Effects of chlorine substituents on the benzene rings of an analogue of the antipsychotic drug butaclamol on the interaction with dopamine and muscarinic receptors in rat brain

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Clozapine has been reported to inhibit psychotic reactions in man without producing extrapyramidal side effects (Angst et al 1971). In animals, clozapine differs from classical neuroleptic drugs in that it does not produce catalepsy, exhibits only weak antagonism of apomorphine and amphetamine stereotype behaviour, and elevates striatal homovanillic acid (HVA) concentration only at high doses (Stille et al 1971). The basis for the difference between clozapine and other neuroleptic drugs has been considered by some investigators to be related to an interaction between the antidopaminergic and antimuscarinic properties of the drugs (Andén & Stock 1973; Miller & Hiley 1974); others do not favour such a hypothesis (Bürki et al 1974).

The antipsychotic drug butaclamol (Hollister et al 1975; Mielke et al 1975) exhibits in animals a profile similar, both biochemically (Lippmann et al 1975) and pharmacologically (Voith & Herr 1975), to various other antipsychotic drugs; all of the activity is associated with the (+)-enantiomer (Humber et al 1975; Lippmann et al 1975; Voith & Cummings 1976). Compound (±)-*5*† is a butaclamol analogue (Table 1) in which the t-butyl group has been replaced by a 2-propyl group. The latter compound, and also (+)-*5*, exhibits a typical neuroleptic profile (Humber et al 1975; Pugsley & Lippmann 1976; Pugsley et al 1976). The purpose of the present study was to determine the effects on the interaction with dopaminergic and muscarinic receptors of compounds synthesized (Humber et al 1978) with chlorine substituents at positions 7, 10, 11 or 12 of the aromatic ring structure in (±)-*5*, and to compare the activities of the compounds with those of butaclamol, (±)-*5*, and clozapine. The studies have been carried out with in vitro and in vivo assay systems, i.e. in vitro dopamine-sensitive adenyl cyclase activity, [^{3}H]haloperidol binding to dopamine receptors, and [^{3}H]quinuclidinyl benzilate (^{3}H-QNB) binding to muscarinic receptors and in vivo striatal homovanillic acid (HVA) content.

Male Sprague Dawley rats (150–180 g; Canadian Breeding Laboratories) were used. The dopamine-sensitive adenyl cyclase in homogenates of rat brain olfactory tubercle was determined in the presence or absence of added dopamine as described previously (Lippmann et al 1975).

The evaluation of the dopamine receptor binding affinity of the test agents was based upon their ability to inhibit the binding of [^{3}H]haloperidol [(G) (sp. act. 13·5 Ci mmol^{-1}, New England Nuclear)] to a rat brain striatal membrane homogenate employing the method of Burt et al (1976). A rat brain striatal homogenate (11·1 mg protein per incubation tube) and the test agents were incubated at 37° C for 10 min with 2 nM [^{3}H]haloperidol in 50 mM tris-HCl buffer, pH 7·1, containing 0·1% ascorbic acid, 10 μM pargyline and various ions (Burt et al 1976). The incubation mixtures were filtered (Whatman GF/B filters) with vacuum, and the filters washed with ice-cold buffer and used for scintillation counting as described by Burt et al (1976). Stereospecific binding for [^{3}H]haloperidol is defined as the difference in binding obtained between incubations conducted in the presence and absence of 0·1 μM (+)-butaclamol.

Muscarinic receptor binding of the test agents was based upon their ability to inhibit the binding of ^{3}H-QNB to rat brain muscarinic receptors as described by Yamamura & Snyder (1974). Rat brain homogenate (whole brain minus cerebellum, 0·25 mg of protein in 50 μl of 0·32 M sucrose) and test agents (100 μl) were incubated for 60 min at 25 °C with 0·23 pmol of ^{3}H-QNB (sp. act. 13 Ci mmol^{-1}, Amersham/Searle) in 2 ml of 0·05 M phosphate buffer, pH 7·4. The incubation mixtures were filtered (Whatman GF/B filters) with vacuum, and the filters washed with ice-cold phosphate buffer and used for scintillation counting as described by Yamamura & Snyder (1974). Specific ^{3}H-QNB binding is defined as the total binding minus the binding in the presence of 100 μM oxotremorine.

In both of the above receptor binding assays, the IC50 is that concentration of test agent which displaces [^{3}H]haloperidol or ^{3}H-QNB binding by 50%, respectively, and was obtained by log-probit plots of the effects of 4 concentrations of each test agent assayed in triplicate.

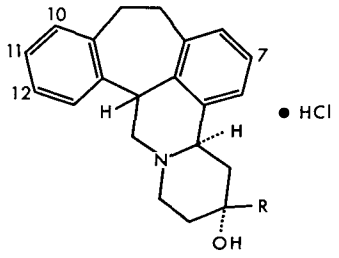
The effect of two of the test agents, each at a single dose, on rat brain striatal HVA content was carried out exactly as described before (Lippmann et al 1975). Protein was determined by the method of Lowry et al 1951).

Compounds *1* and *2* containing a chlorine substituent at position 7 and 10, respectively, exhibited a potency similar to butaclamol and (±)-*5* in inhibiting the dopamine-sensitive olfactory tubercle adenyl cyclase; in contrast, clozapine exhibited a weaker potency (about 3·5 fold less) (Table 1). Compound *4*, with the chlorine at position 12, exhibited a weak activity (about 4·5 times less); due to the limited solubility of *3*, con-

* Correspondence.

† Compound (+)-*5* is also known as dexclamol hydrochloride.

Table 1. Inhibition in vitro of the dopamine-sensitive adenyl cyclase of rat olfactory tubercle, [^{3}H]haloperidol and muscarinic (^{3}H-QNB) binding in rat brain.



Agent	R	Cl pos.	Adenyl cyclase act. IC50 (μM)*	[^{3}H]Hal. bind. IC50 (nM)**	^{3}H-QNB bind. IC50 (μM)***
(±)-*1*	$CH(CH_3)_2$	7	0·42	8·8	>1·0
(±)-*2*	,,	10	0·43	24	>5·0
(±)-*3*	,,	11	—	5	6·2
(±)-*4*	,,	12	1·9	>100	>5·0
(±)-*5*	,,	H	0·43	3·2	24
(+)-*5*	,,	H		1·4	
(−)-*5*	,,	H		>1000	
Butaclamol	$C(CH_3)_3$	H	0·35	3·5	49
(+)-Butaclamol	,,	H		1·4	
(−)-Butaclamol	,,	H		>1000	
Clozapine			1·45	110	0·016

Data in the above assays were obtained by varying the concentration of test agent in the presence of dopamine (20 μM), [^{3}H]haloperidol (2 nM) or ^{3}H-QNB (0·23 pmol).

* Test agent concentration causing 50% inhibition of stimulation of cyclic AMP production by dopamine-sensitive olfactory tubercle adenyl cyclase.

** Test agent concentration required to inhibit by 50% the specific binding of [^{3}H]haloperidol to rat striatal membrane.

*** Test agent concentration required to inhibit by 50% the specific binding of [^{3}H]quinuclidinyl benzilate (^{3}H-QNB) to rat brain homogenates.

taining the chlorine at position 11, the determination of an IC50 could not be achieved. None of these agents affected the basal concentration of adenyl cyclase in the absence of added dopamine. Since the dopamine-sensitive adenyl cyclase has been thought to represent at least part of the "dopamine receptor" (Kebabian et al 1972), and/or coupled to it (Snyder et al 1975), the action of the above mentioned agents is probably indicative of an ability to cause an in vitro blockade of a dopamine-receptor.

Recent studies have indicated that the potencies of various neuroleptic drugs in inhibiting the stereospecific binding of [^{3}H] haloperidol to dopamine receptors in the striatum correlate closely with their clinical and in vivo pharmacological potencies (Snyder et al 1975). As the results indicate (Table 1), the stereospecific binding of [^{3}H]haloperidol to rat striatal membranes was inhibited by butaclamol, (+)-butaclamol, (±)-*5*, and (+)-*5* with the IC50 being 1·4–3·2 nM; the IC50 for clozapine was much greater being about 110 nM. No inhibition was observed even at higher concentrations of (—)-butaclamol and (—)-*5*, i.e., IC50 >1000 nM.

Compounds *1* and *2*, containing a chlorine at position 7 and 10, respectively, were about 3 and 7·5 times less active, respectively, than butaclamol and (±)-*5* with regard to their affinity for the [^{3}H]haloperidol binding sites, although they were similar in activity to butaclamol and (±)-*5* with regard to inhibition of the dopamine-sensitive adenyl cyclase. In contrast, the compound containing the chlorine at position 12 (*4*) exhibited weak activity in each test, i.e., at least 33 and 4·5 times less, respectively. The 11-chloro compound (*3*) was the most potent in inhibiting [^{3}H]haloperidol binding as its activity was within two-fold of that of (±)-*5* (IC50: 5 *vs* 3·1 nM, respectively).

Striatal HVA concentrations in vivo are increased by dopamine receptor antagonists, e.g. neuroleptics, and decreased by dopamine receptor agonists, e.g. apomorphine. Such effects are indicative of an in vivo effect on dopamine turnover presumably owing to antagonism or stimulation of dopamine receptors in the striatum (Carlsson & Lindqvist 1963). Although only the 10- and 12-chloro compounds, i.e. *2* and *4*, were examined (because of limited supply), *4* was ineffective and *2* only weakly effective in elevating striatal HVA compared with butaclamol and (±)-*5* (Table 2). The findings with *4* and *2* on HVA also correlate with the results obtained in the [^{3}H]haloperidol binding study in which these chloro compounds were at least 33 and 7·5 times less potent in comparison, but not entirely with their effect on the dopamine-sensitive adenyl cyclase where *4* was 4·5 times less potent and *2* was equivalent in potency to (±)-*5* and butaclamol.

Other studies have indicated that the pharmacological and clinical potencies of butyrophenones correlate closely with their affinity for [^{3}H]haloperidol binding sites, but much less well with their effects on the dopamine-sensitive adenyl cyclase (Snyder et al 1975). The basis for the latter discrepancy and the discrepancy in the present study between the results obtained with the

Table 2. Effect on rat striatal homovanillic acid (HVA) concentration in vivo. Test agents or saline were injected 3 h (A) or 1 h (B) before the animals were killed. For each treatment there were 5 determinations, each consisting of pooled striata from 4 rats. The content of HVA in saline-treated rats was 0·69 ± 0·05 μg g^{-1}.

Agent	Dose (mg kg^{-1}, i.p.)	Striatal HVA (percent of control ± s.e.)
A.		
Saline	—	100·0 ± 5·6
(±)-*4*	1·2	95·7 ± 5·1
Butaclamol	0·2	283·6 ± 14·7**
B.		
Saline	—	100·0 ± 3·0
(±)-*2*	10	138·0 ± 10·4*
(±)-*5*	0·8	287·2 ± 11·6**

** P <0·001; * P <0·01 compared with saline.

dopamine-sensitive adenyl cyclase and those on dopamine metabolism and [^{3}H]haloperidol binding is not known; it may be related to the "various degrees of coupling of dopamine receptor sites with the adenyl cyclase" (Cuatrecasas 1974; Snyder et al 1975) and/or to the pharmacological specificity as revealed by studies which indicate that the dopamine-sensitive adenyl cyclase corresponds to that of the agonist state of the brain dopamine receptor (Iversen 1977). In addition, other evidence (Leysen & Laduron 1977) based on the different subcellular localization of the neuroleptic receptor and the dopamine-sensitive adenylate cyclase suggests perhaps a different function for the two receptors. Also, whether the basis for the discrepancy between the findings with the chloro compounds on the adenyl cyclase and on the HVA is due to a limited access to the brain of the compounds because of their physical properties and/or metabolism differences is not clear at this time.

One other possible explanation for the lack of effect in vivo on HVA by *2* and *4* is that these compounds may possess antimuscarinic activity. It has been postulated (Andén & Stock 1973; Miller & Hiley 1974) that the antimuscarinic activity of clozapine explains its lack of extrapyramidal side effects and its relatively weak activity in elevating striatal HVA (Stille et al 1971). In the present study, *2* and *4* were greater than 300 times less potent than clozapine in their in vitro affinity for cholinoceptors (Table 1). Thus, it is unlikely that the difference between the potency of *2* and *4* in vivo is due to their possessing an antimuscarinic property. None of the other agents examined (Table 1) exhibited appreciable inhibition of ^{3}H-QNB binding indicating a lack of any significant affinity for rat brain muscarinic receptors.

It is apparent from the present studies that the in vitro and in vivo potencies of (±)-*5* towards dopamine-related mechanisms are generally decreased by the presence of a chlorine in various positions of the ring system. In addition, pharmacological findings (Humber et al 1978) with (±)-*5* and the chloro analogues of (±)-*5* indicative of neuroleptic activity are in general agreement with the present biochemical findings. This is in contrast to that observed with other known neuroleptic agents where the introduction of a chlorine substituent in the ring system of a non-neuroleptic agent, e.g. promazine, yields a compound, chlorpromazine, which exhibits a greatly increased antidopaminergic activity (Snyder et al 1975). Moreover, the position of the chlorine also has an effect on this activity as exemplified by *3* (containing the chlorine at position II) which was the most effective of the chlorine compounds examined in the present study. Furthermore, the relevance of the position of the chlorine substituent is demonstrated by the findings that HF-2046, the "*cis*" isomer of the neuroleptic clozapine, differing from clozapine only in the position of the chlorine substituent relative to the piperazinyl side chain, displays a profile of a typical cataleptic neuroleptic agent in contrast to clozapine, a non-cataleptic neuroleptic agent (Bürki et al 1974; Miller & Hiley 1974).

Thus, the present study demonstrates that introduction of a chlorine substituent at various positions of the ring system of (±)-*5*, an analogue of butaclamol which exhibits similar effects, results in a decrease of activities which are characteristic of various neuroleptic agents and does not confer neurochemically the characteristics of clozapine, a non-cataleptic neuroleptic.

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