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Effects of clozapine on cerebral catecholaminergic neurone systems

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Summary

1. Clozapine, a dibenzodiazepine derivative claimed to possess antipsychotic properties in man without producing extrapyramidal disorders, greatly increased the turnover of cerebral dopamine in the rat.

2. The drug itself was virtually devoid of cataleptigenic activity in rats; however, it antagonized prochlorperazine-induced catalepsy.

3. It is proposed that clozapine causes a blockade of striatal dopamine receptors which is of the surmountable type in contrast to that produced by cataleptigenic neuroleptics. In addition, clozapine may also increase the turnover of cerebral noradrenaline.

Introduction

The classic neuroleptic drugs, e.g. chlorpromazine and haloperidol, are thought to block dopamine receptors in the brain, thereby causing catalepsy in the rat and extrapyramidal disorders in man. Furthermore, these drugs enhance the turnover of cerebral dopamine which is generally assumed to be a direct consequence of the blockade of dopamine receptors (Carlsson & Lindqvist, 1963; Andén, Roos & Werdinius, 1964; Pletscher, Gey & Burkard, 1967; O'Keefe, Sharman & Vogt, 1970). Whether the altered activity of the extrapyramidal brain centres resulting from this blockade (Stille, 1971) is essential for the antipsychotic effect of neuroleptics in schizophrenia has not yet been clarified.

Recently, the tricyclic drug, 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo(b,e)(1,4) diazepine (clozapine) (Stille, Lauener & Eichenberger, 1971), has been reported to possess an antipsychotic action in schizophrenia without producing catalepsy in rats or major extrapyramidal disorders in man (Angst, Jaenicke, Padrutt & Scharfetter, 1971a; Angst, Bente, Berner, Heiman, Helmchen & Hippus, 1971b; Stille & Hippius, 1971). It was therefore of interest to investigate whether clozapine differs from classic neuroleptic drugs in its action on cerebral catecholaminergic neurone systems. So far, only the effect of the drug on homovanillic acid (HVA) in the rat brain has been studied but not definitely established (Stille & Lauener, 1971; Stille, *et al.*, 1971.

Methods

Male albino rats of Wistar origin (Füllinsdorf) weighing 200–300 g, kept at room temperature, were injected with various doses of clozapine i.p. Two hours later, HVA (Murphy, Robinson & Sharman, 1969) and dopamine (Laverty & Taylor,

1968) were determined in the brain (without cerebellum). Another group of animals was kept at an environmental temperature of 32° C which prevented hypothermia. Rectal temperature was measured every hour. These rats received an intraperitoneal injection of 300 mg/kg α-methyl-*p*-tyrosine-methylester hydrochloride (α-MT) alone or 1 h after 50 mg/kg clozapine i.p. Dopamine and noradrenaline (Laverty & Taylor, 1968) were assayed 2 h after the administration of α-MT. Rats injected with 0·9% w/v NaCl solution (saline) and kept at room temperature, served as controls. Catalepsy was measured as follows: The homolateral limbs of the rats were crossed by the experimenter; animals which maintained this imposed abnormal position for more than 10 s were considered to be cataleptic. After i.p. injection of either clozapine or 3-chloro-10-[3-(4-methyl-1-piperazinyl)-propyl] phenothiazine (prochlorperazine) followed by clozapine (see **Results** and Fig. 2), the number of animals which showed catalepsy was counted every 30 min during 6 hours.

Results

Clozapine in a dose range of 10–50 mg/kg i.p. caused a significant ($P>0·01$) dose-dependent increase in the cerebral concentration of HVA in rats kept at room temperature (Fig. 1). The maximal effect was attained 1–2 h following i.p. injection of 30 mg/kg clozapine and after 4 h the HVA concentrations had returned to normal. Similar results were obtained when the HVA measurements were carried out in the striatum. Clozapine (50 mg/kg) did not significantly affect the cerebral dopamine concentrations ($P>0·05$) (Fig. 1). Rats kept at 32° C in order to prevent the hypothermia produced by clozapine showed a somewhat higher rise of HVA than those at room temperature, whereas the dopamine content did not change.

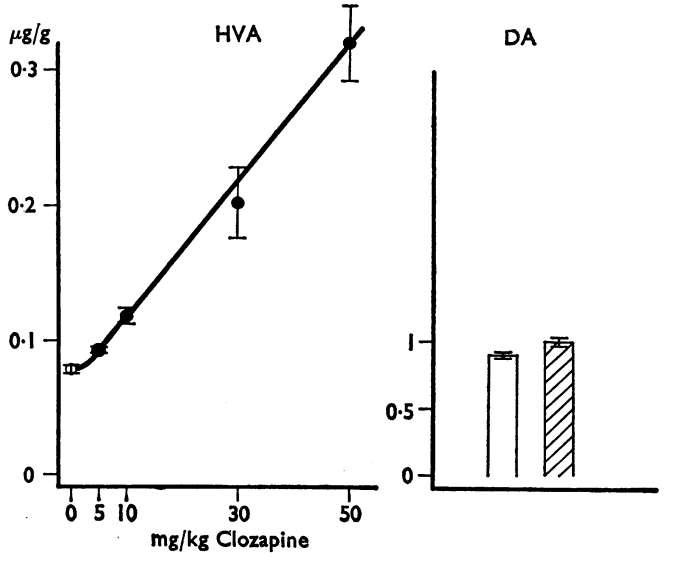

FIG. 1. Content of homovanillic acid (HVA) and dopamine (DA) in rat brain 2 h after i.p. administration of clozapine. In the dopamine experiments the dose of clozapine was 50 mg/kg. The values are expressed in μg/g wet tissue and represent means with S.E.M. of 3–10 and 3 experiments for HVA and dopamine, respectively. The point 0 on the abscissae indicates the concentration of HVA in control rats. Open column—control rats. Cross-hatched column—clozapine-treated rats.

Three hours after i.p. administration of 50 mg/kg of clozapine, the cerebral content of noradrenaline in normothermic animals (kept at 32° C) was decreased to 72·6±1·8% of controls ($P<0·001$) (Table 1). Doses of clozapine as low as 5 mg/kg already had a significant effect (reduction of cerebral noradrenaline to 93·1±1·5% after 1 h) ($P<0·01$).

The concentration of cerebral 5-hydroxytryptamine in normothermic rats was not changed and that of 5-hydroxyindoleacetic acid was slightly increased (120·6± 3·9% of control values) 1 h after administration of clozapine (50 mg/kg).

In normothermic animals the α-MT-induced decrease in cerebral dopamine and noradrenaline was significantly accelerated by clozapine ($P<0·001$) (Table 1).

Following intraperitoneal injection of 50 and 100 mg/kg clozapine, only 7 and 11%, respectively, of the rats were cataleptic. However, clozapine, in a dose-dependent manner, was able to antagonize the catalepsy induced by a dose of prochlorperazine (22·5 mg/kg i.p.) which produced a cataleptic effect in all animals (Fig. 2).

TABLE 1. *Effect of clozapine on the disappearance of cerebral catecholamines induced by α-methyl-p-tyrosine (αMT) in normothermic rats*

Treatment	Dopamine (%)	Noradrenaline (%)
Saline	100·0±1·3	100·0±3·3
Clozapine	107·4±1·7	72·6±1·8*
αMT	53·2±1·4	66·4±2·4
αMT+clozapine	40·5±1·2†	26·9±1·3†

Clozapine (50 mg/kg) and αMT (300 mg/kg) were injected intraperitoneally 3 and 2 h respectively, before the rats were killed. The concentration of dopamine and noradrenaline in control rats were 0·903±0·012 and 0·545±0·018 μg/g wet weight, respectively. Averages with S.E.M. of 3 experiments, each performed with 4–6 rats per group.

* Comparison with control value; $P<0·001$ (Student's t test).

† Comparison with concentration in animals treated with αMT alone; $P<0·001$ (Student's t test).

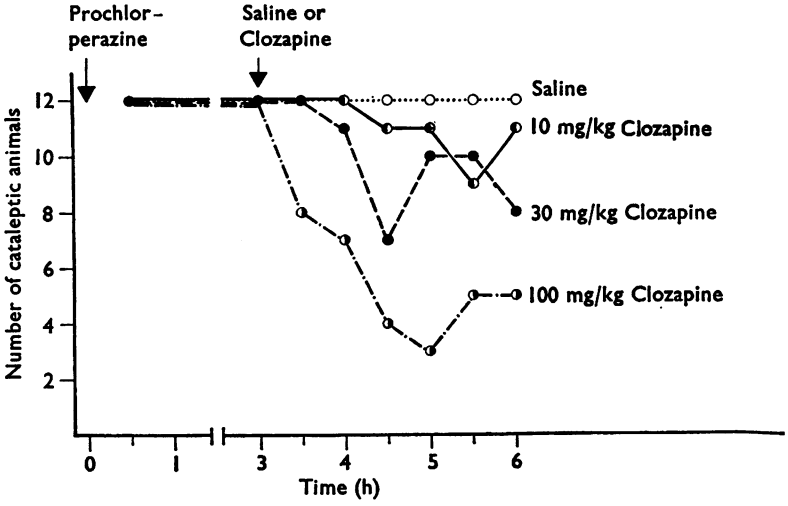

FIG. 2. Antagonism of the prochlorperazine-induced catalepsy by clozapine. Groups of 12 rats were given a dose of prochlorperazine i.p. (22·5 mg/kg) which produced catalepsy in all animals. Three hours later, 0·9% w/v NaCl solution (saline) or clozapine was injected i.p.

Discussion

The increase in the concentration of cerebral HVA observed after the administration of clozapine is probably not due to a reserpine-like release of dopamine from its tissue stores since the drug does not change the concentration of the endogenous cerebral amine. An amphetamine-like liberation of dopamine also seems improbable, as the rise in HVA is too pronounced. Furthermore, clozapine probably does not raise the HVA concentrations by an unspecific inhibition of the outflow of phenolcarboxylic acids from the brain since the drug caused only a slight increase in cerebral 5-hydroxyindoleacetic acid. Therefore, the clozapine-induced elevation of HVA concentrations is likely to be the result of an accelerated dopamine turnover. The enhancement of the α-MT-induced decrease in cerebral dopamine by clozapine supports this view.

The present results demonstrate that clozapine, in contrast to the classic neuroleptics, has little cataleptigenic action even in doses which greatly enhance the turnover of cerebral dopamine, for with doses as high as 50 and 100 mg/kg only slight cataleptic symptoms were observed. The virtual absence of catalepsy in rats treated with clozapine does not seem to be due to a central anticholinergic action of the drug which might antagonize its possible cataleptic effect. In fact, the sinistrotorsion of guinea-pigs elicited by an injection of physostigmine into the right carotid artery (De Jonge & Funcke, 1962) was poorly antagonized by clozapine in contrast to anticholinergic drugs and chlorpromazine (Jalfre, unpublished results).

The surprising observation that clozapine affects the dopaminergic system of the basal ganglia without producing catalepsy, requires further investigation. As a working hypothesis we propose that clozapine, like the classic neuroleptics, causes a blockade of dopamine receptors in the striatum which is assumed to enhance the dopamine turnover by a neuronal feed-back mechanism. However, the blockade of dopamine receptors by clozapine, in contrast to that of classic neuroleptics, is possibly of the surmountable type. On this assumption, the clozapine-induced receptor blockade may be partially overcome by the increased liberation of dopamine at the receptor site resulting from the feed-back activation of dopaminergic neurones, thus preventing catalepsy. This hypothesis is supported by the findings that clozapine antagonizes the cataleptigenic action of high doses of prochlorperazine and, as we have observed in preliminary experiments, has an additive effect with low doses of this potent neuroleptic drug. This suggests that both clozapine and prochlorperazine occupy the same binding sites at the dopamine receptors.

Clozapine greatly accelerated the decrease in noradrenaline induced by α-MT. Since clozapine caused a diminution of endogenous cerebral noradrenaline, a direct amine-releasing action of clozapine cannot be excluded. However, it has been reported that clozapine is a potent peripheral α-adrenoceptor blocking agent (Stille & Hippius, 1971). Therefore, the enhancement of the α-MT-induced noradrenaline decrease by clozapine might also be the result of a blockade of central noradrenaline receptors leading to an increased impulse flow in noradrenergic neurones as a consequence of a feed-back mechanism. It has to be assumed that the enhanced release of noradrenaline due to the increased neuronal activity is not fully compensated for by the synthesis of the amine. This insufficient noradrenaline synthesis may result from an inadequate activity of dopamine-β-hydroxylase rather than of tyrosine-hydroxylase, since the latter is able to maintain the dopamine concentration at a normal level. An inhibition of dopamine-β-hydroxylase by clozapine was not observed in preliminary experiments (Kettler, unpublished results).

Whether the effect on dopamine and/or noradrenaline receptors is related to the reported antipsychotic action of clozapine in schizophrenia (Angst *et al.*, 1971a, b; Stille & Hippius, 1971) remains to be investigated.

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