Responses of rat substantia nigra compacta neurones to L-DOPA

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- 1 The electrophysiological actions of L-DOPA were studied on substantia nigra dopamine-containing neurones by use of intracellular recordings, current and voltage clamp, *in vitro*.
- 2 L-DOPA (3–300 μM), applied by superfusion, decreased the spontaneous firing activity of the neurones, hyperpolarized the membrane potential and produced an outward current.
- 3 These effects of L-DOPA were graded and were antagonized by sulpiride, a D_2 -receptor antagonist.
- 4 The L-DOPA activated membrane hyperpolarization persisted in slices exposed to tetrodotoxin while it was markedly reduced or abolished in solutions with no calcium plus cobalt (2 mM).
- 5 In slices treated with carbidopa, an inhibitor of the aromatic amino acid decarboxylase enzyme, the actions of L-DOPA were greatly reduced.
- 6 We propose that L-DOPA inhibits the firing of substantia nigra zona compacta neurones by being converted to dopamine which is then released by these cells.

Introduction

It is now more than 20 years since L-DOPA was shown to be the most suitable treatment for Parkinson's disease (Cotzias *et al.*, 1969; Barbeau, 1981; Juncos *et al.*, 1987). It is generally believed that the clinical benefits produced by the administration of L-DOPA are due to the drug-induced release of dopamine from the surviving nigro-striatal neurones (Hornykiewicz, 1966; 1973; Melamed *et al.*, 1980; 1984). Previous studies have shown that L-DOPA increases the efflux of dopamine in the striatum (Ng *et al.*, 1970; Hefti & Melamed, 1981; Ponzio *et al.*, 1983; Zetterstrom *et al.*, 1986; Snyder & Zigmond, 1987; Brannan *et al.*, 1989).

An increase in the intracellular and extracellular content of dopamine in the substantia nigra zona compacta has also been reported following the administration of L-DOPA in rats (Bunney *et al.*, 1973; Robertson & Robertson, 1988). It is well known that dopamine, released locally from cell dendrites (Geffen *et al.*, 1976; Korf *et al.*, 1976; Nieuillon *et al.*, 1977; Cheramy *et al.*, 1981), interacts with receptors located on the soma and dendrites of dopamine-containing neurones of the substantia nigra zona compacta, decreasing firing rate by hyperpolarizing the cellular membrane (Aghajanian & Bunney, 1973; 1977; Pinnock, 1983; Lacey *et al.*, 1987).

Bunney *et al.* (1973) have reported that systemic application of L-DOPA reduced the extracellular unitary discharge of rat dopaminergic neurones. However, the cellular mechanisms underlying the neuronal inhibition induced by L-DOPA are still unclear. In this study we have therefore examined in isolation the actions of L-DOPA on dopaminergic neurones using an *in vitro* slice preparation from which we obtained intracellular recordings.

Methods

The methods used have been described previously by Lacey *et al.* (1987). Albino Wistar rats (150–350 g) of either sex were killed by a blow on the chest under ether anaesthesia. The brain was quickly removed and slices of the mesencephalon (300–350 μm thick) which contained the substantia nigra were cut by an Oxford vibratome. A single slice was then trans-

ferred to a recording chamber and continuously superfused at a rate of 2.5 ml min^{-1} with a solution maintained at 36°C and gassed with 95% O_2 /5% CO_2 . The standard solution contained (mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, MgCl_2 1.2, CaCl_2 2.4, glucose 10, NaHCO_3 26. When CoCl_2 (2 mM) was added to the solution NaH_2PO_4 was omitted.

Intracellular recording electrodes were filled with 2 M KCl and had a resistance of 30–100 M Ω . A single electrode voltage clamp amplifier was used (Axoclamp 2A) for both voltage and current recordings. Voltage clamp recordings were made using 2–3.5 Hz switching frequency, while the headstage signal was continuously monitored on a separate oscilloscope. Membrane potentials and currents were displayed on chart by a Gould RS 3400 chart recorder.

Drugs were applied by superfusion, substituting the control solution with one containing the drug at known concentration. Drug solutions entered the recording chamber no later than 20 s after turning a 3 way tap. Complete replacement of the medium in the chamber took 90 s. The following drugs were used: L-DOPA, 1–300 μM , (gift of Dr Amenta), carbidopa, 300 μM , (Merck Sharp & Dhome), dopamine hydrochloride, 10–30 μM , (Sigma), (–)-sulpiride, 1 μM , (Ravizza).

Numerical data were expressed as mean $\pm$ standard error of the mean (s.e.mean).

Results

Data were obtained by intracellular recording from a total of 41 neurones located in the substantia nigra zona compacta. The properties of these cells correspond to those previously described for the principal cells of the substantia nigra zona compacta (Lacey *et al.*, 1987; 1988; 1989a,b; Mercuri *et al.*, 1989a).

Actions of L-DOPA

In all neurones tested, application of L-DOPA (3–300 μM) resulted in a concentration-dependent depression of firing rate and in membrane hyperpolarization (Figure 1). The doses of 30 and 100 μM caused a hyperpolarization of 4 ± 0.4 mV, $n = 4$ and 7.1 ± 1.7 mV, $n = 7$ respectively. Under voltage clamp at –50 to –60 mV holding potential, L-DOPA caused an outward current (Figure 2a). This outward current was dose-dependent and the concentration that caused the half maximal effect (EC_{50}) was 48 μM , as determined by the dose-

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Figure 1 The firing of the substantia nigra dopamine-containing neurones is inhibited by L-DOPA and accompanied by a hyperpolarization. (a) Dose-related reduction of the firing rate produced by increasing doses (3 μ M and 10 μ M) of L-DOPA on a single cell. After 15 min of washing with control solution the firing rate recovered. (b) L-DOPA (100 μ M), superfused for the period indicated by the bar; blocked the firing activity and hyperpolarized the membrane of another dopamine neurone. Broken line indicates -58 mV. (c) Sulpiride (1 μ M) antagonized the membrane hyperpolarization and the block of the action potential discharge, induced by 30 μ M L-DOPA in a different cell. Broken line indicates -57 mV. Full amplitude of the action potentials is not reproduced due to the low frequency response of the pen recorder. Note the different chart speeds in (a) with respect to (b) and (c).

response curve (Figure 2b). The minimal effective concentration was 3 μ M while the maximal effect was obtained with 100 μ M. The effects of L-DOPA took 2–3 min to appear and reached a steady state after superfusion for 4–7 min. Full recovery from the drug could generally be seen after 7–15 min wash out with L-DOPA-free solution. No desensitization was observed with prolonged (more than 20 min) or repeated applications of the drug on the same neurone.

Actions of dopamine

Ten neurones were also exposed to dopamine. This substance (10 and 30 μ M) in the perfusion medium, hyperpolarized the

Figure 2 L-DOPA produces an outward current in the dopamine containing cells which is concentration-dependent. (a) Upper trace, voltage. Lower trace, current. L-DOPA (100 μ M), applied by superfusion during the period indicated by the bar, produced an outward current. The broken line indicates 0 current. (b) Plot of the peak amplitude of the L-DOPA-induced outward current as a function of the concentration applied. Points show mean effect and vertical lines indicate s.e.mean for the number of observations indicated. (The holding potential was between -50 and -60 mV).

substantia nigra zona compacta cells by 5.4 ± 0.9 mV, $n = 5$ and 7 ± 0.8 mV, $n = 5$ respectively. Under voltage clamp these concentrations of dopamine produced an outward current of 45 ± 12 pA, $n = 3$ and 59 ± 10 pA, $n = 5$ (Figure 3).

Antagonism with sulpiride and effects of sodium and calcium blockade

Sulpiride (1 μ M), a dopamine D₂ receptor antagonist, readily reversed the actions of L-DOPA (3 cells) (Figure 1c). Tetradotoxin (TTX) (1 μ M) which blocks the sodium action potentials, did not prevent the hyperpolarizing effect of L-DOPA (Figure 4a).

However, when L-DOPA (100 μ M) was applied to neurones exposed to zero calcium plus cobalt (2 mM), a calcium channel blocker, the effects of the drug were markedly depressed (2 cells) or abolished (3 cells) (Figure 4b).

Pretreatment with carbidopa

Carbidopa (300 μ M), which is a potent inhibitor of the enzyme dopa decarboxylase, was applied for 20–30 min. This agent produced no detectable effects on the properties of the cells but attenuated to $25\% \pm 5.9\%$ of control the amplitude of the outward current caused by 100 μ M L-DOPA ($n = 4$). The cellular responses to L-DOPA from such an experiment are illustrated in Figure 3 before and during the application of carbidopa. However, carbidopa did not prevent the outward current produced by 30 μ M dopamine.

Discussion

The present study has shown that L-DOPA inhibits substantia nigra zona compacta neurones maintained *in vitro* by hyper-

Figure 3 Action of carbidopa on the outward currents caused by L-DOPA and dopamine on a single cell. L-DOPA ($100\ \mu\text{M}$) and dopamine ($30\ \mu\text{M}$) produced an outward current in this neurone. After 25 min exposure to a solution containing carbidopa ($300\ \mu\text{M}$) the effects of L-DOPA disappeared while dopamine was still effective. Note the prolonged application times of L-DOPA and dopamine in the presence of carbidopa. The cell was voltage clamped at $-55\ \text{mV}$. Broken line $0\ \text{pA}$.

polarizing the membrane and that this hyperpolarization was related to an outward current. An outward current similar to the one produced by L-DOPA is mediated through D_2 receptor activation by dopamine and dopamine-like agents in the zona compacta principal neurones (Lacey *et al.*, 1987; 1988; 1989b; Mercuri *et al.*, 1989a).

The most probable interpretation of the present data is that the depression of substantia nigra zona compacta cell activity results from the stimulation of D_2 receptors by dopamine, which has been synthesized from L-DOPA by the endogenous aromatic amino acid decarboxylase enzyme. The evidence supporting such a hypothesis is as follows.

Firstly, sulpiride, a D_2 -receptor antagonist, reversed the effects of L-DOPA as well as those produced by dopamine and dopamine receptor agonists (Pinnock, 1984; Lacey *et al.*, 1987; 1989b; Mercuri *et al.*, 1989a).

Secondly, carbidopa depressed the actions of L-DOPA, confirming the indirect nature of the interaction with dopamine receptors. The efficient and rapid conversion of L-DOPA to dopamine in the substantia nigra, is further supported by the steady state responses for a given concentration of L-DOPA which occurred within about a few minutes of application.

A difference between the effects of L-DOPA and dopamine was that dopamine produced larger hyperpolarizations and outward currents than L-DOPA (see also, Lacey *et al.*, 1987). In addition, the time to peak and the recovery time of the effects of L-DOPA were prolonged in comparison to those produced by dopamine.

The experiments with tetrodotoxin proved that functioning tetrodotoxin-sensitive sodium channels are not required for L-DOPA to act. Similar results were obtained with dopamine, which was not blocked by tetrodotoxin nor were the effects of cocaine and amphetamine on dopamine cells (Lacey *et al.*, 1987; 1989b; Mercuri *et al.*, 1989a).

Calcium-free solutions with added cobalt are capable of reducing or blocking L-DOPA effects, suggesting that the

Figure 4 Effects of tetrodotoxin (TTX) and calcium-free/cobalt solutions on the L-DOPA-induced responses. (a) The membrane hyperpolarization, produced by L-DOPA ($100\ \mu\text{M}$), persists in TTX ($1\ \mu\text{M}$) applied at the point indicated by the arrow. Broken line indicates $-56\ \text{mV}$. (b) Changing the control solution at the point indicated by the arrow to one containing zero calcium and $2\ \text{mM}$ cobalt abolished the membrane effects of L-DOPA in another dopaminergic neurone. Note that the calcium-free, cobalt solution also blocked the spontaneous activity of the cell.

overflow of dopamine may occur from a catecholaminergic pool sensitive to the intracellular level of calcium (Leslie *et al.*, 1985; Woodward *et al.*, 1988). Amphetamine, unlike L-DOPA may induce a calcium independent efflux of dopamine in the substantia nigra (Mercuri *et al.*, 1989a).

Although the intracerebral concentration of L-DOPA reached during the treatment of Parkinsonian patients is unknown, it is worth noting that the plasma level of L-DOPA necessary to improve the motor functions is in the micromolar range (Nutt *et al.*, 1988). This is about the concentration observed to have effects in the substantia nigra.

Thus the treatment of Parkinson's disease with L-DOPA does not exclusively increase the dopaminergic activity in the striatum; it could also augment the effects of dopamine in the substantia nigra. The final outcome of the dopamine substitute therapy is a cellular inhibition both in the striatum (Calabresi *et al.*, 1988) and the substantia nigra pars compacta. The effects of L-DOPA at the compacta cell body level are clear and contrary to current opinion, which considers them as 'unwanted effects'. They could prove to be important in controlling the amount of dopamine released in the target striatal areas (Hoffman *et al.*, 1988). Indeed, newly synthesized dopamine, maintaining the firing rate of the dopamine-containing neurones in a critical range, might play a role in restoring the physiological activity of the residual nigrostriatal pathway in Parkinsonian patients (Mercuri *et al.*, 1989b).

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References

- AGHAJANIAN, G.K. & BUNNEY, B.S. (1973). Central dopaminergic neurones: neurophysiological identification and responses to drugs. In *Frontiers in Catecholamine Research*, ed. Usdin, E. & Snyder, S.H. pp. 643–648. New York: Pergamon Press.
- AGHAJANIAN, G.K. & BUNNEY, B.S. (1977). Dopamine 'autoreceptors': pharmacological characterization by microiontophoretic single cell recordings studies. *Naunyn Schmiedeberg's Arch. Pharmacol.*, **297**, 1–7.

- BARBEAU, A. (1981). The use of L-DOPA in Parkinson's disease: a 20 years follow-up. *Trends Pharmacol. Sci.*, **2**, 297-299.
- BRANNAN, T., KNOTT, P., KAUFFMANN, H., LEUNG, L. & YAHR, M. (1989). Intracerebral dialysis monitoring of striatal dopamine release and metabolism in response to L-DOPA. *J. Neural. Transm.*, **75**, 149-157.
- BUNNEY, B.S., AGHAJANIAN, G.K. & ROTH, R.H. (1973). Comparison of effects of L-DOPA, amphetamine and apomorphine on firing rate of rat dopaminergic neurones. *Nature*, **245**, 123-125.
- CALABRESI, P., BENEDETTI, M., MERCURI, N.B. & BERNARDI, G. (1988). Endogenous dopamine and dopaminergic agonists modulate synaptic excitation in neostriatum: Intracellular studies from naive and catecholamine depleted rats. *Neuroscience*, **27**, 145-147.
- CHERAMY, A., LEVIEL, V. & GLOWINSKI, J. (1981). Dendritic release of dopamine in the substantia nigra. *Nature*, **269**, 537-542.
- COTZIAS, G.C., PAPAVALIOU, G.S. & GELLINE, R. (1969). Modification of Parkinsonism-chronic treatment with L-DOPA. *N. Engl. J. Med.*, **280**, 337-345.
- GEFFEN, L.B., JESSEL, T.M., CUELLO, A.C. & IVERSEN, L.L. (1976). Release of dopamine from dendrites in rat substantia nigra. *Nature*, **260**, 258-260.
- HEFTI, F. & MELAMED, E. (1981). Dopamine release in rat striatum after administration of L-DOPA as studied with *in vivo* electrochemistry. *Brain Res.*, **225**, 333-346.
- HOFFMAN, I.S., TALMACIU, R.K., FERRO, C.P. & CUBEDDU, L.X. (1988). Sustained high release at rapid stimulation rate and reduced functional autoreceptors characterize prefrontal cortex dopamine terminals. *J. Pharmacol. Exp. Ther.*, **245**, 761-772.
- HORNYKIEWICZ, O. (1966). Dopamine and brain function. *Pharmacol. Rev.*, **18**, 925-964.
- HORNYKIEWICZ, O. (1974). The mechanisms of action of L-DOPA in Parkinson's disease. *Life Sci.*, **15**, 1249-1259.
- JUNCOS, J.L., MOURADIAN, M.D., FABBRINI, G., SERRATI, C. & CHASE, T.N. (1987). Levodopa methyl ester treatment of Parkinson's disease. *Neurology*, **37**, 1242-1245.
- KORF, J., ZIELEMAN, M. & WESTERINK, B.C. (1976). Dopamine release in substantia nigra. *Nature*, **260**, 257-258.
- LACEY, M.G., MERCURI, N.B. & NORTH, R.A. (1987). Dopamine acts on D2 receptors to increase potassium conductance in neurones of the rat substantia nigra zona compacta. *J. Physiol.*, **392**, 397-416.
- LACEY, M.G., MERCURI, N.B. & NORTH, R.A. (1988). On the potassium conductance activated by GABA B and dopamine D2 receptors in rat substantia nigra neurones. *J. Physiol.*, **401**, 437-457.
- LACEY, M.G., MERCURI, N.B. & NORTH, R.A. (1989a). Two cell types in rat substantia nigra zona compacta distinguished by membrane properties and the actions of dopamine and opioids. *J. Neurosci.*, **4**, 1233-1241.
- LACEY, M.G., MERCURI, N.B. & NORTH, R.A. (1989b). Cocaine actions on rat dopamine neurones *in vitro*. *Br. J. Pharmacol.*, (in press).
- LESLIE, S.W., WOODWARD, J.J. & WILCOX, R.E. (1985). Correlation of rates of calcium entry and endogenous dopamine release in mouse striatal synaptosome. *Brain Res.*, **325**, 99-105.
- MELAMED, E., HEFTI, F., LIEBMANN, J., SCHLOSBERG, A.J. & WURTMAN, R. (1980). Serotonergic neurones are not involved in action of L-DOPA in Parkinson's disease. *Nature*, **283**, 772-774.
- MELAMED, E., HEFTI, F., BITTON, V. & GLOBUS, M. (1984). Suppression of amphetamine-induced circling in rats with nigral lesions by blockade of central dopa-carboxylase: implications for mechanism of action of L-DOPA in Parkinsonism. *Neurology*, **34**, 1566-1570.
- MERCURI, N.B., CALABRESI, P. & BERNARDI, G. (1989a). The mechanism of amphetamine-induced inhibition of rat substantia nigra compacta neurones investigated with intracellular recording *in vitro*. *Br. J. Pharmacol.*, **98**, 127-134.
- MERCURI, N.B., CALABRESI, P. & BERNARDI, G. (1989b). Physiology and pharmacology of dopamine D₂-receptors: Their implications in dopamine-substitute therapy of Parkinson's disease. *Neurology*, **39**, 1106-1108.
- NIEULLON, A., CHERAMY, A. & GLOWINSKI, J. (1977). Release of dopamine *in vivo* from cat substantia nigra. *Nature*, **266**, 375-377.
- NG, K.J., CHASE, T.N., COLBURN, R.W. & KOPIN, I.J. (1970). L-DOPA induced release of cerebral monoamines. *Science*, **170**, 76-77.
- NUTT, J.C., GANCHER, S.T. & WOODWARD, W.R. (1988). Does an inhibitory action of levodopa contribute to motor fluctuations? *Neurology*, **38**, 1553-1557.
- PINNOCK, R.D. (1983). Sensitivity of compacta neurones in the rat substantia nigra slices to dopamine agonists. *Eur. J. Pharmacol.*, **96**, 269-276.
- PINNOCK, R.D. (1984). The action of antipsychotic drugs on dopamine receptors in the rat substantia nigra. *Br. J. Pharmacol.*, **81**, 631-635.
- PONZIO, F., ACHILLI, G., PEREGO, C., RINALDI, G. & ALGERI, S. (1983). Does acute L-DOPA increase active release of dopamine from dopaminergic terminals? *Brain Res.*, **273**, 45-51.
- ROBERTSON, G.S. & ROBERTSON, H.A. (1988). Evidence that the substantia nigra is a site of action for L-DOPA. *Neurosci. Lett.*, **89**, 204-208.
- SNYDER, G.L. & ZIGMOND, M.J. (1987). L-DOPA-induced increase in DA release from striatum: *in vitro* studies on mechanism of action. *Soc. Neurosci.*, **13**, 1642.
- WOODWARD, J.J., CHANDLER, L.J. & LESLIE, S.W. (1988). Calcium-dependent and -independent release of endogenous dopamine from rat striatal synaptosomes. *Brain Res.*, **473**, 91-98.
- ZETTERSTROM, T., HERRERA-MARCHITZ, M. & UNGERSTEDT, U. (1986). Simultaneous measurement of dopamine release and rotational behavior in 6-hydroxydopamine denervated rats using intracerebral dialysis. *Brain Res.*, **376**, 1-7.

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