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THE ACTION OF MICRO-ELECTROPHORETICALLY APPLIED L–3,4-DIHYDROXYPHENYLALANINE (DOPA) ON SINGLE CORTICAL NEURONES

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1 The technique of microelectrophoresis was used in order to compare the actions of L-3,4-dihydroxyphenylalanine (DOPA) and noradrenaline on single neurones in the cerebral cortices of cats and rats.

2 DOPA could both excite and depress cortical neurones. Cells excited by DOPA were also excited by noradrenaline and cells depressed by DOPA were also depressed by noradrenaline.

3 In the case of both excitatory and depressant responses, DOPA appeared to be less potent than noradrenaline.

4 Responses to DOPA and noradrenaline could be antagonized by phentolamine and propranolol. Responses to acetylcholine were not affected.

5 Responses to acetylcholine, but not responses to DOPA, were antagonized by atropine.

6 The results indicate that locally applied DOPA may mimic the actions of noradrenaline on cortical neurones. Possible mechanisms for these effects of DOPA are discussed.

Introduction

L-3,4-Dihydroxyphenylalanine (DOPA) is widely used in the treatment of Parkinson's disease. DOPA is the metabolic precursor of dopamine, and it is generally assumed that DOPA exerts its therapeutic effect via the synthesis and release of dopamine in the caudate nucleus (Hornykiewicz, 1974). However, DOPA is also a precursor of noradrenaline (NA), and it has been suggested that in structures receiving a NA innervation, exogenously administered DOPA may cause the release of NA from presynaptic terminals and thus mimic the actions of NA on post-synaptic cells (Andén, Carlsson & Häggendal, 1969). In the experiments described here we compared the effects of microelectrophoretically applied DOPA and NA on single neurones in the cerebral cortex, since this structure is known to be innervated by NA-containing neurones (Fuxe, 1965).

Some of these results have been reported to the British Pharmacological Society (Bevan, Bradshaw & Szabadi, 1975a).

Methods

The experiments were conducted on adult cats of either sex (2–3.5 kg) and on male albino Wistar rats (250–300 grams). The animals were anaesthetized with halothane (0.5–1.2%). Our methods for the surgical preparation of the animals and the manufacture of six-barrelled glass micropipettes for extracellular recording and microelectrophoretic drug application, have been described previously (Bradshaw, Roberts & Szabadi, 1973a; Bradshaw, Szabadi & Roberts, 1973b; Bevan, Bradshaw & Szabadi, 1975b).

Micropipettes having tip diameters of 3–5 μm were used. Two barrels of each micropipette contained 4 M NaCl, one barrel for recording action potentials, the other for use in current balancing (Roberts & Straughan, 1967). The remaining barrels contained drug solutions. The following drug solutions were used: L-3,4-dihydroxyphenylalanine methylester hydrochloride (0.05 M, pH 5.0), (–)-noradrenaline bitartrate (0.05 M, pH 3.0–3.5), acetylcholine chloride (0.05 M, pH 3.5–4.0), phentolamine mesylate (0.01 M, pH 4.5–5.5), propranolol hydrochloride (0.01 M, pH 4.5–5.5) and atropine sulphate (0.01 M, pH 5.5–6.0).

Only spontaneously active neurones were studied in these experiments. All the drugs were applied by microelectrophoresis. When a suitable unit was encountered, the agonists were applied in a regular cycle. Between successive drug applications retaining

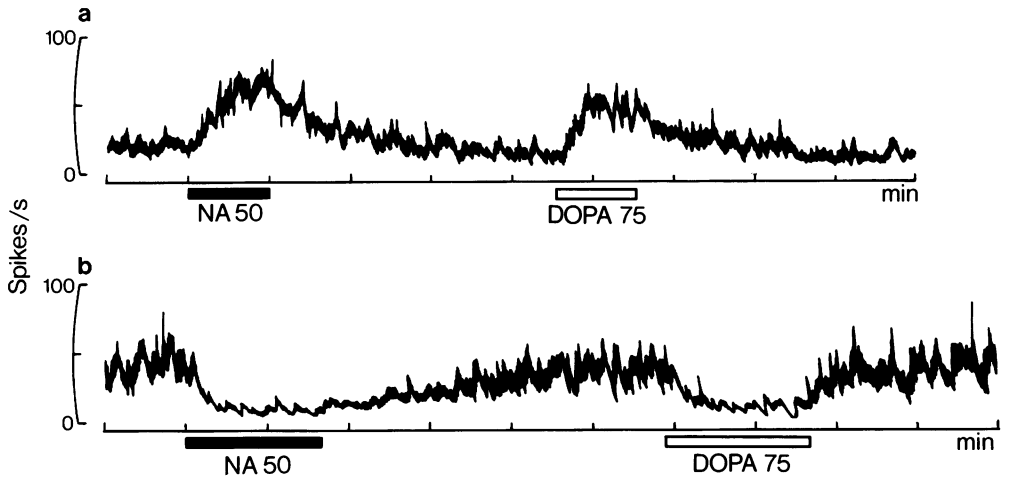

Figure 1 Correlation between effects of L-3,4-dihydroxyphenylalanine (DOPA) and noradrenaline (NA). Ratemeter recordings of the firing rates of two cortical neurones in the rat (a and b). Ordinates: firing rate (spikes/s); abscissae: running time (min). Horizontal bars indicate microelectrophoretic drug applications; numbers refer to intensities of ejecting currents (nA). (a) A cell which was excited by both DOPA and NA; (b) a cell which was depressed by both DOPA and NA.

currents of −10 nA were passed. Intervals between successive applications of the same agonist were kept constant in order to standardize the effects of the retaining current upon drug release during the ejection periods (Bradshaw *et al.*, 1973a; 1973b). The sizes of the neuronal responses to the agonists were expressed as the total number of action potentials produced in response to each drug application ('total spike number'). The total spike number was calculated by measuring the number of spikes generated between the onset of the drug application and the recovery of the baseline firing rate, and subtracting the number of spikes generated during an equivalent control period (Bradshaw *et al.*, 1973b; Bevan, Bradshaw, Roberts & Szabadi, 1974).

The effects of antagonists were evaluated in the following way. When stable responses to the agonists had been obtained, the antagonist was applied continuously, either by removal of the retaining current (thus allowing the drug to diffuse out from the micropipette) or by the passage of a weak ejecting current (5 to 10 A), and the time-course of the developing antagonism was followed. If necessary, the intensity of the ejecting current was increased until antagonism was observed. Then the application of the antagonist was terminated and the time-course of recovery was followed. The response to an agonist was regarded as antagonized if there was at least a 50% reduction in the total spike number (Bevan *et al.*, 1974).

Results

Agonistic effect of DOPA

Responses of cortical neurones to DOPA. Both excitatory and depressant responses to DOPA were observed in these experiments. The effect of DOPA (25–150 nA, applied for 25–100 s) was tested on 51 neurones in the rat; 44 were excited and 7 were depressed by DOPA. Eleven neurones were studied in the cat; of these 10 were excited and one depressed by DOPA. The predominance of excitatory responses in both species is statistically significant (binomial test, $P<0.01$ in both cases).

Direction of responses to DOPA and noradrenaline. Responses to both DOPA and NA were obtained from 40 cells (cat 10 cells; rat 30 cells). All of these cells responded in the same direction to the two drugs, cells excited by DOPA being also excited by NA and cells depressed by DOPA being also depressed by NA (Figure 1). This positive correlation between the effects of the two drugs is statistically significant (χ^2 test, $P<0.001$).

Comparison of apparent potencies of DOPA and noradrenaline. In 34 of the 40 cells on which the effects of DOPA and NA were compared, DOPA appeared to be less potent than NA (binomial test, $P<0.01$). When DOPA and NA were applied to the

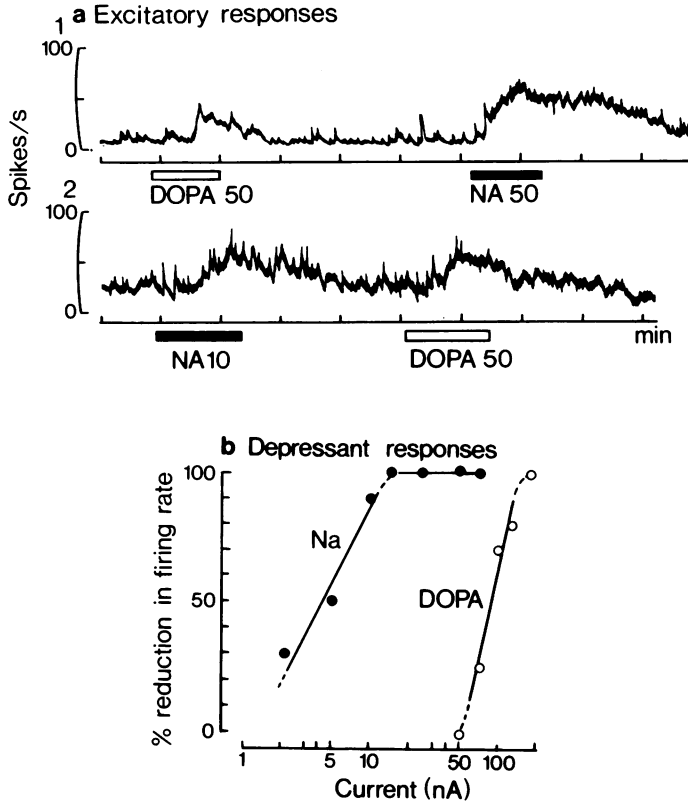

Figure 2 Comparison of apparent potencies of noradrenaline (NA) and L-3,4-dihydroxyphenylalanine (DOPA). (a) Excitatory responses. Ratemeter recordings of firing rates of single cortical neurones in cat (trace 1) and rat (trace 2). (1): DOPA evoked a smaller response than NA when both drugs were applied with identical ejecting pulses; (2) in order to evoke approximately equivalent responses, a higher current was needed to apply DOPA than NA. (b) Depressant responses. Current-response curves for suppression of firing rate, measured at equilibrium, plotted against intensity of ejecting current, on a log scale. Data were obtained from a single cortical neurone in the rat. Each point represents one individual response. DOPA was apparently less potent than NA; the equipotent current ratio at 50% response was 18.

same cell with identical ejecting pulses the size of the response (see Methods section) evoked by NA was 1.8–3.4 times greater (inter-quartile range) than the response evoked by DOPA. In order to obtain approximately equivalent responses to the two drugs, the current needed to apply DOPA was 2.0–3.0 times greater (inter-quartile range) than that needed to apply NA. The lower potency of DOPA than NA was apparent in the case of both excitatory and depressant responses. Examples are shown in Figure 2.

Comparison of latencies to onset of responses to DOPA and noradrenaline. The latencies to onset of responses to DOPA and NA were compared in 12 cells to which the two drugs were applied with identical ejecting currents. The latencies of responses to DOPA were 1.7–2.3 times greater (inter-quartile range) than those of responses to NA.

Effects of antagonists on responses to DOPA

In these experiments the effects of the antagonists on responses to DOPA were compared with their effects on responses to NA and acetylcholine (ACh).

Phentolamine. The effects of phentolamine were studied on 12 cells excited by DOPA (cat: 6 cells; rat: 6 cells). In all the cells tested, phentolamine (0–40 nA, applied for 10–60 min) reversibly antagonized excitatory responses to DOPA; excitatory responses to NA were also antagonized. These effects of phentolamine occurred at times when responses to ACh were not affected (see Figure 3). However, more prolonged applications of phentolamine were sometimes accompanied by loss of spike amplitude and reduced sensitivity to ACh.

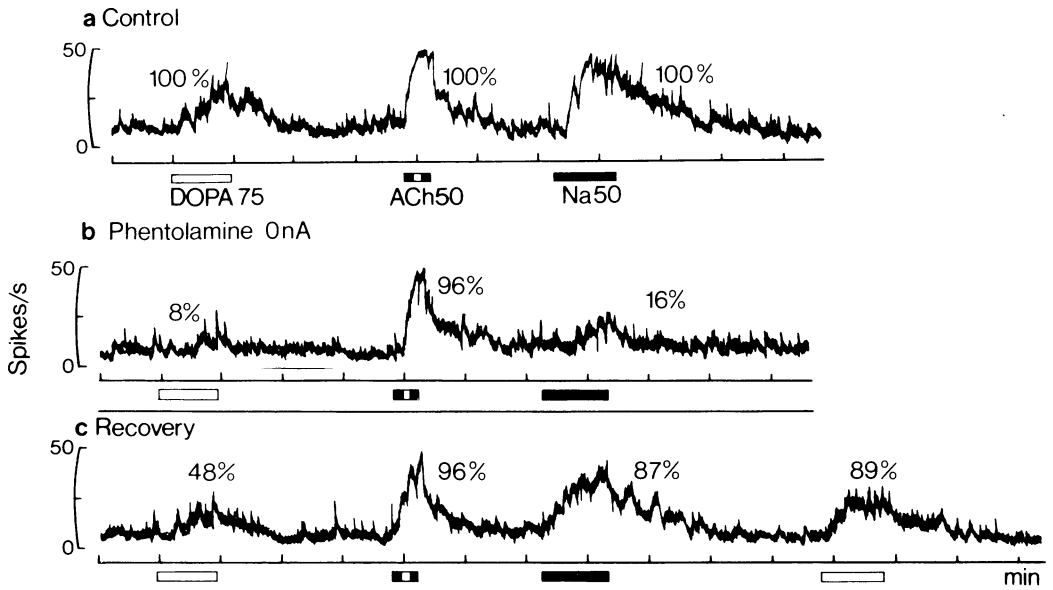

Figure 3 Effect of phentolamine on excitatory responses to L-3,4-dihydroxyphenylalanine (DOPA), noradrenaline (NA) and acetylcholine (ACh). Ratemeter recording of firing rate of single cortical neurone in the rat. Figures above the traces indicate total spike numbers (%) taking the sizes of the control responses to each agonist as 100%. (a) Control responses to the agonists; (b) responses to the agonists during the continuous application of phentolamine. Phentolamine was applied by removal of the retaining current and at the start of trace (b) had been applied continuously for 21 minutes. The responses to NA and DOPA, but not the response to ACh, were antagonized. (c) Recovery of responses, 16 min after the application of phentolamine had been terminated.

In two cells in the rat, phentolamine failed to antagonize depressant responses to DOPA and NA.

Propranolol. The effects of propranolol on excitatory responses to DOPA, NA and ACh were tested on 11 cells in the rat. In 8 of these cells propranolol (0–10 nA, applied for 10–40 min) reversibly antagonized the responses to DOPA and NA without affecting responses to ACh (see Figure 4). In the remaining 3 cells, propranolol produced a reduction in spike amplitude which precluded successful drug interaction studies.

Atropine. The effects of atropine on excitatory responses to DOPA and ACh were tested on 6 cells in the rat. In all the cells tested, atropine (0–10 nA, applied for 10–30 min) reversibly antagonized the responses to ACh with little effect on responses to DOPA. An example of a cell on which the effects of both atropine and propranolol were studied is shown in Figure 5. On one cell atropine reversibly abolished

Figure 4 Effect of propranolol on excitatory responses to L-3,4-dihydroxyphenylalanine (DOPA), noradrenaline (NA) and acetylcholine (ACh). Ratemeter recording of firing rate of a single cortical neurone in the rat (as in Figure 3). (a) Control responses to the agonists; (b) responses to the agonists during the continuous application of propranolol. At the start of trace (b) propranolol (10 nA) had been applied continuously for 11 minutes. The responses to NA and DOPA, but not the response to ACh, were antagonized. (c) Recovery of responses, 6.5 min after the application of propranolol had been terminated.

Figure 5 Comparison of effects of propranolol and atropine on excitatory responses to L-3,4-dihydroxyphenylalanine (DOPA) and acetylcholine (ACh). Ratemeter recording of firing rate of single cortical neurone in the rat (as in Figure 3): (a) and (b) are from two studies conducted on the same neurone. (a) Effect of propranolol: (i) control responses to DOPA and ACh; (ii) responses to the agonists during the continuous application of propranolol. At the start of trace (ii), propranolol (10 nA) had been applied continuously for 7.5 minutes. The response to DOPA, but not the response to ACh, was antagonized. (iii) Recovery of responses, 8 min after the application of propranolol had been terminated. (b) Effect of atropine: (i) control responses to DOPA and ACh; (ii) responses to the agonists during the continuous application of atropine. At the start of trace (ii), atropine (10 nA) had been applied for 8 minutes. The response to ACh was antagonized, but the response to DOPA was not greatly affected. (iii) Recovery of the responses 12 min after the application of atropine had been terminated.

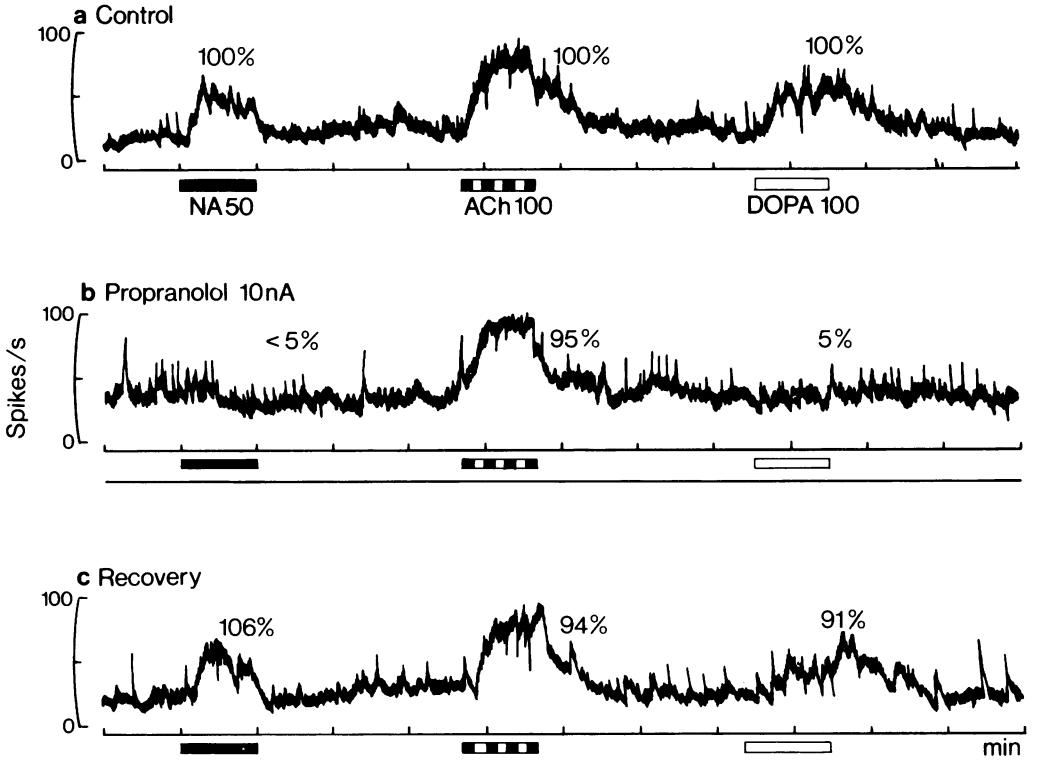

Figure 4

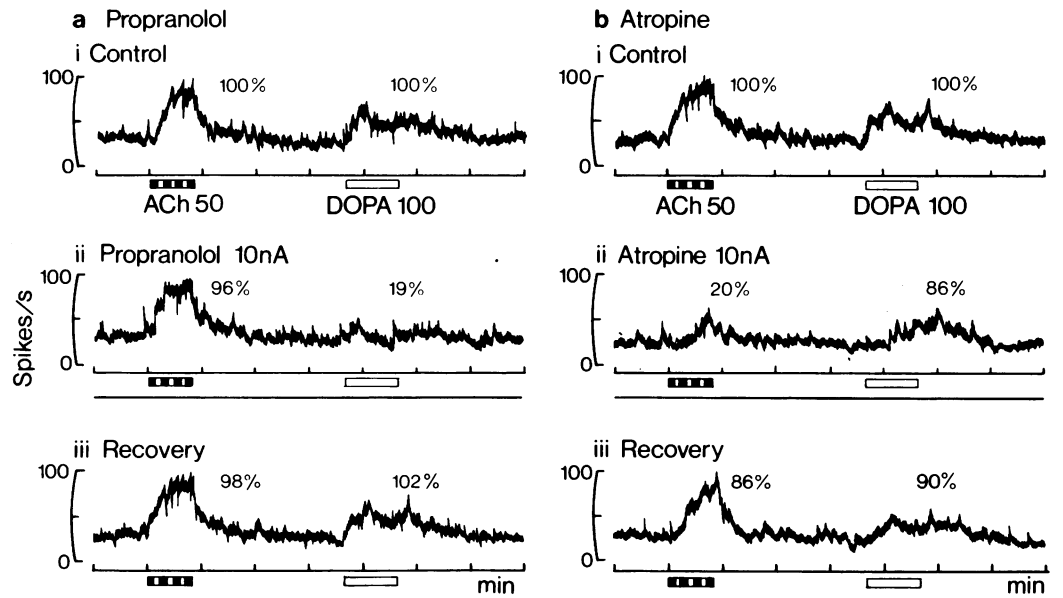

Figure 5

the excitatory effects of ACh without affecting depressant responses to DOPA.

Discussion

The results described here indicate that the effects of DOPA on single cortical neurones are very similar to those of NA. Firstly, it was found that cells invariably responded in the same direction to the two drugs. This contrasts with much lower correlations between the effects of NA and ACh, or NA and 5-hydroxytryptamine on cortical neurones (Johnson, Roberts, Sobieszek & Straughan, 1969; Bradshaw, Roberts & Szabadi, 1971; Bevan *et al.*, 1974). Secondly, the excitatory effects of both DOPA and NA could be abolished by phentolamine and propranolol when responses to ACh remained unaffected, while responses to DOPA were not affected by atropine when responses to ACh were completely abolished. The selectivity of these antagonists with respect to NA and ACh in our experiments is in agreement with the observations of Johnson *et al.* (1969). The present finding of a close correlation between the effects of DOPA and NA seems to be in contrast with the observations of Krnjević & Phillis (1963) who reported that DOPA had a weak excitatory action on cortical neurones while NA was predominantly depressant. However, these authors did not report within-cell comparisons of the effects of the two drugs.

A possible explanation for the agonistic effects of DOPA seen in our experiments is the release of NA from noradrenergic nerve terminals. There is evidence that such a mechanism underlies the facilitatory effects of DOPA on spinal reflexes (Andén *et al.*, 1969; Andén, Engel & Rubenson, 1972a) and the stimulant effects of DOPA on locomotor activity (Corrodi, Fuxe, Ljungdahl & Ögren, 1970). Since these actions of DOPA coincide with raised levels of NA in the CNS, and can be blocked by drugs which inhibit the synthesis of NA from DOPA, it is unlikely that DOPA exerts a direct action of its own in these test systems. However, in the present experiments, the possibility cannot be excluded that DOPA acted directly on post-synaptic NA receptors. The observation that the latency to onset of responses to DOPA was greater than that of responses to NA might be taken as evidence in favour of an indirect action of DOPA; however the dependence of response latency upon physical factors such as transport number, diffusion coefficient and the parameters of the ejecting and retaining currents (Bradshaw *et al.*, 1973a, b; Szabadi & Bradshaw, 1974) makes such observations very difficult to interpret.

An alternative possibility is that agonistic actions of DOPA were mediated by dopamine rather than NA. Recent evidence suggests that there are dopamine-containing nerve terminals in the cerebral cortex (Thierry, Hirsch, Tassin, Blanc & Glowinski, 1974; Fuxe, Hökfelt, Johansson, Jonsson, Lidbrink & Ljungdahl, 1974) and that dopamine can be synthesized in these terminals from systemically administered DOPA (Hökfelt, Ljungdahl, Fuxe & Johansson, 1974). Moreover, DOPA may be decarboxylated in 5-hydroxytryptamine-containing nerve terminals, with the subsequent release of dopamine (Butcher, Engel & Fuxe, 1970). If, in the present experiments, DOPA acted either directly or indirectly on dopamine receptors, then the antagonistic effects of phentolamine and propranolol on responses to DOPA and NA would indicate that dopamine receptors are highly similar to NA receptors on cortical neurones.

Finally, some comment is needed about the apparently lower potency of DOPA than NA seen in our experiments. Such a difference in potency might reflect physical factors, such as differences between the transport numbers and diffusion coefficients of the two drugs (Szabadi & Bradshaw, 1974). However, the lower potency of DOPA might also be due to biological factors, such as the extra-neuronal accumulation of DOPA (Butcher *et al.*, 1970; Andén *et al.*, 1972b), the retention of newly synthesized catecholamines in presynaptic stores after DOPA administration (Butcher *et al.*, 1970), or, if the primary action of DOPA is post-synaptic, differences between the affinities and/or intrinsic activities of the two drugs at NA receptors.

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