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A COMPARISON OF STEREOSPECIFICITY AT CENTRAL AND PERIPHERAL 'MUSCARINE-SENSITIVE' ACETYLCHOLINE RECEPTORS: OBSERVATIONS WITH THE ENANTIOMERIC FORMS OF PROCYCLIDINE AND TRICYCLAMOL

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1 Procyclidine resembles hyoscine in enhancing the effects of amphetamine on ipsiversive turning by mice with a unilateral central dopamine lesion.

2 The stereospecific index for procyclidine is not greater than 10, in contrast to 173 for acetylcholine receptors in ileum from the same mice.

3 This suggests that although the central effects of procyclidine in this test involve acetylcholine receptors similar to those at peripheral sites, they cannot be identical with them unless there are differences at some secondary site, for example, if the weaker enantiomer were a stronger inhibitor of dopamine uptake or if there were a stereoselective uptake process for procyclidine itself.

Introduction

The effects of anticholinergic drugs such as procyclidine in the control of Parkinson's disease are thought to involve a blocking action at 'muscarine-sensitive' acetylcholine receptors in the central nervous system. If these receptors are the same as in peripheral tissues, the stereospecificity should be the same but it does not appear to have been measured for these central actions. We have attempted to obtain some idea of central stereospecificity in mice with a unilateral central dopamine lesion (Von Voigtlander & Moore, 1973). In these animals amphetamine produces ipsiversive turning and Pycock, Milson, Tarsy & Marsden (1978) found that this was significantly potentiated by hyoscine and benztropine and depressed by physostigmine, arecoline and pilocarpine. Because the stereospecificity in these tests appeared to be much less than had been found for the same compounds on guinea-pig ileum (Barlow, 1971), experiments were also carried out with procyclidine on ileum taken from some of the mice.

Methods

Compounds

The samples of the resolved forms of procyclidine (HCl) and tricyclamol (iodide) were those used by Duffin & Green (1955) and by Barlow (1971). Their high stereospecific index on the guinea-pig ileum indicates their high stereochemical purity (Barlow, Franks & Pearson, 1972).

Carbachol chloride and hexamethonium bromide were obtained from Sigma Ltd.

Mouse isolated ileum

The mouse isolated ileum was set up in aerated Tyrode solution containing hexamethonium 0.28 mM, as with the guinea-pig ileum (Edinburgh Staff, 1974). The temperature was 37°C and contractions were recorded isotonically with a load of about 0.25 g. Carbachol was used as agonist but the tissue was much less sensitive than guinea-pig ileum and satisfactory control responses were usually obtained with 0.25 and 1×10^{-5} M carbachol (instead of 1 and 2×10^{-7} M). The same automated apparatus was used as with the ileum, with the agonist allowed to act for 30 s and applied every 90 s. The method of calculating dose-ratios is described by Edinburgh Staff (1974).

In each experiment the (+)- and (−)-enantiomers of procyclidine were tested in concentrations of 2.46×10^{-6} M on the same preparation and the dose-ratios were estimated. The mean dose-ratios were calculated but the stereospecific index could also be estimated in each experiment from the value of (dose-ratio for (−) minus 1)/(dose-ratio for (+) minus 1); the mean of these was also calculated.

Unilaterally lesioned mice

Mice were prepared by injecting 6-hydroxydopamine (HBr, Sigma; 16μg in 4 μl saline containing 0.1% ascorbic acid) freehand into the right striatum of

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LAC/G female mice under ether anaesthesia, as described previously (Von Voigtlander & Moore, 1973; Pycock, Tarsy & Marsden, 1975). Animals were tested 2 weeks after this operation, and those showing regular turning towards the lesioned side with (+)-amphetamine sulphate (Smith, Kline & French; 5 mg/kg i.p.) and turning away from the lesioned side with apomorphine hydrochloride, (Macfarlan Smith; 1 mg/kg s.c.) at a rate > 5 turns per min, were selected for the experiments with procyclidine and tricyclamol.

Turning behaviour was assessed from the number of net complete turns made in any one direction in a 1 min period at a predetermined time after drug injection. During testing periods mice were observed in individual Perspex boxes measuring 12 × 12 cm. The spontaneous turning was measured and after 10 min the animals were given amphetamine sulphate (5 mg/kg, i.p.) and turning behaviour was assessed 30 min later. Experiments were done in sets in which each animal received either saline or a low dose or high dose of either enantiomer. Tests were made every third day and continued until each animal had received all 5 treatments. Where possible the same animals were used in a subsequent set with different

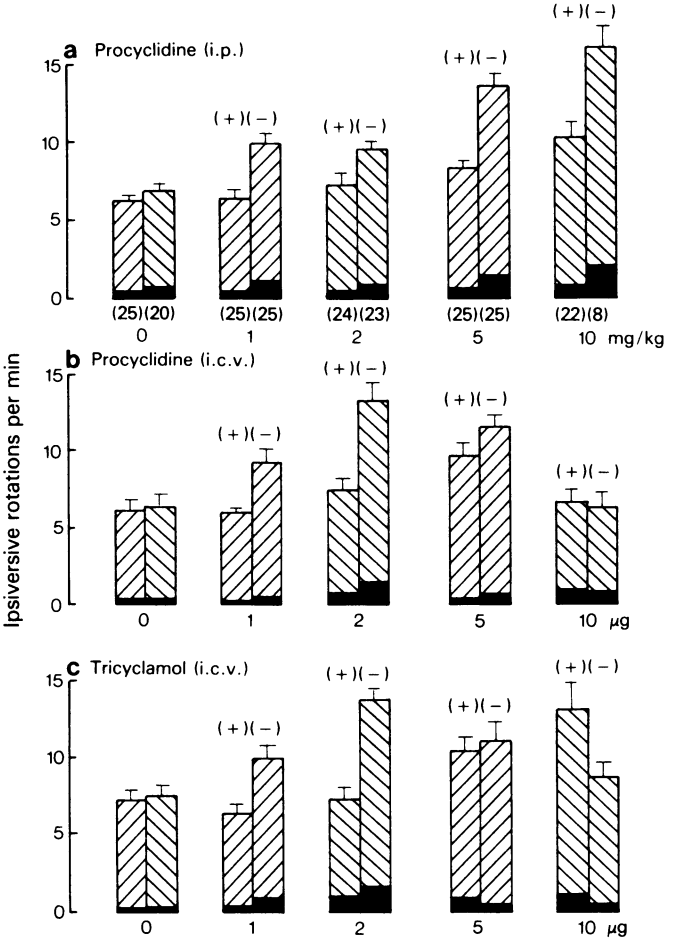

Figure 1 Effects on mice with a unilateral central lesion. The histograms indicate the mean number of ipsiversive rotations/min; the bars indicate the standard errors and the numbers of results are shown in parentheses: in (b) and (c) there were 12 results in all groups. Solid blocks show spontaneous ipsiversive turning before the injection of amphetamine. The hatching indicates groups which formed part of the same set. In (a) results are shown for the enantiomers of procyclidine given intraperitoneally; (b) shows the results when they are given intracerebroventricularly and (c) the corresponding results for the tricyclamol enantiomers. Note that the effects increase with dose but then decrease and that roughly comparable effects are produced by doses of the (+)-enantiomers which are 5 times those of the (−)-enantiomers.

doses of the compounds. A latin-square design was used to randomize the order in which the mice were selected for each treatment.

The procyclidine enantiomers were administered intraperitoneally in the range 1 to 10 mg/kg or intracerebroventricularly in the range 1 to 10 μg in 5 μl saline. The enantiomers of tricyclamol were given only by the latter route in the same dose-range. Intracerebral injections were made into the aqueductus cerebri in conscious mice as described by Brittain & Handley (1967).

Results and Discussion

The results are shown in Figure 1. When given intraperitoneally in doses from 1 to 10 mg/kg the enantiomers of procyclidine had very little effect on the turning of the unilaterally lesioned mice. However, in this dose-range, the ipsiversive turning effects of amphetamine were potentiated (Figure 1a). Qualitatively similar results were obtained by Pycock *et al.* (1978) with 1 mg/kg doses of hyoscine. With the larger doses there is stereotyped behaviour, sniffing and grooming, which reduces turning; this was particularly noticeable in the experiments with the drugs given intracerebroventricularly (Figure 1b and c). The dose-response curve appears to be bell-shaped and cannot be used to obtain a precise estimate of the stereospecific index but it seems that the effects produced by 5 to 10 mg/kg of (+)-procyclidine are comparable with those of 1 to 2 mg/kg of (−)-procyclidine, which implies that the stereospecific index cannot be greater than 10.

It is just possible that the low stereospecificity may be due to transport problems associated with the intraperitoneal route of injection. Although the relevant physicochemical properties of the enantiomers should be identical, the more active form might be metabolized more rapidly. A similar low stereospecificity was found, however, when the compounds were given intracerebroventricularly: this was also observed with the tricyclamol isomers. Low stereospecificity in these central effects is further confirmed by the appearance of stereotyped behaviour with the higher doses of the (+)-enantiomers; these are less than 10 times the doses of the (−)-enantiomers which have such effects.

These results are in marked contrast to the stereospecific index of 375 for procyclidine and 87 for tricyclamol observed on the guinea-pig ileum (Barlow, 1971). This is not a difference due to species. In the experiments on mouse intestine the mean estimate of the stereospecific index was 173 ± 20 (s.e., 4 estimates). The mean dose-ratios were 3.45 ± 0.6 for the (+)-enantiomer and 402 ± 91 for the (−)-enantiomer (tested in concentrations of 2.46×10^{-6} M): the mean estimates of log K were 5.95 and 8.18, compared with 5.69 and 8.27 for the (+)- and (−)-compounds, respectively, in the experiments on the guinea-pig ileum.

There is a difference of at least a factor of 10 between the relative binding of the enantiomers to the receptors in the ileum (assessed from dose-ratios) and their relative activities in effects on amphetamine-induced turning in centrally lesioned mice. From the work of Pycock *et al.* (1978) these central effects are likely to involve an acetylcholine receptor and the present findings could simply be explained by supposing that this receptor, though similar to that in peripheral tissues such as ileum, is not identical with it. (Perhaps the term 'muscarine-sensitive' should be avoided unless it is clear that it covers a variety of receptors rather than a single type). Otherwise it is necessary to suppose that the stereospecificity is less because some secondary process is involved in the test for central activity, for example the weaker enantiomer might be a more powerful inhibitor of dopamine uptake or there might be some stereospecific uptake process for procyclidine itself. However, such processes, would involve procyclidine binding groups and so constitute a type of receptor.

Although our model may not be valid for the treatment of Parkinson's disease, any evidence suggesting the existence of more than one type of receptor blocked by hyoscine or procyclidine is important in relation to the design and testing of potentially useful drugs.

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