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THE ACTION OF MICROELECTROPHORETICALLY APPLIED (3,4-DIHYDROXY-PHENYLAMINO)-2-IMIDAZOLINE (DPI) ON SINGLE CORTICAL NEURONES

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1 The technique of microelectrophoresis was used in order to compare the actions of the imidazoline derivative, (3,4-dihydroxy-phenylamino)-2-imidazoline (DPI), with those of dopamine and phenylephrine on single neurones in the cerebral cortex of the rat anaesthetized with halothane.

2 DPI and phenylephrine were almost exclusively excitatory, whereas dopamine could evoke both excitatory and depressant responses.

3 In the case of excitatory responses, DPI appeared to be more potent than dopamine, and was approximately equipotent with phenylephrine.

4 The dopamine antagonist, haloperidol, could discriminate between excitatory responses to DPI and dopamine: responses to dopamine were abolished, whereas responses to DPI, and to a control agonist, acetylcholine, were unaffected.

5 The α-adrenoceptor antagonist, phenoxybenzamine, antagonized equally excitatory responses to DPI and phenylephrine. Responses to acetylcholine were not affected.

6 It is concluded that DPI does not stimulate dopamine receptors on cortical neurones; the excitatory responses of these cells to DPI may be mediated by α-adrenoceptors.

Introduction

Noradrenaline (NA) applied by microelectrophoresis can evoke both excitatory and depressant responses on single cortical neurones (Johnson, Roberts, Sobieszek & Straughan, 1969; Bevan, Bradshaw, Roberts & Szabadi, 1974). Recent evidence indicates that these responses are mediated by pharmacologically distinct receptors: the excitatory responses by α-adrenoceptors, and the depressant responses by β-adrenoceptors (Bevan, Bradshaw & Szabadi, 1977). Dopamine, like NA, can both excite and depress cortical neurones (Bevan, Bradshaw & Szabadi, 1975; Stone, 1976; Bunney & Aghajanian, 1976; Sharma, 1977). Evidence has recently been obtained suggesting that the excitatory responses to the catecholamines may be mediated by two populations of receptors: α-adrenoceptors and specific excitatory dopamine receptors (Bevan, Bradshaw, Pun, Slater & Szabadi, 1978). However, it is not clear whether in addition to β-adrenoceptors, there is also a separate population of inhibitory dopamine receptors.

The imidazoline derivative (3,4-dihydroxy-phenylamino)-2-imidazoline (DPI) has been reported to be a specific agonist at inhibitory dopamine receptors on molluscan ganglion cells (Struyker Boudier, Teppema, Cools & van Rossum, 1975). On the basis of behavioural experiments, it was suggested that DPI may also stimulate inhibitory dopamine receptors in the mammalian CNS (Cools, Honig, Pijnenburg & van Rossum, 1976a; Cools, Struyker Boudier & van Rossum, 1976b).

In the present experiments, we have used the technique of microelectrophoresis to examine the hypothesis that DPI stimulates inhibitory dopamine receptors on single cortical neurones.

Methods

Adult male albino Wistar rats (250 to 350 g) were used. The animals were anaesthetized with halothane (0.8 to 1.0%). The oxygen–halothane mixture was administered through a face-mask throughout the experiment. The methods for the surgical preparation

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of the animals, and the manufacture of multi-barrelled glass micropipettes for extracellular recording and microelectrophoretic drug application, have been described elsewhere (Bradshaw, Roberts & Szabadi, 1973a; Bradshaw, Szabadi & Roberts, 1973b; Bevan *et al.*, 1975).

Six- or seven-barrelled micropipettes of tip diameter 3 to 5 μm were used. Two barrels of each micropipette contained 4 M NaCl, one for recording action potentials, the other for current balancing. The remaining barrels contained drug solutions. The following drug solutions were used: dopamine hydrochloride (0.05 M, pH 4.0–4.5); (3,4-dihydroxy-phenylamino)-2-imidazoline (0.05 M, pH 4.5); acetylcholine chloride (0.05 M, pH 3.5–4.0); phenylephrine hydrochloride (0.05 M, pH 5.0–5.5); haloperidol (0.01 M dissolved in 0.01 M tartaric acid, pH 4.0); phenoxybenzamine hydrochloride (0.01 M or 0.005 M, pH 3.0).

Spontaneously active neurones in the cerebral cortex were studied (stereotaxic coordinates, according to König & Klippel (1963); A 4.8–6.5, L 0.9–2.4). A small hole was drilled in the skull with a dental burr; the dura was penetrated directly with the micropipette. All the drugs were applied by microelectrophoresis. When a suitable unit was encountered, the agonists were applied in a regular cycle. Between successive applications of agonists, retaining currents of −10 nA were passed. Retaining currents of −25 nA were used for the antagonists. 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 period (Bradshaw *et al.*, 1973a, b). The effects of antagonists were evaluated by the procedures described previously (Bevan *et al.*, 1977; 1978). The response to an agonist was regarded as antagonized if its size ('total spike number', see Bradshaw *et al.*, 1973b) was reduced by at least 50%.

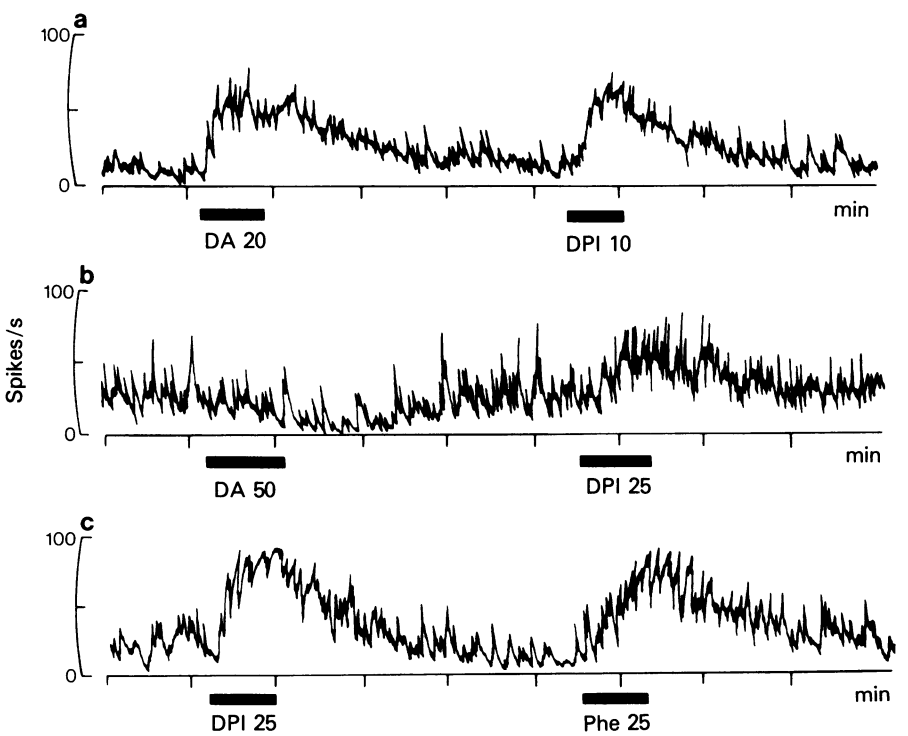

Figure 1 Correlation between the effects of DPI, dopamine and phenylephrine. Ratemeter recordings of the firing rates of three cortical neurones (a, b and c). Ordinates: firing rate (spikes/s); abscissae: running time (min). Horizontal bars indicate mircoelectrophoretic drug applications; numbers refer to intensities of ejecting current (nA). (a) A cell which was excited by both dopamine (DA) and DPI. (b) A cell which was depressed by dopamine, but was excited by DPI. (c) A cell which was excited by both DPI and phenylephrine (Phe).

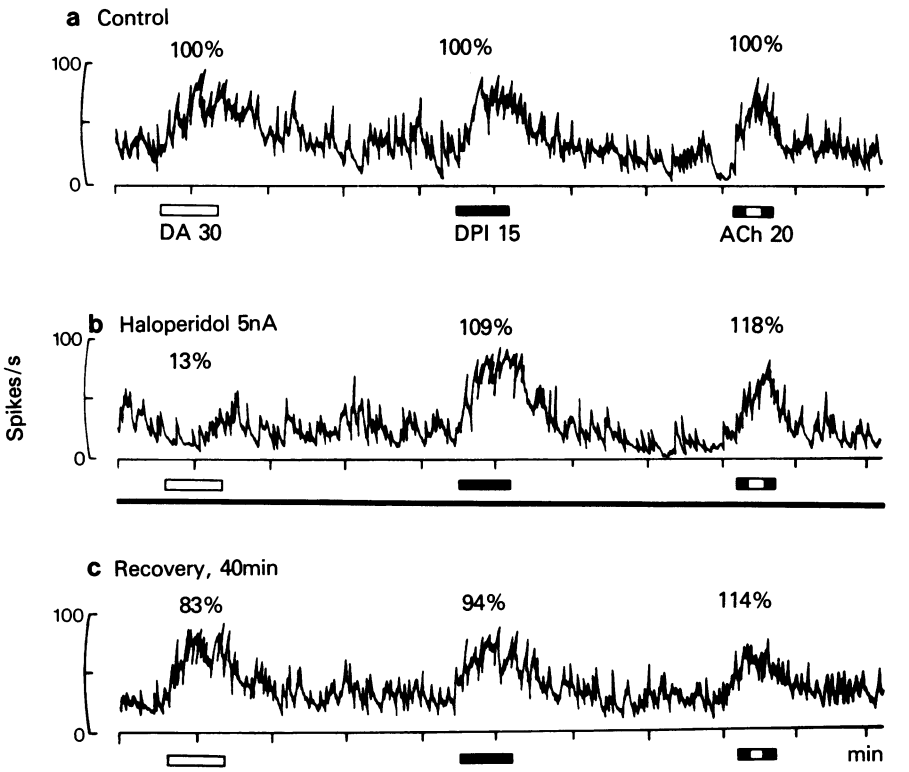

Figure 2 Effects of haloperidol on excitatory responses to dopamine, DPI and acetylcholine. Excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in Figure 1). Figures above the traces indicate total spike numbers (%), taking the size of the control response to each agonist as 100%. (a) Control responses to the agonists. (b) Responses to the agonists during the continuous application of haloperidol (5 nA). At the start of trace (b), haloperidol had been applied continuously for 22 min. The response to dopamine (DA) was antagonized, while the responses to DPI and acetylcholine (ACh) were unaffected. (c) Recovery of the response to dopamine 40 min after the application of haloperidol had been terminated.

Results

Comparison of effects of dopamine and DPI

Responses of cortical neurones to dopamine and DPI The directions of the responses (excitation or depression) evoked by dopamine and DPI was compared on 135 neurones. Dopamine evoked both excitatory and depressant responses, 113 cells being excited, and 21 cells being depressed. DPI excited 134 cells and depressed one cell (this cell was also depressed by dopamine). Examples of these observations are shown in Figure 1.

Apparent potencies of dopamine and DPI The relative potencies of dopamine and DPI on neurones excited by both agonists were assessed either by comparing the sizes of the responses evoked when the two drugs were applied with identical ejecting currents, or in terms of the equipotent current ratio. The equipotent current ratio was defined as the ratio of the ejecting currents (current for dopamine/current for DPI) needed in order to evoke responses of approximately equal magnitude to the two drugs (i.e. total spike numbers not differing by more than 20%; see Bevan *et al.*, 1977; 1978). In general, DPI appeared to be approximately twice as potent as dopamine. On 26 cells where both agonists evoked approximately equal responses, the mean equipotent current ratio ($\pm$s.e. mean) was 2.2 ± 0.1. On 13 cells where both drugs were applied with currents of equal intensity and duration, the mean ratio of the sizes of the responses ($\pm$s.e. mean) (response to DPI/response to dopamine) was 2.0 ± 0.2.

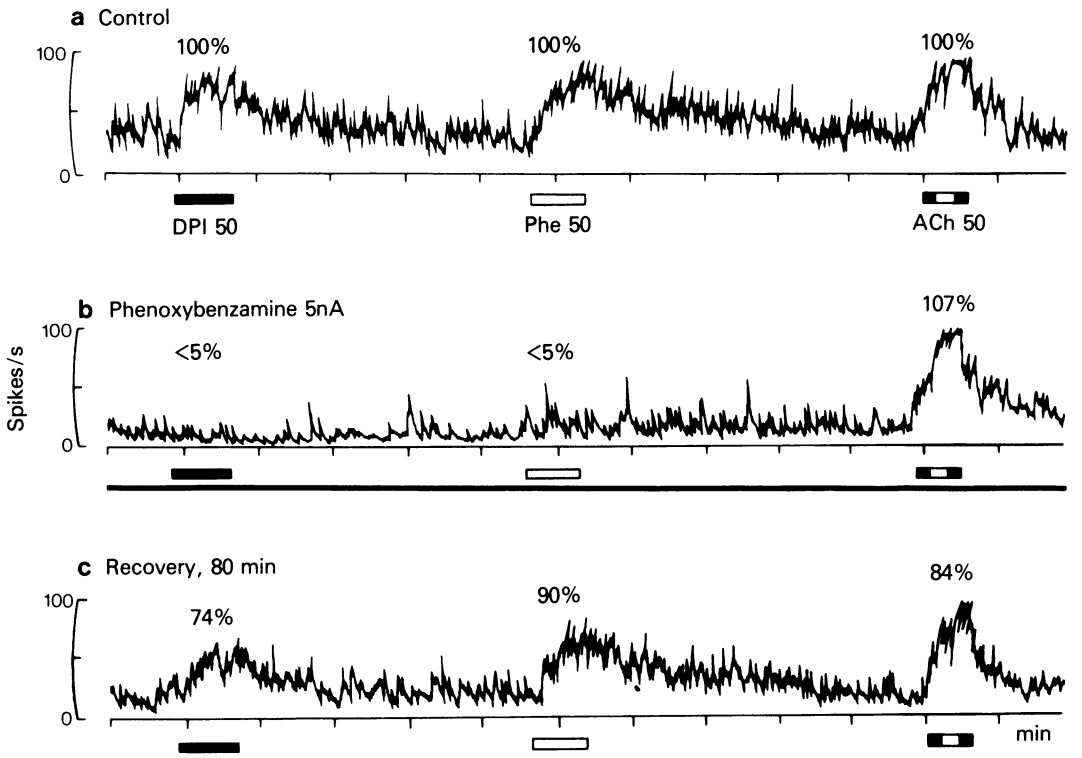

Figure 3 Effects of phenoxybenzamine on excitatory responses to DPI, phenylephrine and acetylcholine. Excerpts from the ratemeter recording of the firing rate of a single cortical neurone (as in Figures 1 and 2). (a) Control responses to the agonists. (b) Responses to the agonists during the continuous application of phenoxybenzamine (5 nA). At the start of trace (b), phenoxybenzamine had been applied continuously for 2 min. The responses to DPI and phenylephrine (Phe) were antagonized, while the response to acetylcholine (ACh) was unaffected. (c) Recovery of the responses to DPI and phenylephrine 80 min after the application of phenoxybenzamine had been terminated.

Effects of haloperidol on excitatory responses to dopamine and DPI The effects of haloperidol were examined on 11 cortical neurones excited by both dopamine and DPI. On every cell studied acetylcholine (ACh) was used as a control agonist. On 10 cells, haloperidol (0 to 10 nA) could discriminate between the responses to dopamine and DPI: on all these cells the response to dopamine was abolished, while the response to DPI was unaffected. On the remaining cell, the excitatory responses to dopamine and DPI were equally antagonized. Responses to ACh were not affected by haloperidol. An example of the effects of haloperidol on excitatory responses to dopamine and DPI is shown in Figure 2.

Comparison of effects of phenylephrine and DPI

Responses of cortical neurones to phenylephrine and DPI The direction of the responses evoked by phenylephrine and DPI was compared on 74 neurones. All of these cells were excited by both drugs (Figure 1).

Apparent potencies of phenylephrine and DPI The relative potencies of phenylephrine and DPI on neurones excited by both agonists were assessed on 11 cells excited by both agonists (for methods, see above). In general, phenylephrine and DPI appeared to be of similar potency on cortical neurones. On

4 cells from which approximately equal responses to both agonists were obtained, the mean equipotent current ratio (±s.e. mean) (current for phenylephrine/current for DPI) was 0.9 ± 0.2. On 7 cells where both drugs were ejected with currents of equal intensity and duration, the mean ratio of the sizes of the responses (±s.e. mean) (response to phenylephrine/response to DPI) was 1.23 ± 0.06.

Effects of phenoxybenzamine on excitatory responses to phenylephrine and DPI The effects of phenoxybenzamine were studied on 8 cortical neurones excited by both phenylephrine and DPI. On all the cells tested the responses to both drugs were equally antagonized by phenoxybenzamine (0 to 5 nA), whilst responses to ACh were not affected (Figure 3).

Discussion

The results reported here indicate that DPI has a powerful excitatory action on cortical neurones in the rat. DPI had a greater apparent potency than dopamine, and was approximately equipotent with phenylephrine. However, the relative potencies of different drugs applied by microelectrophoresis may reflect physical rather than biological factors (see Curtis, 1964; Szabadi & Bradshaw, 1974), and it therefore remains to be determined whether the present results reflect the true biological potency of DPI on cortical neurones.

Previous studies with DPI indicate that this drug is a selective agonist at inhibitory dopamine receptors on molluscan neurones (Struyker Boudier *et al.*, 1975). Furthermore, on the basis of behavioural experiments, it has been suggested that DPI may also stimulate inhibitory dopamine receptors in the nucleus accumbens (Cools *et al.*, 1976a) and in the caudate nucleus (Cools *et al.*, 1976b) in the mammalian brain. However, the results described here indicate that this is not the case on rat cerebral cortical neurones. Firstly, DPI evoked excitatory responses on all but one of the 135 neurones studied, whereas dopamine both excited and depressed cortical neurones. Secondly, the dopamine receptor blocking agent, haloperidol, could discriminate between excitatory responses to dopamine and DPI: while responses to dopamine were antagonized, responses to DPI were not affected. Thus, it is unlikely that the responses to DPI are mediated by dopamine receptors.

In the present experiments, the α-adrenoceptor antagonist, phenoxybenzamine, antagonized equally responses to phenylephrine and DPI, while responses to the control agonist, ACh, were not affected. These results are consistent with the hypothesis that the excitatory responses to DPI are mediated by α-adrenoceptors. It is noteworthy that Ruffolo, Miller & Patil (1978) have reported that DPI stimulates α-adrenoceptors in the rabbit aortic strip preparation.

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