membranes by increasing the concentration gradient. The results reported here show that non-specific binding of chlorpromazine to brain constituents can occur, and will therefore account to some extent for the distribution of drug to the brain.

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Clozapine's anti-acetylcholine property modulates its antistereotypic action in the mesolimbic sytem

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Clozapine is a unique neuroleptic agent. Compared with the classical neuroleptics such as haloperidol, it possesses strong anti-acetylcholine properties (Miller & Hiley 1974; Snyder et al 1974) and lacks antistereotypic and cataleptic actions in animals (Stille et al 1971) as well as extrapyramidal side effects in man (Simpson 1974). In an attempt to explain this, it has been suggested that the neuroleptic has a selective antidopaminergic effect on the mesolimbic system (Andén & Stock 1973; Zivkovic et al 1975; Bartholini 1976). Since the latter system seems also to be associated with (+)-amphetamine-induced stereotypies (Pijnenburg et al 1975; Costall et al 1977), we have studied the action of clozapine alone or in combination with various acetylcholine-like and anti-acetylcholine agents given bilaterally into the nucleus accumbens septi on (+)-amphetamine-induced stereotyped behaviour. The action of central injections of low doses of haloperidol alone into the same region on (+)-amphetamine stereotypies has also been investigated.

Male Wistar rats, 200–250 g, were used in groups of 4–6, anaesthetized with halothane and implanted bilaterally with stainless steel cannulae into the nucleus accumbens septi (A = 9, V = 2, L = ± 1·5) (De Groot coordinates). 1–2 weeks after surgery the animals were challenged with a submaximal stereotypic dose of (+)-amphetamine sulphate (SKF) (3 mg kg^{-1} i.p.). After 20 min of (+)-amphetamine treatment, rats were restrained manually and given an intracerebral injection of either 0·9% NaCl (saline) or vehicle (control groups), haloperidol (Searle), clozapine (Sandoz) or a combined injection of clozapine together with oxotremorine sesquifumarate (Aldrich), physostigmine salicylate (BDH), scopolamine HBr (BW) or atropine sulphate (BDH). A 10 μl Hamilton syringe was used to deliver 2μl bilaterally at a rate of 1μl min^{-1}. All drugs were dissolved in saline except haloperidol (Serenace ampoules), and clozapine which was dissolved in saline acidified with 0·1 M HCl, and adjusted to pH 3·5 with 0·1 M NaOH.

Stereotypies were scored in individual animals at 15 min intervals for 90 min after the intracerebral injection according to the following rating scale: 1 = periodic mild sniffing, 2 = continuous sniffing, 3 = intermittent licking, gnawing or biting, 4 = continuous licking, gnawing or biting. Animals were used once and the site of injection was confirmed histologically. Statistical analysis was by the Mann-Whitney U-test.

Bilateral application of haloperidol, 2·5–5 μg into the nucleus accumbens septi (NAS) effectively antagonized (+)-amphetamine stereotypies (Fig. 1a). The antagonism appeared most pronounced at 45–90 min during which time very little stereotypic activity was observed. The application of 10 μg of clozapine into the same site produced no significant change in the stereotyped behaviour, while increasing the dose to 25 and 50 μg produced a potentiation (Fig. 1b), at both 75 and 90 min after injection.

The combination of 25 μg of clozapine together with the cholinergic agents oxotremorine (5 μg) or physostigmine (10 μg) effectively antagonized the stereotypies (Fig. 2a). The antagonism was rapid and lasted throughout the scoring period during which the animals appeared similar to those pre-treated with haloperidol. On the other hand, the combination of a subthreshold

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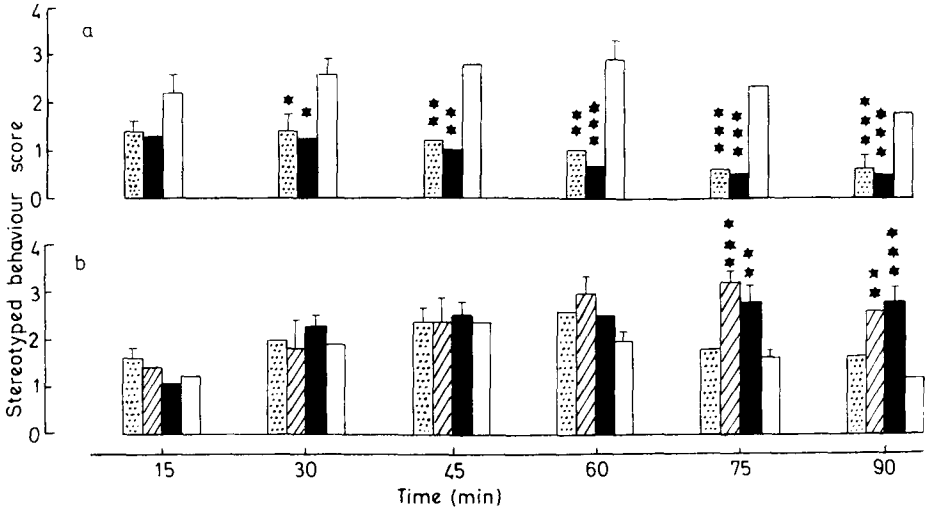

FIG. 1. The effect of bilateral injections of haloperidol and clozapine into the NAS on (+)-amphetamine stereotypies. (a), haloperidol 2·5 (stippled) and 5 (solid) μg; (b) clozapine 10 (stippled), 25 (hatched) and 50 (solid) μg were given 20 min after (+)-amphetamine administration (3 mg kg^{-1} i.p.). Open columns represent control groups. *$P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

10 μg dose of clozapine with the anti-acetylcholine agents scopolamine (20 μg) or atropine (15 μg) resulted in a significant potentiation of (+)-amphetamine stereotypies (Fig. 2b). The animals showed persistent licking and/or gnawing behaviour immediately after the bilateral injections. No significant effect on stereotypies was observed after the injection of oxotremorine or physostigmine alone into the NAS while the injection of scopolamine or atropine produced a slight although significant potentiation at the doses used.

Much evidence indicates that the stereotyped behaviour elicited by (+)-amphetamine is mediated by an effect on central nervous system dopamine (Randrup & Munkvad 1966; Weissman et al 1966; Scheel-Kruger & Randrup 1967). Studies with chemically induced as well as electrolytic lesions point towards the caudate/putamen as the target for (+)-amphetamine stereotypies (Naylor & Olley 1972; Asher & Aghajanian 1974; Creese & Iversen 1974). A detailed examination by Kelly et al (1975) and by Costall et al (1977) have shown that the NAS is involved in at least (+)-amphetamine-induced sniffing behaviour.

The (+)-amphetamine stereotypies were reduced, in the present study, by injections of haloperidol into the NAS. Inhibition of (+)-amphetamine as well as apomorphine stereotypies by injections of similar doses of haloperidol into the NAS and caudate/putamen was reported earlier by Pijnenberg et al (1975). Our results with the atypical neuroleptic agent clozapine were rather different from those obtained following haloperidol. Only high doses of the former were able to modify (+)-amphetamine stereotypies in which a potentiation was produced. The stereotypies potentiated by the 25 μg dose of clozapine were profoundly antagonized by a small dose of the muscarinic agonist oxotremorine and by the cholinesterase inhibitor physostigmine. It seems, therefore, that the anti-acetylcholine component of clozapine plays a critical modulatory role in its mesolimbic antidopaminergic action; when the anti-acetylcholine component is antagonized, the neuroleptic clearly acquires a marked antistereotypic action. The potentiation of stereotypies by the co-administration of the anti-acetylcholine agents scopolamine or atropine with an ineffective stereotypic dose of clozapine (10 μg) would also indicate a strong cholinergic-dopaminergic interaction within the NAS and is in agreement with systemic injection studies in which it has been shown that anti-acetylcholine agents potentiate (+)-amphetamine stereotypies (Klawans et al 1972).

Whilst the effect on (+)-amphetamine stereotypies induced by haloperidol, clozapine or the combined injections of clozapine with the acetylcholine-like and anti-acetylcholine agents given into the NAS most probably reflects interactions with dopamine and/or acetylcholine receptors within this region, it cannot be excluded that a diffusion of the solution from the area of disposition may influence associated structures. This may be particularly relevant to areas within the caudate/putamen which may be penetrated via the spread of injected solution along the cannula track. However, it has been observed that injection of the same higher doses of clozapine into the caudate/putamen tends to antagonize (+)-amphetamine stereotypies (unpublished observations). This would indicate that the net effect on stereotypies after NAS administration is the result of interactions within this region. It could also be suggested

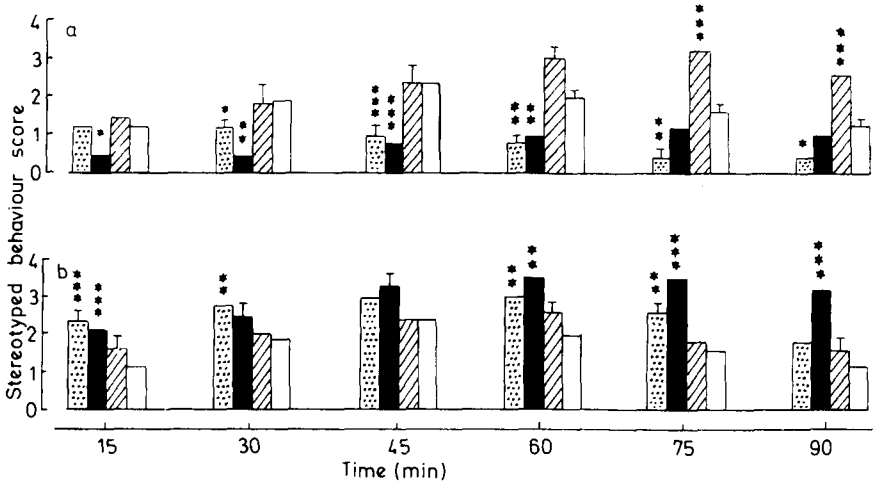

FIG. 2. The effect of combined bilateral injections of clozapine and acetylcholine-like/antiacetylcholine agents into the NAS on (+)-amphetamine stereotypies. (a) Clozapine (25 μg) plus oxotremorine (5 μg) (stippled), clozapine (25 μg) plus physostigmine (10 μg) (solid), and clozapine (25 μg) alone (hatched); (b) clozapine (10 μg) plus scopolamine (20 μg) (stippled), clozapine (10 μg) plus atropine (15 μg) (solid), and clozapine (10 μg) alone (hatched). Open columns represent control groups. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

that the reported inability of systemic injections of clozapine to modulate (+)-amphetamine stereotypies indicates an interaction at a different site within the dopaminergic mesolimbic and nigrostriatal systems following this route of administration.

Finally, it has been possible to show that the atypical neuroleptic agent clozapine can exhibit the same anti-dopaminergic properties as the typical neuroleptics in the mesolimbic system when its anti-acetylcholine component is removed.

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