride antagonizes the action of dopamine and quinpirole. *A*, selected records from one cell of outward currents produced by superfusion of dopamine in the concentrations indicated. Upper row, controls. Second row, in (−)-sulpiride (100 nM). Third row, in (−)-sulpiride (1 μM). Bottom row, after washing out (−)-sulpiride. Cocaine (10 μM) was present throughout; note the inward current produced by (−)-sulpiride. Holding potential −57 mV. Dashed line indicates zero current. *B*, concentration–response curve for all the applications of dopamine made to the cell shown in *A*. *C*, Schild transformation of data in *B* measured at a response level of 50 pA. The linear portion of the Schild plot (omitting the value at 30 nM) has a slope of 0·95 and indicates a value of 52 nM for the equilibrium dissociation constant for (−)-sulpiride. *D*, concentration–response curves for outward currents caused by quinpirole in the absence and presence of various concentrations of (−)-sulpiride (a different cell to that shown in *A–C*). Quinpirole concentration was increased cumulatively for the control applications and within each (−)-sulpiride concentration, but quinpirole was washed out after control and between each sulpiride concentration. *E*, Schild plot for data in *D* measured at response level of 80 pA. The line has a slope of 1·0 and provides a value of 13 nM for the equilibrium dissociation constant for (−)-sulpiride.

SCH23390, a selective D_1 receptor antagonist (Iorio, Barnett, Leitz, Houser & Korduba, 1983) had no effect on the firing rate or membrane potential of seven neurones at concentrations of 100 nM–10 μM. Dopamine hyperpolarizations and outward currents in these neurones were unaffected by SCH22390 at concentrations less than 10 μM; 10 μM-SCH22390 produced small reductions in the dopamine hyperpolarization in two out of five cells tested.

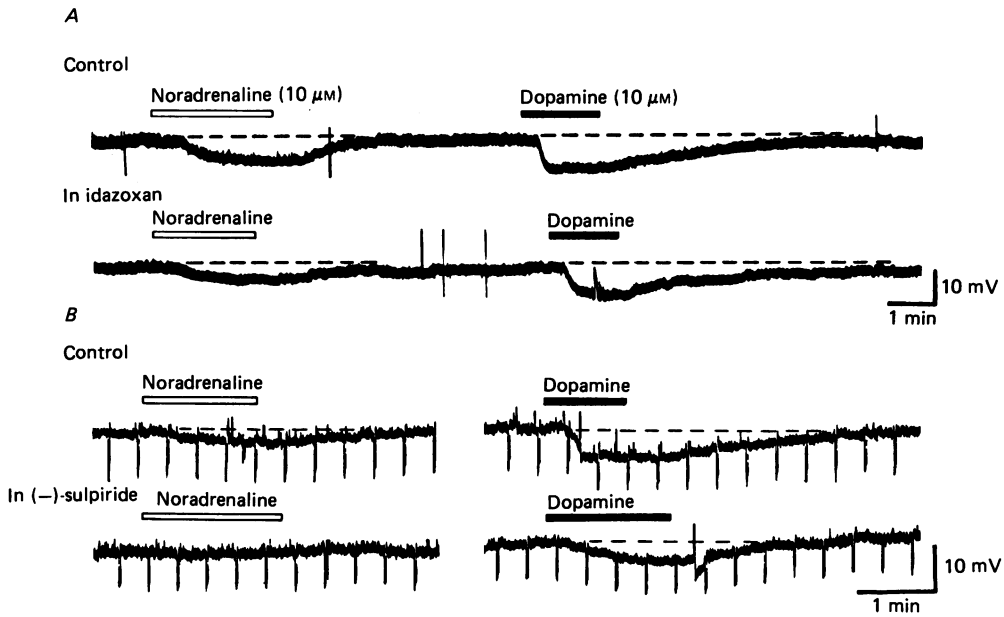

Fig. 10. Noradrenaline acts on dopamine receptors. *A*, both noradrenaline and dopamine hyperpolarize the neurone, although dopamine is more potent. Idazoxan (1 μM) reduces only slightly the response to noradrenaline, and also reduces the response to dopamine. This concentration of idazoxan is about 100 times greater than its dissociation equilibrium constant at α_2-adrenoceptors. Dashed lines indicate −59 mV. *B*, another cell which was hyperpolarized by both noradrenaline and dopamine. (−)-Sulpiride (30 nM) completely blocked the response to noradrenaline and also reduces the effect of dopamine. Dashed line indicates 63 mV.

Noradrenaline (10 μM) produced hyperpolarizations in four cells; these were unaffected by idazoxan (1 μM, Fig. 10*A*), the selective α_2-adrenoceptor antagonist (Doxey, Roach & Smith, 1983), but blocked by (−)-sulpiride (30–300 nM, three cells; Fig. 10*B*). Responses to dopamine were unaffected by idazoxan (1 μM) in two cells tested (Fig. 10*A*).

DISCUSSION

Dopamine increases potassium conductance

The critical evidence that dopamine increases the potassium conductance of substantia nigra neurones is the finding that the potential change or current flow caused by dopamine reverses its polarity at a membrane potential which changes according to the Nernst equation when the external potassium concentration is altered (Fig. 5). Other findings which are consistent with this interpretation are (1) the increase in

membrane conductance (Figs 1 and 4) and (2) the reduction of the response by barium and high concentrations of TEA (Fig. 6). On the basis of the present results, it is possible to say that a significant part of the action of dopamine occurs at a part of the neurone which is electrotonically close to the site of recording (which was within the zona compacta and, presumably, in the cell soma). One cannot readily determine if a part of the effect of dopamine also takes place at electrotonically more distant sites such as dendrites reaching into the pars reticulata. Such a dendritic component might account for the variation of reversal potential among cells. Also compatible with this interpretation is the finding that low concentrations of TEA (which increased input resistance and may serve to make the neurone more electrotonically compact) potentiated the current evoked by dopamine.

TTX blocked spontaneous action potentials, but its lack of effect on dopamine hyperpolarizations and outward currents suggests that the action of dopamine on the neurones is direct. If dopamine was releasing another hyperpolarizing transmitter from surrounding neurones, then such release must be TTX insensitive. Superfusion with cobalt and 'calcium-free' solutions (containing 0·5 mM-EGTA and 0·5–2 mM-cobalt) rapidly blocked rhythmic depolarizations both in the presence and absence of TTX (Fig. 7). If it is assumed that such solutions would block transmitter release, the failure to block the dopamine outward current is evidence for a direct action of dopamine. The independence of the dopamine outward current on calcium entry also indicates that the dopamine-operated potassium conductance does not depend on any inward calcium current in the short term.

It has been suggested (Grace & Bunney, 1985) that the cessation of firing and accompanying hyperpolarization of substantia nigra compacta neurones following systematic administration of apomorphine *in vivo* was the result of blockade of an inward current. It was proposed that the slow depolarization preceding spontaneous action potentials was primarily due to sodium influx and that it was through blockade of this conductance that apomorphine had its effect (Grace & Bunney, 1985). The present study indicates that an enhancement of an outward potassium current is a more likely interpretation of these results.

Dopamine acts on D_2 receptors

The inability of the selective D_1 antagonist SCH23390 to reduce significantly dopamine outward currents or hyperpolarizations at concentrations up to 10 μM indicates a lack of involvement of D_1 receptors in this effect. Stimulation of adenylate cyclase in rat striatal homogenates by D_1 receptor agonists is blocked by SCH23390 with the concentrations producing half-maximal inhibition (IC_{50}) being 10 or 4 nM (Iorio *et al.* 1983; Hyttel, 1984). The K_D for [^{3}H]SCH23390-specific binding in the striatum has been reported as being 2 nM (Dawson, Gehlert, McCabe, Barnett & Walmsley, 1986; Savasta, Dubois & Scatton, 1986); thus the concentrations of SCH23390 used in the present study should occupy 99·99% of D_1 receptors. The [^{3}H]SCH23390 binding sites in rat substantia nigra (Dawson *et al.* 1986) therefore may not reside on the principal cells of the zona compacta, or their occupancy by agonists may have effects which do not obviously change the membrane properties of the neurones.

The lack of effect of the selective D_1 receptor agonist SKF38393 at concentrations

up to 10 μM supports this conclusion. The effective concentrations of this compound at responses mediated by D_1 receptors are lower than this. (Increased efflux of cyclic adenosine 3′,5′-monophosphate from blocks of rat striatum produced by SKF38393 was half-maximal at a concentration of 200 nM (Stoof & Kebabian, 1981), stimulation of neurone-specific synaptic vesicle proteins in rat pituitary gland by SKF38393 was maximal at 5 μM (Treiman & Greengard, 1985) and adenylate cyclase stimulation in carp retinae by SKF38393 was half-maximal at 1 μM (Watling & Dowling, 1981).) The small effects seen at the higher concentrations used in the present study may result from an action at D_2 receptors. Our failure to find effects attributable to D_1 receptor activation is consistent with receptor distributions following lesions of the intrinsic neurones of the striatum or 6-hydroxydopamine lesions of dopamine-containing neurones of the substantia nigra. Nigral lesions markedly reduce nigral D_2 receptor binding (Quik, Emson & Joyce, 1979; Walmsley, Filloux, Gehlert & Dawson, 1986, but leave D_1 receptor-stimulated adenylate cyclase (Gale, Guidotti & Costa, 1977; Quik *et al.* 1979) and binding density (Walmsley *et al.* 1986) unaffected. Conversely, striatal lesions reduce D_1 receptor-stimulated cyclase (Gale *et al.* 1977; Quik *et al.* 1979) and D_1 receptor binding (Walmsley *et al.* 1986) in the ipsilateral substantia nigra, leaving D_2 receptor binding unaffected (Quik *et al.* 1979; Walmsley *et al.* 1986). These studies all indicate that nigral D_2 receptors are solely on dopamine-containing neurones which are themselves devoid of D_1 receptors, most of which reside on terminals of neurones projecting from the striatum to the nigra.

Quinpirole, which is a selective D_2 receptor agonist (Tsuruta *et al.* 1981), mimicked the action of dopamine at concentrations of 100 nM–3 μM. The effective concentrations can be compared with those which cause half-maximal inhibition of [^{3}H]acetylcholine release from striatal slices (55 nM: Hyttel, 1984), inhibition of stimulated [^{3}H]dopamine release from rabbit retinae (about 100 nM: Dubocovich & Hensler, 1986), or inhibition of adenylate cyclase activity in striatum (3·5 μM: Onali, Olianas & Gessa, 1985).

Efforts to determine the antagonist K_D for (−)-sulpiride met with two difficulties. Because cocaine increased the amplitude of the response to dopamine it must be assumed that, in the absence of cocaine, the concentration of dopamine close to the receptor is less than that in the superfusion solution. The inclusion of cocaine in such experiments is therefore necessary, but unfortunately introduces further complications. The cocaine itself produces an effect by preventing reuptake of endogenously released dopamine. It can never be known if a given concentration of cocaine totally blocks the uptake of added dopamine; indeed, there may be significant (non-neuronal) uptake which is insensitive to cocaine. Pinnock (1984) met with similar difficulties in his efforts to apply Schild analysis to the results of experiments using extracellular recording from nigral neurones and dopamine as the agonist.

To avoid the problems of uptake, quinpirole was used as the agonist. In this case, a semicumulative method of applying the agonist concentrations was necessary because the effect of quinpirole was slow to reverse on washing. None the less, the Schild plot from this experiment (Fig. 9*E*) was consistent with simple competition between quinpirole and sulpiride. The estimates of K_D for (−)-sulpiride obtained in the present study (10–50 nM) compare favourably with that seen in studies reporting K_D values of 27 nM (Theodorou, Crockett, Jenner & Marsden, 1979) and 7 nM

(Woodruff & Freedman, 1981) for [^{3}H]sulpiride binding to brain membranes. Thus, although the attempts to determine antagonist K_D in our experiments were less than fully satisfactory, there remains little doubt that the receptor involved is the D_2 type.

Hyperpolarizing responses to dopamine have been reported previously in a variety of cells (*Aplysia* neurones (Ascher, 1972; Sawada, Enomoto, Maeno & Blankenship, 1980), *Lymnaea stagnalis* neurones (de Vlieger, Lodder, Stoof & Werkman, 1986), hippocampal pyramidal cells (Benardo & Prince, 1982*a*, *b*) and neurones of the nucleus accumbens (Uchimura, Higashi & Nishi, 1986)) which in most cases were attributable to an increase in potassium conductance. Only the effect reported by de Vlieger *et al.* (1986) was pharmacologically comparable to that of the present study because it was blocked by (−)-sulpiride (1 μM). The results of Benardo & Prince (1982*b*) and of Uchimura *et al.* (1986) suggested the involvement of D_1 receptors in the dopamine-induced hyperpolarization. Uchimura *et al.* (1986) additionally found that dopamine *decreased* the potassium conductance of some nucleus accumbens neurones, and that this was blocked by (−)-sulpiride.

Functional implications

The significance of the dopamine-mediated inhibition of firing of zona compacta neurones in the overall extrapyramidal control of movement has been extensively discussed since the original findings of Bunney *et al.* (1973*a*). The likely source of the dopamine under physiological conditions is dendrites of the same or neighbouring substantia nigra neurones (Cheramy *et al.* 1981). What then are the further implications of the present findings that the mechanism of inhibition is a hyperpolarization resulting from an increase in membrane potassium conductance? First, this hyperpolarization prevents spontaneous firing of action potentials, both in the presence and absence of TTX. However, it appears that these neurones are capable of generating action potentials in regions other than the cell soma (Llinás *et al.* 1984; Kita *et al.* 1986) and one such phenomenon, the calcium-dependent 'low-threshold spike', is made more likely by hyperpolarization (Llinás *et al.* 1984; and our unpublished observations), presumably by the removal of inactivation. Although we have not observed that such spikes are induced by dopamine during the hyperpolarization which it produces while recording from the cell soma, such spikes might be occurring undetected in electrotonically distant regions of the cell such as the dendrites extending into the zona reticulata. The removal of inactivation of a calcium current by the dopamine hyperpolarization would be expected to influence neuronal discharge in response to excitatory inputs as seen, for example, in the thalamus (Jahnsen & Llinás, 1984) following hyperpolarization by acetylcholine (McCormick & Prince, 1986).

The second functional implication relates to mechanisms of transmitter release from the nigral neurones. The finding that the D_2 receptor is linked to a potassium conductance in the neuronal somata most likely implies that the same linkage occurs elsewhere in the neurone. D_2 receptors are known to be present on the terminals of the nigral cells in the striatum, where their activation inhibits the release of dopamine (Starke, Reimann, Zumstein & Hertting, 1978). Testing of excitability of the striatal terminal regions of nigral neurones indicates that D_2 receptor agonists cause localized

hyperpolarization (Tepper, Nakamura, Young & Groves, 1984). It is likely that transmitter release is reduced because of the increase in membrane potassium conductance. Dopamine is also released from nigral dendrites by depolarization in a calcium-dependent manner (Geffen, Jessell, Cuello & Iversen, 1976) and the potassium conductance increase induced by dopamine would be expected to inhibit this. However, if the 'low-threshold spike' contributes to the dendritic release of dopamine, a hyperpolarization might *enhance* release, particularly if accompanied by excitatory afferent input.

The results and interpretations of the present study invite comparisons with similar findings on the neurones of the other major monoamine-containing nuclei, the locus coeruleus and raphe dorsalis. As in the substantia nigra zona compacta, the principal transmitter synthesized by those neurones is able to act directly on the neurones themselves and inhibit firing through a hyperpolarization. These hyperpolarizations (brought about by activation of α_2-adrenoceptors and 5-hydroxytryptamine (5-HT_1) receptors) also result from an increase in potassium conductance (Egan, Henderson, North & Williams, 1983; Yoshimura & Higashi, 1985; Williams, Henderson & North, 1985; Sprouse & Aghajanian, 1987). Other similarities between these three monoamine systems are evident from studies of the actions of their respective transmitters upon their own release from nerve terminals *in vitro*. As for dopamine, noradrenaline (Starke & Montel, 1973; Wemer, van der Lugt, de Langen & Mulder, 1979) and 5-hydroxytryptamine (Mounsey, Brady, Carroll, Fisher & Middlemiss, 1982; Middlemiss, 1985), acting on receptors identical or very similar to those involved in somatic hyperpolarizations, will inhibit transmitter release. Furthermore, the decrease in terminal excitability in noradrenergic neurones following local infusion of α_2-adrenoceptor agonists suggests, as in the case of dopamine in the nigro-striatal pathway, that noradrenaline hyperpolarizes the terminals of noradrenergic nerves (Nakamura, Tepper, Young & Groves, 1981).

A coherent picture of self-modulation through receptors relatively specific for each of dopamine, noradrenaline and 5-hydroxytryptamine, positively coupled to a potassium conductance increase, is emerging for monoaminergic central neurones. Activation of these receptors (sometimes referred to as 'autoreceptors') will inhibit transmitter release at nerve terminals ('presynaptic inhibition') and, following dendritic release of transmitter, inhibit firing at the level of the cell body ('autoinhibition'). The present study places firmly within this scheme the D_2 receptor and the inhibitory mechanism in substantia nigra zona compacta neurones to which it is coupled.

This work was supported by grants from the U.S. Department of Health and Human Services MH40416 and DA03161.

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