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General Pharmacology of Clozapine

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Clozapine shows neuroleptic-like inhibition of locomotor activity and conditioned avoidance responding in rodents, although tolerance develops on repeated treatment. EEG-based studies show strong arousal-inhibiting activity of clozapine as well as neuroleptic-like effects on both caudate spindle duration and rat sleep–waking patterns. Effects such as apomorphine blockade, catalepsy and strong increases of plasma prolactin levels are not seen, however, and chronic treatment does not lead to dopamine D_2 receptor supersensitivity. Binding studies show clozapine's highest affinities to be for dopamine D_4, 5-HT_{1C}, 5-HT_2, α_1, muscarinic and histamine H_1 receptors, but moderate affinity is also seen for many other receptor subtypes. Microdialysis studies indicate a preferential interaction with striatal D_1 receptors, whereas autoradiographical studies indicate upregulation of D_1 and downregulation of 5-HT_2 receptors after chronic clozapine. Clarification of the mechanisms underlying clozapine's special attributes is often hampered by a failure to examine compounds which show a close chemical relationship to clozapine, but which produce extrapyramidal side-effects in man, such as clothiapine, loxapine and amoxapine.

Clinical studies showing that use of the dibenzazepine clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e]-[1,4]diazepine) to treat schizophrenia was associated with a lower incidence of extrapyramidal side-effects (EPS) than with similarly effective agents (Angst *et al*, 1971; Stille & Hippius, 1971), led to the introduction of the term 'atypical neuroleptic' some 20 years ago. However, while clozapine continues to be the reference standard in this regard, newer findings show that its attributes range far beyond this low EPS liability. Thus, in schizophrenic subjects shown to be unresponsive to conventional neuroleptics, clozapine leads to a significant improvement of both positive and negative symptoms in approximately one-third of those treated for six weeks (Kane *et al*, 1988). These findings have emphasised the need for greater understanding of the pharmacological mechanisms underlying clozapine's unique properties, a situation made more acute by the increased risk of agranulocytosis associated with its use (see Krupp & Barnes, 1989). This paper presents an overview of clozapine's major pharmacological properties and compares and contrasts them with other clinically and chemically related agents.

Behavioural actions of clozapine

As with many conventional neuroleptics, acute administration of clozapine to mice or rats leads to dose-dependent sedation and a corresponding reduction of motor and locomotor activity. Its potency is approximately one-tenth that of haloperidol and somewhat greater than that of chlorpromazine (Table 1). Clozapine also shares an ability with these agents to inhibit conditioned avoidance responding of rats, but is strikingly weak in this regard (Table 1).

Table 1
Comparison of the ability of clozapine and other agents to induce catalepsy (CAT) and to inhibit locomotor activity (LMA), conditioned avoidance (CAR) and apomorphine-induced gnawing (APO) in mice (M) or rats (R) (ED_{50} in mg/kg)

Drug	ED_{50}LMA (M, p.o.)	ED_{50}CAR (R, p.o.)	ED_{50}APO (R, s.c.)	ED_{50}CAT (R, s.c.)
Clozapine	2.5[1]	20.0[1]	Inactive	Inactive
Clothiapine	0.6	–	0.3	0.7[1]
Octoclothepin	–	–	0.1	0.4[1]
Haloperidol	0.3	0.4	0.1	0.2[1]
Chlorpromazine	3.5	4.1	2.6	3.8[1]

Data derived from Schmutz (1975) and Stille *et al* (1971).
1.Tolerance developed on repeated administration.

In contrast to the situation with classical neuroleptics, both of these actions of clozapine exhibit complete tolerance on repeated administration (Stille *et al*, 1971; White, 1979; Sanger, 1985; Menon *et al*, 1988).

Clozapine also differs from conventional neuroleptics in two other areas considered to be of relevance to antipsychotic and EPS liability: the ability to inhibit apomorphine- or amphetamine-induced stereotypies and to induce catalepsy. Thus, whereas classical neuroleptics block the behavioural effects of apomorphine or amphetamine in the rat and induce catalepsy, clozapine is essentially devoid of such actions (Table 1). More recent studies have shown that clozapine in fact modifies certain dopamine-agonist-induced behaviours in the rat, but the use of selective D_1 and D_2 agonists is necessary for this to be seen. For example, Murray & Waddington (1990) demonstrated that clozapine inhibits the intense grooming induced by the selective

D_1 agonist SK&F 77434, and, in a less consistent manner, hyperactivity resulting from administration of the D_2 agonist LY 163502.

The failure of clozapine to induce catalepsy or block apomorphine-induced gnawing in rats is probably related to a further distinction between this agent and classical neuroleptics, namely, a failure to induce supersensitivity of dopaminergic mechanisms within the basal ganglia on repeated administration. In studies examining apomorphine-induced ipsilateral circling in unilaterally caudate-lesioned rats, for example, heightened responsiveness to apomorphine was observed after withdrawal from chronic treatment with haloperidol, but not after clozapine (Sayers *et al*, 1975). A similar difference between clozapine and conventional drugs is observed when they are examined for the ability to modify GABA sensitivity within the substantia nigra as judged by the contralateral circling response to intranigral application of the GABA agonist muscimol (Coward, 1982). The latter two findings are consistent with biochemical studies examining changes in the density of D_2 and GABA receptors within the basal ganglia in response to chronic neuroleptic administration (see below).

Biochemical properties of clozapine

As with chlorpromazine and many other neuroleptics, radioligand binding studies using homogenates from different mammalian brain areas or, in some cases, cell lines expressing cloned receptors, show that clozapine interacts with many neurotransmitter binding sites. In terms of absolute affinity, clozapine's most striking interactions are with α_1, 5-HT_{1C}, 5-HT_2, muscarinic and histamine H_1 receptors (Table 2), as well as with the newly described dopamine D_4 receptor (Van Tol *et al*, 1991; Table 3). Moderate and possibly clinically relevant activity is also seen with regard to several other binding sites, including D_1, D_2, D_3 (Sokoloff *et al*, 1990) and D_5 (Sunahara *et al*, 1991) receptors (Tables 2 and 3).

Table 3

Relative affinities of clozapine, its congener octoclothepin-R and the butyrophenones haloperidol and spiperone for various dopamine receptor subtypes as measured in brain, anterior pituitary or CHO/COS-7 cell lines ($pK_i = -\log K_i$, where K_i is inhibition constant in nM)

Drug	Receptor affinity (pK_i)				
	D_1	D_5	D_2	D_3	D_4
Clozapine	6.85	6.60	–	–	–
	–	–	7.75	6.74	–
	–	–	6.89	–	8.05
Haloperidol	7.6	7.3	–	–	–
			9.3	8.0	8.3
Spiperone	6.66	5.35	–	–	–
	–	–	10.15	9.21	10.30
Octoclothepin-R	–	–	7.87	–	8.80

Data derived from Sokoloff *et al* (1990); Sunahara *et al* (1991); Van Tol *et al* (1991).

However, allowing for the possibility of variations between *in vitro* and *in vivo* binding activity (see Andersen *et al*, 1986; Leysen *et al*, 1988), it should be noted that there are close similarities between clozapine's binding profile and that of chemically related but classical neuroleptics, such as clothiapine and loxapine, and the antidepressant/neuroleptic amoxapine (Table 2). Although the affinities of these agents for the newly described D_4 receptor are not yet known, findings with the related clozapine congeners, (S)- and, especially, (R)-octoclothepin (Table 3; see Van Tol *et al*, 1991) suggest that these compounds might also be expected to share clozapine's high D_4 affinity.

In keeping with the behavioural studies of Sayers *et al* (1975), chronic administration of clozapine does not result in an increase of D_2 receptors within the corpus striatum (Rupniak *et al*, 1985; LaHoste *et al*, 1991). This is in contrast to the characteristic effects of classical neuroleptics (Burt *et al*, 1976), and may be linked to the apparent failure of clozapine to induce tardive dyskinesia (Seeman, 1980). While there is no information as yet about the possible

Table 2

Relative affinities of clozapine and comparative agents for various neurotransmitter receptor subtypes as measured *in vitro* using homogenates prepared from calf, pig or rat brain ($pK_i = -\log K_i$, where K_i is inhibition constant in nM)

	Receptor pK_i							
	D_1	D_2	α_1	α_2	5-HT_{1A}	5-HT_{1C}	5-HT_2	5-HT_3
Clozapine	6.6	6.3	9.0	7.1	6.7	8.2	8.2	7.3
Clothiapine	8.8	8.8	7.9	6.1	5.5	7.6	9.0	6.8
Haloperidol	6.5	8.2	8.5	<6.0	<6.0	<6.0	6.6	–
Spiperone	<6.0	~10.0	8.7	6.1	7.2	<6.0	9.5	–
Amoxapine	6.8	7.2	7.4	5.8	6.1	8.5	8.8	–

Data generated by S. Urwyler and D. Hoyer, Sandoz Pharma Ltd.

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influence of chronic clozapine exposure on D_3, D_4 or D_5 receptor subtypes, there is clear evidence for adaptive changes of D_1 and 5-HT_2 receptor populations. For example, quantitative autoradiographical studies by Marshall and colleagues (LaHoste *et al*, 1991) show that treatment with clozapine (30 mg/kg/day, i.p.) for three weeks causes a 30–40% increase in D_1 receptor density in various basal ganglia and mesolimbic regions of rat brain. In the same clozapine-treated animals, 5-HT_2 receptor densities were significantly reduced (by approximately 30%) in various cortical regions, and in the ventral striatum. In contrast, comparable treatment with haloperidol (1.0 mg/kg/day) caused the expected increase of D_2 receptor density but failed to alter either D_1 or 5-HT_2 function. When more appropriate comparison drugs are examined with regard to the question of whether clozapine's influences on D_1 and 5-HT_2 receptor densities might make it unique, the situation changes slightly. For example, drugs such as clothiapine, loxapine and amoxapine downregulate cortical 5-HT_2 receptors in a similar fashion to clozapine (Lee & Tang, 1984; Matsubara & Meltzer, 1989). In contrast, various studies with classical neuroleptics considered to exhibit strong D_1 and D_2 blockade, such as *cis*-flupenthixol and fluphenazine, indicate the expression of surprisingly little D_1 blockade on an *in vivo* and/or chronic basis (Andersen, 1988; Hess *et al*, 1988).

Biochemical studies of the functional consequences of clozapine's monoamine receptor interactions in rats also reveal both similarities and differences between this agent and conventional neuroleptics. As with many classical neuroleptics, clozapine administration leads to increased concentrations of the major noradrenalin, dopamine and serotonin metabolites, 3-methoxy-4-hydroxyphenylethyleneglycol, homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) respectively, within the CNS (Buerki *et al*, 1975). However, whereas tolerance develops to clozapine's elevation of noradrenalin turnover, this is not so for its increase in HVA levels (Buerki *et al*, 1974). Together with the fact that striatal dopamine concentrations are increased rather than decreased after clozapine administration (Buerki *et al*, 1975), this indicates a qualitatively different interaction of this agent with dopamine control mechanisms to that produced by classical neuroleptics. Recent microdialysis studies in conscious rats have started to throw more light on this issue. Thus, there is considerable evidence that clozapine's elevation of striatal HVA concentrations arises from increased release of dopamine (Imperato & Angelucci, 1988), and that this is the result of a preferential interaction with D_1 rather than D_2 receptors. This conclusion stems from the fact that increases of extracellular dopamine and HVA concentrations produced by low doses (1–5 mg/kg s.c.) of clozapine are reduced after pre-treatment with selective D_1 agonists, whereas there is no evidence for D_2 blockade with these doses of clozapine, as judged by their failure to inhibit the decrease of dopamine release produced by direct intrastriatal application of the D_2 agonist LY 171555. At high doses of clozapine (20.0 mg/kg s.c.), LY 171555's effects are blocked, which indicates additional D_2 blockade (see Coward *et al*, 1989; Imperato & Angelucci, 1988).

In addition to their well known effects on monoamine release and turnover, neuroleptics can bring about secondary changes in neurotransmitter or neuromodulator function. Examples of this are the increase of striatal acetylcholine turnover which results from the diminished dopaminergic tone they produce, as well as related alterations of cholecystokinin (CCK) and neurotensin (NT) content within the basal ganglia or mesolimbic system (Govoni *et al*, 1980; Meyer & Krauss, 1983; Frey *et al*, 1986). Clozapine resembles haloperidol in its ability to increase central NT levels after acute administration, but differs from conventional neuroleptics in that this action exhibits tolerance after chronic exposure (Frey *et al*, 1986). Subsequent studies showed that this difference is not attributable to clozapine's concomitant anticholinergic properties (Frey *et al*, 1988). Examination of clozapine's indirect effects on basal ganglia GABA turnover reveals further differences between this agent and classical neuroleptics. For example, whereas haloperidol and similar agents reduce GABA turnover within the substantia nigra, clozapine enhances it (Marco *et al*, 1976; Mao *et al*, 1977). This characteristic of classical neuroleptics is reflected in the fact that chronic treatment with them leads to the induction of compensatory GABA-ergic supersensitivity within the substantia nigra (Gale, 1980). These effects may presage the eventual loss of striatonigral nerve terminals within this structure if treatment is continued on a long-term basis, which may be relevant to the emergence of tardive dyskinesias (Gunne & Haggstrom, 1983; Gunne *et al*, 1984).

Electrophysiological effects of clozapine

Clozapine's effects upon the normal or modified EEG of rats and rabbits are pronounced, and played a major role in its early pre-clinical characterisation. For example, clozapine leads to a marked increase of spindling (10–20 Hz, spindle-like bursts lasting 0.3–1.0s) in the basic EEG of the rabbit at a dose of 0.6 mg/kg i.v., an effect much greater than that produced by corresponding doses of haloperidol,

chlorpromazine, fluphenazine or loxapine (Stille *et al*, 1971). Similarly, clozapine is much stronger than classical neuroleptics as an inhibitor of EEG arousal reactions in the rabbit, regardless of whether they are elicited by arecoline administration or electrical stimulation of the ascending reticular formation (Stille *et al*, 1971). In rats, clozapine shares the ability of classical neuroleptics to prolong the rhythmic after-discharges known as caudate spindles which result from single, short-lasting stimulation of this nucleus, and also induces an atypical dozing pattern in the sleep-EEG which is considered to be indicative of likely antipsychotic potential (Stille *et al*, 1971).

Microiontophoresis studies using single cell recording show that clozapine and some (thioridazine, methiothepin) but not all (chlorpromazine, haloperidol) classical neuroleptics alter the firing rate of serotonergic neurons whose cell bodies are to be found within the dorsal raphe nucleus (Gallager & Aghajanian, 1976). This action could underlie or contribute towards clozapine's increase of 5-HIAA levels and, interestingly, appears to reflect a primary interaction with noradrenergic mechanisms (Gallager & Aghajanian, 1976). More recent studies have focused on the similarities and differences of the effects of clozapine and classical neuroleptics regarding the alteration of dopamine neuron firing rates within the substantia nigra (A9) and ventral tegmental (A10) areas. While acute administration of both classical neuroleptics and clozapine increases the firing rate of dopaminergic neurons in both these regions, differences arise after chronic administration. Thus, clozapine shares the ability of classical neuroleptics to induce a state of chronic depolarisation blockade within the A10 population, but fails to produce a similar effect on A9 neurons (Chiodo & Bunney, 1983; White & Wang, 1983). The failure of chronic clozapine to eventually depress A9 firing rates is shared by thioridazine, however, and may reflect these agents' intrinsic anticholinergic activity (Chiodo & Bunney, 1985).

Effects on prolactin

Clozapine produces a dose-related increase of prolactin levels in rats, but this effect is much weaker and shorter-lasting than that seen with classical neuroleptics (Meltzer *et al*, 1975). This may be due to a compensatory increase of dopamine synthesis in tuberoinfundibular neurons not seen with classical neuroleptics (Gudelsky *et al*, 1989). However, unlike classical neuroleptics, clozapine does not block dopamine-induced inhibition of prolactin release from cultured rat pituitary cells (Lamberts *et al*, 1990). Taken together, these findings suggest that both the initial releasing effect of clozapine and the mechanism(s) underlying its rapid attenuation are to be found at the suprapituitary level.

Discussion

The actions of clozapine after acute and chronic administration show only partial overlap with those of many conventional neuroleptics. Similarities after acute administration include its gross behavioural and EEG effects, as well as its inhibition of conditioned avoidance responding. However, rapid tolerance develops to some of these actions, making their clinical relevance difficult to assess. This situation is made more complex by the fact that clozapine's superiority over conventional neuroleptics with regard to EPS liability and efficacy in non-responders is large enough (see Kane *et al*, 1988) to suggest the existence of qualitative differences between these agents at the mechanistic level. While these do appear to exist, it is usually a question of what clozapine does not do in comparison with classical neuroleptics rather than what it does in addition. Examples of this are its failure to block apomorphine activity, cause catalepsy or induce either A9 neuronal block or supersensitivity of D_2 mechanisms after chronic administration (Table 4).

The lack of catalepsy and A9 blockade could arise from clozapine's intrinsic anticholinergic activity, but this would not explain its failure to inhibit apomorphine or cause D_2 supersensitivity (Sayers *et al*, 1976). Some light may be thrown on the latter issues by the discovery that clozapine shows a preferential interaction with D_1 rather than D_2 mechanisms in rats (Imperato & Angelucci, 1988;

Table 4
Qualitative similarities and differences between clozapine and conventional neuroleptics as seen in preclinical studies

Parameter	Clozapine	Other neuroleptics
Apomorphine inhibition	–	+
Catalepsy	–	+
Strong D_2 blockade	–	+
Induction of D_2 receptor supersensitivity	–	+
Preferential D_4 and D_1 receptor blockade	+	–
Depolarisation block of dopamine neurones[1]		
A-9	–	+
A-10	+	+
Strong 5-HT_2 receptor blockade	+	+[2]

1. After chronic administration.
2. Some conventional agents.

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Coward *et al*, 1989; Murray & Waddington, 1990), and produces lower D_2 but greater D_1 receptor occupancy in the CNS of schizophrenic patients than do classical neuroleptics (Farde *et al*, 1989; Farde & Nordström, this supplement, pp. 30–33). In addition to the possibility that D_1 antagonism might contribute towards clozapine's superior efficacy (see Coward *et al*, 1989), the finding of reduced levels of D_2 blockade might explain some of the above observations. Thus, the degree of D_2 receptor blockade produced by clozapine may be insufficient to bring about an increase in D_2 receptor numbers, or its association/dissociation pattern at this receptor may be sufficiently different from that of other neuroleptics (e.g. rapid) as to preclude the induction of compensatory receptor upregulation. Support for the first possibility stems from the fact that D_2 supersensitivity in animals is only produced by classical neuroleptics at much higher doses than those required to produce strong apomorphine blockade or catalepsy, suggesting the need for maximal or supramaximal D_2 receptor blockade. In the case of haloperidol, for example, strong apomorphine blockade and catalepsy induction are seen at oral doses of 0.2–0.5 mg/kg (Coward *et al*, 1990) whereas the induction of D_2 supersensitivity requires daily doses of 1.0–3.0 mg/kg (e.g. Sayers *et al*, 1975). With regard to the second possibility, positron emission tomography (PET) studies in monkeys indicate that clozapine may be more loosely bound to D_2 receptors than butyrophenone-type agents (Hartvig *et al*, 1986), whereas other findings show that several different principles appear to play a role in determining the manner and extent to which receptor upregulation occurs (Van Tol *et al*, 1990).

One way in which the consequences of individual quantitative differences between clozapine and other drugs might be amplified would be if they were to interact in an additive or synergistic manner. This principle has been suggested in connection with clozapine's strong 5-HT and weak D_2 blocking properties, where its overlap with other strong 5-HT_{1C}, 5-HT_2 and 5-HT_3 antagonists such as spiperone, loxapine and clothiapine (Hoyer *et al*, 1989; Meltzer *et al*, 1989; Canton *et al*, 1990) could be offset by the fact that these agents also produce strong D_2 blockade. However, on this basis, *in vitro* binding data (Table 2) indicate that the antidepressant amoxapine (a desmethylated form of loxapine) should exhibit clozapine-like activity in the clinic. In fact, although data from therapy-resistant schizophrenics are unavailable, amoxapine is at least known to cause classical neuroleptic-like EPS at high doses (e.g. Sunderland *et al*, 1983).

Similar reasoning can be advanced with regard to clozapine's newly described interaction with D_4 receptors. Thus, notwithstanding the possibility that classical agents such as loxapine, clothiapine and amoxapine might share clozapine's preferential affinity for the D_4 dopamine receptor – as octoclothepin appears to do (Van Tol *et al*, 1991) – it must be argued that the expression of the potentially beneficial effects of D_4 blockade produced by a classical drug such as spiperone (Table 3) is offset by its equally strong action at D_2 receptors (see Van Tol *et al*, 1991). A need for low D_2 blockade may also apply with regard to clozapine's blockade of D_1 receptors, although in this case there is no evidence that any classical neuroleptic consistently achieves the degree of D_1 occupancy in patients shown by clozapine (Farde & Nordström, this supplement, pp. 30–33). If further studies support this notion, it would represent not only a truly qualitative difference between clozapine and other drugs, but one where clozapine does something that other neuroleptics do not.

While it is to be expected that efforts to further clarify clozapine's mode(s) of action will intensify, aided by technological advances such as *in vivo* microdialysis, voltammetry, PET and single-photon emission computed tomography (SPECT), history suggests that this will be no easy task. In analogy to the difficulties of interpretation posed by early findings of clozapine-induced increases of striatal HVA concentrations in the absence of apomorphine blockade, for example, discrepancies are already arising between the interpretation of data derived from some microdialysis, voltammetry and microiontophoresis studies. Whereas some microdialysis (Chen *et al*, 1991) and voltammetry (Blaha & Lane, 1987) studies are supportive of chronic clozapine's ability to induce A10 depolarisation blockade, for example, others have not seen this correlation (Ichikawa & Meltzer, 1990). Also, as emphasised earlier, few studies examine the most appropriate comparison agents for clozapine, namely clothiapine, loxapine and amoxapine. It is to be hoped that greater awareness and consideration of this issue will contribute towards a more rapid clarification of clozapine's presently unique attributes.

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