Changes in dopamine-dependent motor behaviour caused by propranolol and its isomers

B. COSTALL*, R. J. NAYLOR, V. NOHRIA, R. T. OWEN, *Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, U.K.*

Doses of propranolol that have been shown to modify catecholaminergic mechanisms (Laverty & Taylor, 1968; Andén & Strombom, 1974) are generally considered inactive on cerebral dopamine systems, although dopamine turnover is modified when larger doses of propranolol are used (Wiesel, 1977), and the work of Fuxe, Bolme & others (1976) indicates the limbic system as a locus of action. The functional significance of such an action of propranolol is not known, but the mesolimbic dopamine system is considered to play an essential role in the modulation of psychomotor activity (see Stevens, 1973; Cools, 1976, Costall & Naylor, 1976a,b) and this focusses attention on preliminary clinical observations that propranolol, generally administered in high doses, may induce motor effects resembling those of dopamine receptor antagonists (Crawford, 1977) and may augment neuroleptic action in the treatment of schizophrenia (Yorkston, Gruzelier & others, 1977) and chorea (Martin, 1977).

On the basis of the clinical data, we have carried out a series of experiments to examine the neuropsychopharmacological profile of propranolol and its isomers using animal models of enhanced dopamine-like activity; dyskinetic phenomena caused by intrastriatal dopamine or 2-(*NN*-dipropyl)amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene in the guinea-pig, climbing behaviour induced by apomorphine in the mouse, and hyperactivity induced by dopamine injected into the mesolimbic nucleus accumbens of the rat.

Dyskinesias (biting, gnawing, limb and body movements) induced by the bilateral intrastriatal administration of dopamine 100 μg in 1 μl to guinea-pigs pretreated with nialamide (75 mg kg^{-1}, i.p., 2 h) (see Costall, Naylor & Pinder, 1975 for experimental details) were antagonized by two agents shown to have clinical antidyskinetic activity, oxiperomide and tiapride (Lhermitte, Agid & others, 1977; Bédard, Parkes & Marsden, 1978; Brugmans, personal communication) (for oxiperomide 1/6–6/6 animals inhibited in the dose range 0·5–2 mg kg^{-1} (i.p.), onset 2·5 min, duration 20–51 min. For tiapride 1/6–6/6 animals inhibited in dose range 80–160 mg kg^{-1} (i.p.), onset 95–140 min, duration 104–240+ min). (±)-, (+)- and (—)-propranolol all failed to antagonize the dopamine dyskinesias in doses of 10–40 mg kg^{-1} (i.p.). Abnormal peri-oral movements induced by injecting 2-(*NN*-dipropyl)amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene, 25 μg in 2 μl of nitrogen bubbled distilled water, bilaterally into the striata of guinea-pigs (see Costall, Naylor & Owen, 1977, for experimental details) were antagonized dose-dependently by haloperidol (0·5–2 mg kg^{-1}, i.p.), and by oxiperomide (0·13–0·5 mg kg^{-1}, i.p.), but not by the propranolol isomers (10–40 mg kg^{-1}, i.p.)

Climbing behaviour was induced in mice by apomorphine (1 mg kg^{-1}, s.c.) and measured in cages lined with wire mesh (see Costall, Naylor & Nohria, 1978, for experimental details). Briefly, the apomorphine 'response' generally had an onset of 5–7 min and lasted for 40–50 min: during this time animals persistently climbed the wire mesh 'walls' using all four limbs. One method of

Table 1. *Antagonism of the climbing behaviour induced by apomorphine in the mouse.* Climbing behaviour was induced by apomorphine 1·0 mg kg^{-1}, s.c. The climbing index represents the percentage of time spent climbing during the 30 min after the first climb. The mean ± s.e.m. are given. n = 6–8.

Drug (pretreatment)	Dose (mg kg^{-1}, i.p.)	Antagonism of climbing: Climbing index
Haloperidol (20 min)	0	74·6 ± 2·9
	0·006	73·7 ± 4·2
	0·013	52·3 ± 3·7**
	0·025	41·4 ± 7·5**
	0·05	10·7 ± 2·1***
	0·1	0***
Sulpiride (60 min)	0	78·6 ± 2·4
	0·63	75·2 ± 1·7
	1·25	70·6 ± 2·9
	2·5	62·7 ± 5·2*
	5	22·1 ± 4·3***
	10	0***
(±)-Propranolol (30 min)	0	78·2 ± 4·1
	10	77·6 ± 3·4
	20	65·1 ± 5·4
	40	0***
(+)-Propranolol (30 min)	0	79·3 ± 3·7
	2·5	76·4 ± 4·9
	5	74·9 ± 6·9
	10	40·2 ± 5·3**
	20	0***
(−)-Propranolol (30 min)	0	74·0 ± 3·1
	2·5	72·9 ± 3·6
	5	71·5 ± 4·9
	10	71·4 ± 7·1
	20	71·5 ± 5·6
	40	62·6 ± 9·2

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (Student's *t*-test).

* Correspondence.

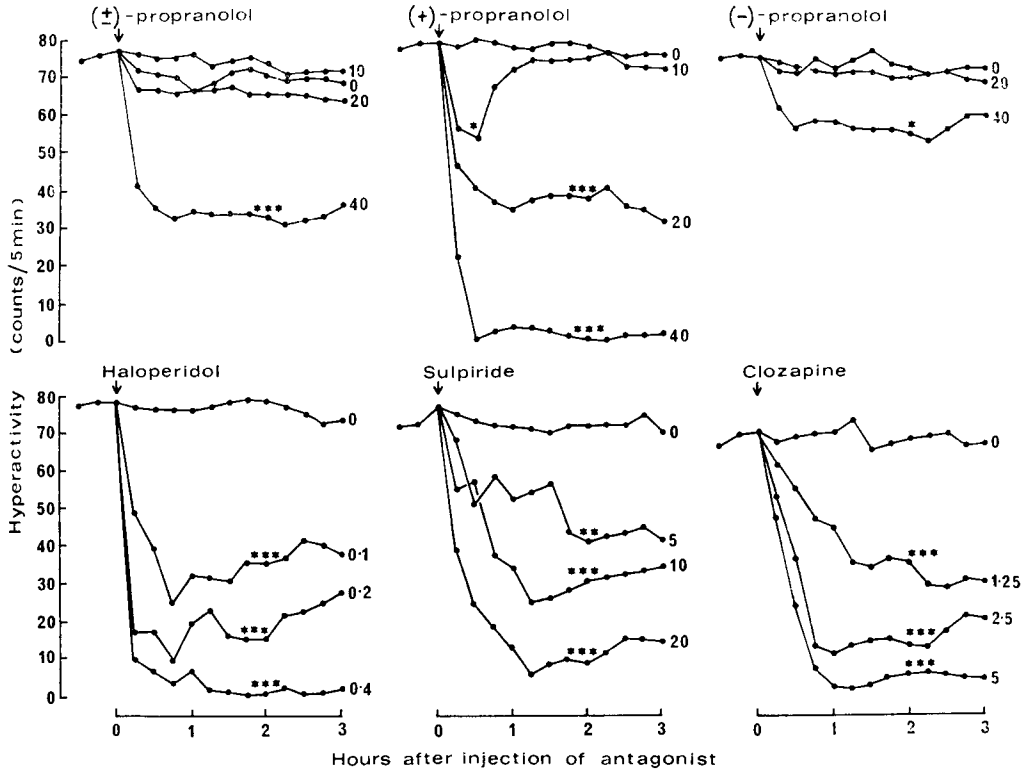

FIG. 1. Antagonism by (±)-, (+) and (−)-propranolol, haloperidol, sulpiride and clozapine of the hyperactivity induced by 50 μg dopamine injected bilaterally into the nucleus accumbens of rat following pretreatment with nialamide (100 mg kg^{-1}, i.p., 2 h). The arrow indicates the time at which the antagonists were given by the intraperitoneal route (2·5 h after dopamine). 5–10 rats were used at each dose of drug. Standard errors are less than 22% of the means. *P <0·05, ** P <0·01, *** P <0·001 (Student's t-test).

measuring this behaviour was to determine the percentage of time each animal spent climbing during the 30 min *after* the first climb: this measure has been termed the 'climbing index'. Pharmacologically low doses of haloperidol and sulpiride each antagonized apomorphine climbing in a dose-dependent manner (Table 1). (±)- and (+)-Propranolol also antagonized the mouse climbing behaviour at pharmacologically acceptable dose-levels and in a dose-dependent manner, the (+)-isomer being more active. The (−)-isomer was ineffective at the doses used (Table 1).

Hyperactivity was induced in the rat by 50 μg dopamine injected bilaterally into the nucleus accumbens following pretreatment with nialamide (100 mg kg^{-1}, i.p., 2 h) (see Costall & Naylor, 1976a, for experimental details). Haloperidol, sulpiride and clozapine each caused dose-dependent reductions in the hyperactivity (Fig. 1). (±)-(+)- and (−)-Propranolol also antagonized the hyperactivity in a dose range of 10–40 mg kg^{-1} (i.p.), but the effects of (+)-propranolol were the more marked (Fig. 1). The depression of hyperactivity recorded at 40 mg kg^{-1} (i.p.) (±)- or (+)-propranolol (and to a lesser extent, (−)-propranolol) was accompanied by marked sedation and muscular hypotonia, in which state animals were slow to respond to external stimuli.

The injection of dopamine and other catecholamines into the nucleus accumbens causes a marked increase in locomotor activity in the rat (Pijnenburg & van Rossum, 1973). This effect is considered to result from a stimulation of dopamine receptors since small doses of neuroleptic agent will inhibit the response whilst it is resistant to α-adrenoceptor blockers (piperoxan, phenoxybenzamine and phentolamine) and β-adrenergic antagonism ((±)-propranolol) (Jackson, Andén & Dahlstrom, 1975; Pijnenburg, Honig & van Rossum, 1975; Costall, Naylor & Pinder, 1976). In the present work, in which higher doses of (±)- and (+)-propranolol (40 mg kg^{-1}) were used, marked reductions or an abolition of the dopamine-induced hyperactivity was observed, although there was a concomitant decrease in general reactivity and muscle tone. The antagonistic effect of (+)-propranolol was also apparent at lower doses: (±)-propranolol was less active and (−)-propranolol exerted least activity. Two conclusions which may be drawn from these studies are, firstly, that the

relatively low efficacy of (−)-propranolol, the more potent β-receptor antagonist, suggests a relatively unimportant role for β-adrenergic antagonism in the modulation of the dopamine response and, secondly, that although the antagonistic effects could not be dissociated from 'non-specific' depressant or muscular changes when larger doses were used, a specific antagonism was recorded at 'lower' doses of (+)-propranolol. It is at the dose levels used in the present study that (±)-, (+)- and (−)-propranolol have been shown to cause significant decrease in dopamine fluoresecence in the nucleus accumbens, similarly to the neuroleptic agents (Fuxe & others, 1976).

In a further model, climbing behaviour was induced in the mouse by apomorphine and, again, this is a response which has been shown to be specifically antagonized by the neuroleptic drugs including 'atypical agents' such as sulpiride and clozapine (Protais, Costentin & Schwartz, 1976; Costall & others, 1978). The model has, indeed, been put forward as a useful test procedure for the detection of antipsychotic action. In the present study (+)-propranol was shown to be approximately twice as potent as (±)-propranolol in antagonizing apomorphine and the effect was clearly dissociated from any non-specific sedative action. (−)-Propranolol was effective at 4 times the dose of (+)-propranolol and, even then, caused only a modest reduction in climbing, which would lead us to suggest a non-essential role for β-adrenergic mechanisms in the control of this climbing phenomenon. Whilst the neuroleptic agents may exert a direct blocking effect on the apomorphine-sensitive receptor, it is less likely that this is the case for propranolol (Seeman, Lee & others, 1976).

Other models were used in this work to study the effects of propranolol and its isomers on abnormal motor function. Dyskinesias induced in the guinea-pig may be antagonized by selected neuroleptic agents, in particular oxiperomide and tiapride (Costall & Naylor, 1978). These latter two agents have been indicated as clinically active antidyskinetic drugs. (±), (+)- and (−)-Propranolol failed to antagonize the dopamine or 2-amino-1,2,3,4-tetrahydronaphthalene-induced dyskinesias: it would therefore appear unlikely that propranolol, in any isomeric form, is able to block *that* dopamine receptor or mechanism which is influenced by oxiperomide and tiapride.

It would be interesting to correlate the present observations with the preliminary clinical indications of a usefulness for propranolol in schizophrenia and mental disorders. Further clinical confirmation would make this a useful exercise but at the present time we put forward our findings to provide information of drug action on cerebral dopamine systems which may subsequently allow a better understanding of the clinical effects of propranolol in the treatment of schizophrenia and neurological disease.

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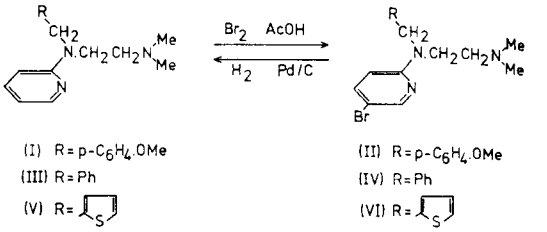
A convenient synthesis of [^{3}H]mepyramine and certain related [^{3}H]antihistamines

D. H. MARRIAN, S. J. HILL*, J. K. M. SANDERS†, J. M. YOUNG*‡, *Department of Haematological Medicine, *Department of Pharmacology, and †University Chemical Laboratory, University of Cambridge, Cambridge, CB2 2QD, U.K.*

In recent years valuable information on the numbers, distribution and biochemical properties of a wide range of drug receptors has been obtained from studies on the binding of receptor ligands. The success of this approach is dependent on the availability of high-affinity receptor-specific ligands radioactively labelled, usually with tritium, to high specific activity, and consequently the preparation and purification of such compounds is of particular importance. We have recently demonstrated that [^{3}H]mepyramine is a suitable ligand for the study of histamine H_1 receptors in guinea-pig small intestine (Hill, Young & Marrian, 1977) and brain (Hill & Young, 1978; Hill, Emson & Young, 1978). Similar results have been obtained by Chang, Tran & Snyder (1978) in rat brain. In this communication we describe in detail a convenient method for the preparation of [^{3}H]mepyramine. The synthetic route adopted has the advantages that it (a) starts from commercially available material, (b) involves one simple chemical reaction to produce the bromoderivative required for catalytic reduction, (c) yields a tritiated product of high specific activity with the tritium in a known position and (d) proceeds via an intermediate which has a lower affinity for the H_1 receptor than the final product [^{3}H]mepyramine. The last point, (d), is an important consideration if the problems which could arise from incomplete purification of the tritiated product are to be minimised.

The same synthetic route can be used for other non-halogenated antihistamines containing the 2-aminopyridyl residue, but for two we have examined, tripelennamine and methapyrilene, the particular advantage (d) above does not hold.

The route adopted (Scheme 1) turns on the ease with which 2-aminopyridines can be brominated to yield the corresponding 5-bromo-derivative. Thus mepyramine (I) on treatment with bromine in acetic acid yields the monobromo-derivative (II) which is easily and quantitatively converted back to the parent compound (I) by catalytic reduction in ethanolic triethylamine solution. The structure of the bromomepyramine (II) was established from the ^{1}H nmr spectrum, which clearly showed



that bromination had taken place in the 5-position of the aminopyridine ring, leaving the *p*-methoxyphenyl group intact.

The ready bromination of mepyramine (I) to give the 5-bromopyridyl derivative (II) suggested that this reaction should be applicable to other 2-aminopyridyl antihistamines. This was confirmed by the isolation of the 5-bromopyridyl-derivatives as the major reaction products from the bromination of tripelennamine (III) and methapyrilene (V). The nmr spectra of the bromo-componds (IV & VI) were again consistent with the structure assigned. Both were reduced to the parent compounds (III & V, respectively) with the consumption of the theoretical amount of hydrogen gas, but the reduction of bromomethapyrilene (VI) was slow, presumably because of the effect of the sulphur on the catalyst.

Bromomepyramine (II). Mepyramine maleate (5 g, May & Baker) was dissolved in water, treated with 5M NaOH (6·5 ml) and the free base formed extracted into ether. The ethereal layer was washed, dried over Na_2SO_4 and evaporated under vacuum. The residue in acetic acid (90 ml) was stirred at room temperature and bromine (2·0 g, i.e. equimolar quantity) in acetic acid (90 ml) added slowly. The resulting solution was evaporated and the residue taken up in a little warm water. The solid which separated on cooling was recrystallized from water (20 ml) yielding impure *N-2-(5-bromo)pyridinyl-N-[(4-methoxyphenyl)methyl]-N'N'-dimethyl-1,2-ethanediamine hydrobromide* (II) (4 g, m.p. 169–170°), which after recrystallization three times from methanol formed colourless prisms, m.p. 177–178° [Found (in material dried over P_2O_5 at room temp.): C, 45·8; H, 5·2; N,

‡ Correspondence.