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ON THE MECHANISM OF ACTION OF CLOZAPINE ON THE ADRENERGIC NEURONE

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1 The antipsychotic drug, clozapine, lowered noradrenaline and metaraminol (MA) concentrations in the rat heart. This action was blocked by the presence of a ganglionic blocking drug.

2 Other α-adrenoceptor blocking drugs (phenoxybenzamine, phentolamine) did not significantly lower heart amine concentrations. An inhibitor of neuronal amine uptake (desipramine) caused only a slight lowering. The combination of phentolamine and desipramine showed considerable activity, and this action was blocked by ganglionic blockade.

3 Clozapine had little or no action in blocking amine uptake, yet greatly potentiated amine release caused by the phentolamine-desipramine combination.

4 Other antipsychotic drugs (haloperidol, chlorpromazine, thioridazine) or other agents (propranolol, atropine) did not share this action of clozapine.

5 Ganglionic blockade markedly delayed amine release induced by reserpine administration.

6 It is suggested that clozapine may have an incomplete reserpine-like effect specifically on the adrenergic neurone, facilitating impulse-induced amine release.

Introduction

The antipsychotic drug, clozapine, is of considerable current interest since it causes little or no extrapyramidal dysfunction (Stille, Lauener & Eichenberger, 1971; Angst, Bente, Berner, Heimann, Helmchen & Hippius, 1971; Angst, Jaenicke, Padrutt & Scharfetter, 1971; Stille & Hippius, 1971), even though the drug, like other antipsychotics, enhances dopamine turnover in the corpus striatum and limbic dopamine centres (Bartholini, Haefely, Jalfre, Keller & Pletscher, 1972; Andén & Stock, 1973). In addition to its other actions, clozapine exerts α-adrenoceptor blocking effects and shows considerable cholinolytic activity (Stille *et al.*, 1971). In fact, it has been postulated that the latter effect may be responsible for the lack of overt striatal dysfunction after clozapine administration (Andén & Stock, 1973; Snyder, Banerjee, Yamamura & Greenberg, 1974).

Clozapine has also been shown to cause a marked depletion of brain and spinal cord noradrenaline (NA), an action which is blocked by high doses of the α-adrenoceptor agonist, clonidine (Bartholini, Keller & Pletscher, 1973). It has been suggested therefore, that the NA-lowering effect of clozapine is due to marked α-adrenoceptor blockade followed by compensatory adrenergic neurone hyperactivity such as to release the adrenergic transmitter faster than synthesis can maintain normal concentrations. In agreement with this concept is the observation that clozapine depletes spinal cord NA cranial, but not caudal, to the site of a spinal transection (Bartholini *et al.*, 1973).

The present study examines the effect of clozapine on peripheral adrenergic neurones of the rat heart. It is shown that the drug lowers the concentration of heart NA and also releases the non-metabolizable false adrenergic transmitter, metaraminol (MA) by a nerve impulse-dependent mechanism. It is shown, however, that unlike other α-adrenoceptor blocking drugs or antipsychotic drugs tested, clozapine appears to exert a further action directly on the adrenergic neurone so as to facilitate impulse-dependent amine release.

Methods

Female Sprague Dawley rats (200-250 g) were used. In experiments in which the release of the false adrenergic transmitter, metaraminol (MA) was examined, (Shore, Busfield & Alpers, 1964; Crout, Alpers, Tatum & Shore, 1964) the amine was loaded in tissues by administration of the MA precursor, DL-α-methyl-*m*-tyrosine (100 mg/kg s.c.; Regis Chemical Co.) 16-18 h before the experiments were started (Shore *et al.*, 1964).

Animals were killed by chloroform asphyxiation, hearts were removed and analyzed for NA or MA by fluorimetric methods described previously (Shore &

Alpers, 1964; Neff & Costa, 1966). Aqueous solutions of the following drugs were used: clozapine (Sandoz), haloperidol (McNeil Labs), phentolamine and chlorisondamine (Ciba), mecamylamine (Merck, Sharp-Dohme), (–)-metaraminol (Regis Chemical Co.), atropine (Sigma Chemical Co.), propranolol (ICI), chlorpromazine (Smith, Kline & French), and thioridazine (Sandoz). Phenoxybenzamine (Smith, Kline & French) was dissolved in a small volume of propylene glycol and diluted with water. Doses and routes of administration are indicated in the results section. Doses refer to free base forms of the drugs. The dose of clozapine used in this study (20 mg/kg) was selected as it is within the dose range studied in previous investigations (Bartholini *et al.*, 1972, 1973).

The marked hypothermia caused by clozapine administration was prevented by keeping clozapine-treated rats in a ventilated chamber at 32°C (Bartholini *et al.*, 1972).

Results

Administration of clozapine (20 mg/kg s.c.) every 2 h for 6 h resulted in a significant lowering of heart NA (Table 1), a lowering which was blocked by prior treatment with the ganglionic blocking drug, chlorisondamine. These results are qualitatively similar to the action of clozapine seen in brain and spinal cord and the effect of spinal transaction (Bartholini *et al.*, 1973).

This regimen of clozapine also lowered, but to a much greater extent (76%), the concentration of metaraminol (MA) in hearts of rats pretreated with the MA precursor. Pretreatment with chlorisondamine largely blocked MA release following clozapine. The greater effect of clozapine on MA than on NA is probably due to the fact that synthesis of NA tends to maintain the endogenous concentration of this amine while MA is not further synthesized. Since MA and NA responded in a qualitatively similar manner, further studies on the action of clozapine on the heart adrenergic neurone were carried out using MA measurement as the index of amine releasing action.

The experiments described above indicate that clozapine causes NA and MA release from heart by a nerve impulse-dependent mechanism. If clozapine were acting solely by virtue of its α-adrenoceptor blocking action so as to enhance reflexly adrenergic nerve traffic, then other α-blockers should mimic the action of clozapine. However, as shown in Table 1, neither phenoxybenzamine nor phentolamine caused a significant lowering of heart MA, suggesting that clozapine exerts an action in addition to that of α-blockade.

One possibility for a second action of clozapine would be inhibition of re-uptake of amine by the adrenergic neurone following release. However, according to Stille *et al.* (1971), clozapine has only a weak inhibitory action on the neuronal amine pump. Verification of this conclusion was obtained in experiments described in Table 2. In these experiments, the retention by the heart of MA given to rats was measured in the presence of a ganglionic blocking drug so as to eliminate the factor of impulse flow. The hearts of control rats accumulated considerable MA. Desipramine (desmethyl-imipramine), a potent inhibitor of the adrenergic neurone amine pump, markedly inhibited MA accumulation but clozapine had only a slight effect, indicating that clozapine has little effect on the amine uptake system.

Table 1 Action of clozapine and other α-adrenoceptor blocking agents on heart noradrenaline (NA) or metaraminol (MA) and effect of ganglionic blockade

	NA ($\mu g/g \pm$ s.e.)		*MA* ($\mu g/g \pm$ s.e.)	
Control	1.38 ± 0.06	(4)	0.49 ± 0.01	(11)
Clozapine	1.08 ± 0.09^{a}	(7)	0.12 ± 0.01^{c}	(10)
Phenoxybenzamine	–		$0.43 \pm 0.05^{b,e}$	(4)
Phentolamine	–		$0.50 \pm 0.01^{b,e}$	(4)
Chlorisondamine	–		$0.52 \pm .05^{d,e}$	(7)
Clozapine + chlorisondamine	1.24 ± 0.07^{b}	(8)	0.41 ± 0.01^{b}	(6)

NA or MA were measured 6 h after treatment of rats with clozapine (20 mg/kg, s.c. every 2 h), phentolamine (20 mg/kg, s.c. every 3 h) or phenoxybenzamine (15 mg/kg i.p.). Chlorisondamine, when used was given (5 mg/kg, s.c.) 6 h before killing animals (30 min before clozapine). Numbers in parentheses denote number of experiments.

(*a*) differs from control value ($P < 0.025$); (*b*) no significant difference from control; (*c*) differs from control value ($P < 0.001$); (*d*) differs from control ($P < 0.05$); (*e*) differs from clozapine ($P < 0.001$).

Despite these findings, we were concerned with the possibility that even a low degree of amine uptake blockade, when coupled with marked adrenergic impulse flow might be responsible for the clozapine-induced amine release. Experiments in which both phentolamine and desipramine were given to rats showed the expected finding that, over a 6 h period, a marked depletion of heart MA resulted, a depletion which was blocked by a ganglionic blocking agent, in this case, mecamylamine (Table 3).

Since relatively little MA was released in a 1 h period even by the combination of phentolamine and desipramine (Table 4), experiments were carried out at this short time to investigate whether clozapine would show an additional releasing action in the presence of the other drugs. As shown in Table 4, a single dose of clozapine caused a further lowering of heart MA, suggesting that the drug exerts an action directly on the adrenergic neurone, an action over and above that of enhanced impulse flow or possible uptake inhibition. Other antipsychotic drugs, haloperidol, chlorpromazine or thioridazine did not share this action of clozapine (Table 4). The β-adrenoceptor blocking drug, propranolol, did not facilitate release, nor did atropine (Table 4), suggesting that the direct neuronal action of clozapine was not associated with blockade of a presynaptic β-adrenoceptor or muscarinic site. Atropine, in fact, inhibited release of MA, possibly by inhibition of ganglionic transmission.

The experiments described thus far indicate that, in contrast to some other antipsychotic drugs and α-adrenoceptor blocking agents, clozapine has a direct facilatory action on amine release working in concert with enhanced nerve impulse flow. This action appears not to be associated with presynaptic α- or β-adrenoceptor sites or with a muscarinic site. One other possible mechanism for clozapine's action might be a

Table 2 Effect of desipramine and clozapine on the adrenergic neurone amine uptake system

		Heart MA (μg/g ± s.e.)	*Inhibition (%)*
Control	(8)	0.50 ± 0.02	–
Desipramine	(4)	0.12 ± 0.01*	76
Clozapine	(4)	0.42 ± 0.02†	16

Rats were given chlorisondamine (5 mg/kg, s.c.). Desipramine (3 mg/kg, s.c.) was given 30 min later. Clozapine (20 mg/kg, s.c.) was given 90 min before and 30 min after chlorisondamine. (–)-Metaraminol (MA) (50 μg/kg, i.v.) was given 1 h after chlorisondamine and rats were killed 1 h later. Numbers in parentheses denote numbers of experiments.

*Differs from control or clozapine ($P < 0.001$); †Differs from control ($P < 0.025$).

Table 3 Release of heart metaraminol (MA) by the combination of phentolamine and desipramine. Inhibition of release by ganglionic blockade

Treatment		*Heart MA (μg/g ± s.e.)*
Control	(8)	0.51 ± 0.03
Phentolamine	(8)	0.50 ± 0.01 NS
Desipramine	(4)	0.44 ± 0.03*
Phentolamine + desipramine	(4)	0.17 ± 0.03†
Phentolamine + desipramine + mecamylamine	(4)	0.47 ± 0.03

Rats were given desipramine (3 mg/kg s.c.) or phentolamine (20 mg/kg s.c.). When used in combination, phentolamine was given 30 min before desipramine. Mecamylamine, where used was given 30 min (5 mg/kg i.p.) before desipramine. Rats were killed 6 h after phentolamine. Numbers in parentheses denote numbers of experiments.

NS No significant difference from control; *Differs from control ($P < 0.05$); †Differs from other values ($P < 0.001$).

Table 4 The effect of clozapine and other agents on heart metaraminol (MA) when given to rats that had also received phentolamine and desipramine

Treatment		*Heart MA (μg/g ± s.e.)*
Control (Phentolamine + desipramine)	(20)	0.32 ± 0.01
Clozapine (20 mg/kg)	(12)	0.15 ± 0.02*
Haloperidol (2 mg/kg)	(4)	0.32 ± 0.03 NS
Chlorpromazine (20 mg/kg)	(11)	0.28 ± 0.02 NS
Thioridazine (20 mg/kg)	(11)	0.34 ± 0.03 NS
Propranolol (2 mg/kg)	(4)	0.37 ± 0.04 NS
Atropine (20 mg/kg)	(4)	0.47 ± 0.05†

Rats were given phentolamine (20 mg/kg s.c.) and desipramine (3 mg/kg s.c.). These rats served as controls. Other rats also received, at the same time, clozapine or one of the other agents listed. All agents were given subcutaneously. Rats were killed 1 h after drug treatment. Numbers in parentheses denote numbers of experiments.

*Differs from control ($P < 0.001$); NS No significant difference from control; †Differs from control ($P < 0.005$).

direct and selective effect on adrenergic storage granules so as to facilitate impulse-related release. Consistent with this possibility are previously described studies showing that reserpine-induced release of peripheral catecholamines is delayed by interruption of efferent adrenergic impulse flow (Holzbauer & Vogt, 1956; Brodie, Olin, Kuntzman & Shore, 1957; Hertting, Potter & Axelrod, 1962). This phenomenon was verified in the system described in the present studies. Thus, Table 5 shows that the release of MA from rat heart, when measured a short time after reserpine, was greatly inhibited by the presence of a ganglionic blocking drug.

Discussion

The present results show that administration of clozapine brings about the lowering of NA or MA concentrations in rat heart and that the effect is prevented by ganglionic blockade, indicating that the action requires neuronal impulse flow. This finding is in accord with the described effect of this drug on brain and spinal cord and inhibition of release by blockade of impulse flow, an observation which led to the suggestion that the depleting action of clozapine resides in its ability to block α-adrenoceptors, with a consequent feedback activation of NA neurones to such an extent that synthesis of the transmitter cannot keep pace with release (Bartholini *et al.*, 1972, 1973). However, it would seem that if this were the case, other α-receptor blocking drugs should share this action of clozapine, but neither phentolamine nor phenoxybenzamine significantly altered heart MA concentrations, unless given in combination with the amine uptake bocker, desipramine. It seems clear from the experiments described in the present study that clozapine has little or no effect on amine uptake mechanisms in the adrenergic neuronal membrane. Clozapine caused pronounced further lowering of MA, even in the presence of phentolamine and desipramine. This finding would seem to exclude the possibility that compensatory activation of neuronal firing by α-receptor blockade following clozapine administration could alone cause the marked lowering of heart amine levels, but that there must exist some additional action of clozapine.

There are now many reports of the existence of α- or dopamine receptors at the adrenergic nerve terminal which regulate impulse-induced NA release (See review by Langer, 1974). It is tempting to suggest that an action of clozapine may be the blockade of one of these receptors. However, our *in vivo* experiments gave no support to this possibility, as we were unable to demonstrate a clozapine-like action on heart MA release by the administration of other α-receptor or dopamine receptor blocking drugs.

Another possible site of action of clozapine could be at adrenergic amine storage granules to facilitate amine release. Although clozapine does not share reserpine's pronounced depleting action on 5-hydroxytryptamine and dopamine, it may have an incomplete reserpine-like action selectively on the adrenergic neurone. This possibility is strengthened by the demonstration that the depletion of heart MA by reserpine is greatly slowed by interruption of nerve traffic. Clozapine thus may exert a labilizing effect on adrenergic storage vesicles so as to facilitate amine release by the nerve impulse.

Table 5 The effect of reserpine alone and in combination with phentolamine and chlorisondamine on heart metaraminol (MA)

Treatment		*Heart MA (μg/g ± s.e.)*
Control	(6)	0.62 ± 0.02
Reserpine	(8)	0.32 ± 0.01*
Reserpine + phentolamine	(9)	0.30 ± 0.02*
Reserpine + phentolamine + chlorisondamine	(9)	0.50 ± 0.03†

Rats were given reserpine (2 mg/kg s.c.). Others were given reserpine and phentolamine (20 mg/kg i.p.) simultaneously. In other rats the combination was given 30 min after treatment with chlorisondamine (5 mg/kg s.c.). Rats were killed 1 h after reserpine. Numbers in parentheses denote numbers of experiments.
* Differs from control ($P < 0.001$); † Differs from reserpine or reserpine + phentolamine ($P < 0.001$).

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