# Inhibition of turning behaviour by clozapine in mice with unilateral destruction of dopaminergic nerve terminals

Clozapine (8-chloro-11-[4-methyl-1-piperazinyl]-5H-dibenzo[b,e][1,4]diazepine) is a novel neuroleptic agent. Unlike classical neuroleptics, it produces little or no catalepsy in animals and only weakly antagonizes apomorphine or amphetamine stereotypies (Stille, Lauener & Eichenberger, 1971; Stille & Hippius, 1971; Costall & Naylor, 1974). Yet clozapine is an effective anti-psychotic drug in man, which has little or no propensity to cause extrapyramidal side effects (Berzewski, Helmchen & others, 1969; Gross & Langner, 1969; De Maio, 1972). The outstanding biochemical effect of neuroleptics is their capacity to increase cerebral dopamine turnover (Carlsson & Lindqvist, 1963; Nybäck & Sedvall, 1968; Andén, Butcher & others, 1970) due, it is believed, to their ability to block central dopamine receptors leading to feedback excitation of dopaminergic neurons (Bunny, Walters & others, 1973). Such an increase in dopamine turnover causes a rise in cerebral homovanillic acid (HVA) concentrations (Andén & others, 1970), but Stille & others (1971) originally found that clozapine (in oral doses of up to 20 mg $kg^{-1}$) had little effect on HVA concentrations in the brain. These behavioural and biochemical studies might suggest that clozapine does not act by blockade of cerebral dopamine receptors, and in particular that it may not effect striatal dopamine activity. We, therefore, studied the effect of clozapine on circling behaviour in mice with a unilateral destruction of one nigro-striatal dopamine pathway (von Voigtlander & Moore, 1973). In such animals apomorphine causes circling towards the intact side, interpreted as due to preferential stimulation of the denervated striatal dopamine receptors, while amphetamine causes circling towards the lesioned side, due to release of endogenous dopamine from the intact nigro-striatal terminals (Ungerstedt, 1971).

Male Swiss 'S' strain mice (20–25 g) were anaesthetized with ether, and 16 $\mu$g of 6-hydroxydopamine in 4 $\mu$l chilled saline was injected free-hand into the right striatum according to von Voigtlander & Moore (1973). Ten days later animals showing strong contraversive circling to apomorphine (2 mg $kg^{-1}$, i.p.) and strong ipsilateral circling to amphetamine (5 mg $kg^{-1}$, i.p.) were selected. Clozapine was administered 45 min before apomorphine and 30 min before amphetamine in a dose range of 2·5–80 mg $kg^{-1}$ (i.p.). An incomplete Latin square design was used to randomize the distribution of the various doses of clozapine, and included a control series of experiments with saline. Fifteen min after administration of apomorphine and 30 min after amphetamine, the number of full circles completed by each animal was counted and compared with the rate of circling in control saline-treated animals.

Clozapine caused a dose-dependent inhibition of the intensity of both apomorphine and amphetamine-induced circling (Fig. 1). The number of mice that failed to turn in response to either drug was expressed as a percentage of the total number treated for each dose of clozapine, transformed into probits, and plotted against the log-dose of clozapine. The ED50 for inhibition of apomorphine-induced turning was 17 mg $kg^{-1}$, while that for inhibition of amphetamine-induced turning was 13 mg $kg^{-1}$.

These data indicate that clozapine, like other neuroleptic drugs is capable of blocking striatal dopamine receptors. Bartholini, Haefely & others (1972) found that clozapine in doses of 10–50 mg $kg^{-1}$ (i.p.), caused a dose-dependent increase in rat brain HVA and Dorris & Shore (1974) demonstrated a similar rise in rat striatal HVA. Andén & Stock (1973) found that clozapine (5 mg $kg^{-1}$, i.v.) caused a rise of HVA in corpus striatum and an even greater rise in the limbic areas of the rabbit brain. Thus the more recent biochemical evidence also suggests that clozapine is capable of blocking dopamine receptors in striatum, as well as in mesolimbic areas. Kelly, Miller &

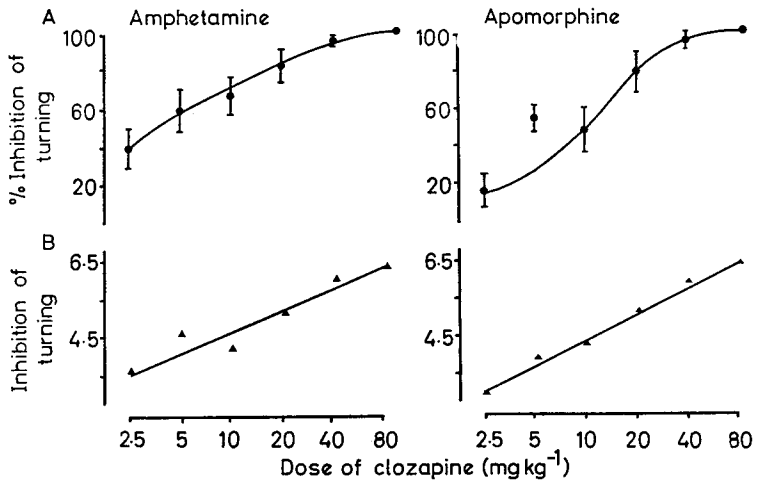


FIG. 1. A. Dose-response curves for inhibition of intensity of turning to amphetamine (5 mg $kg^{-1}$, i.p.) or apomorphine (2 mg $kg^{-1}$, i.p.) by clozapine. Mean turning rate for control animals 30 min after amphetamine alone was 8·0 ± 0·6 (1 s.e.m.) per min (n = 12), and 15 min after apomorphine alone was 8·7 ± 0·7 (1 s.e.m.) per min (n = 12). The reduction in mean number of turns per min for each dose of clozapine from that in saline-treated control animals is expressed as % inhibition of turning. (Means ± s.e.m. for 12 observations at each dose level are shown).

B. Probit-dose plots for abolition of turning to amphetamine or apomorphine. Each point was obtained from the average from 12 observations.

Sahakian (1974) reported that clozapine in doses of up to 18 mg $kg^{-1}$ did not effect the turning behaviour produced by methamphetamine in rats with 6-hydroxydopamine lesions of the substantia nigra. But our results indicate that clozapine does antagonize this behavioural effect of striatal dopamine receptor stimulation in the mouse model. The fact that clozapine has been shown to inhibit striatal dopamine-sensitive adenyl cyclase *in vitro* (Clement-Cormier, Kebabian & others, 1974; Miller & Hiley, 1974) provides direct evidence that clozapine blocks cerebral dopamine receptors.

The failure of clozapine, unlike most other neuroleptics, to cause frequent or clear-cut extrapyramidal side effects in man may be due to its inherent potent antimuscarinic properties (Miller & Hiley, 1974). The anti-muscarinic action of clozapine also may explain why the drug fails to cause catalepsy, only weakly antagonizes drug-induced stereotypy, and inhibits apomorphine- and amphetamine-induced turning in relatively high doses. Catalepsy produced by neuroleptics is antagonized by antiacetylcholine drugs (Costall & Olley, 1971), while amphetamine-induced stereotypy (Arnfred & Randrup, 1968), and circling behaviour (Pycock, Tarsy, Milson & Marsden, unpublished data) are both potentiated by antiacetylcholine drugs.

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# Urinary 3-methoxy-4-hydroxyphenylglycol production in mice and rats following intraventricular 6-hydroxydopamine

The longitudinal study of noradrenaline metabolism in the mammalian brain is hampered by the inaccessibility of the most relevant tissue. A major metabolite in the dog (Maas & Landis, 1968), the cat (Mannarino, Kirshner & Nashold, 1963), the rat (Schanberg, Schildkraut & others, 1968) and the rabbit (Rutledge & Jonason, 1967) is 3-methoxy-4-hydroxyphenylglycol (MOPEG). In particular, the sulphate conjugate of MOPEG has been shown to increase in the brains of rats when noradrenaline synthesis is stimulated by neuroleptic drugs (Keller, Bartholini & Pletscher, 1973) and to increase or decrease after stimulation or destruction, respectively, of the locus coeruleus (Korf, Aghajanian & Roth, 1973). The measurement of urinary MOPEG has therefore been proposed as a possible indicator of brain metabolism (Maas & Landis, 1968; Gitlow, Mendlowitz & others, 1971).

Intraventricular administration of 6-hydroxydopamine (6-OH-DA) results in a prolonged depletion of brain noradrenaline (Breese & Traylor, 1970; Uretsky & Iversen, 1970), a chronic reduction of tyrosine hydroxylase (Fibiger, Fibiger & Zis, 1973) and ultrastructural damage in brain regions rich in adrenergic terminals (Bloom, Algeri & others, 1969). There are, however, conflicting reports on how this specific destruction of catecholamine neuronal processes in rat brain (Breese, Prange & others, 1972; Karoum & Costa, 1974; Hoeldtke, Rogawski & Wurtman, 1974; Bareggi, Marc & Morselli, 1974) and primate brain (Breese & others, 1972; Maas, Dekirmenjian & others, 1972) is reflected by urinary MOPEG.