Effect of clozapine on the turnover of dopamine in the corpus striatum and in the limbic system

Antipsychotic and extrapyramidal actions are often considered to be the main properties of neuroleptic drugs (Bobon, Janssen & Bobon, 1970). Recently, a compound, clozapine,* has been described to efficiently inhibit psychotic reactions in man without producing any clearcut extrapyramidal side-effects (Berzewski, Helmchen & others, 1969; Gross & Langner, 1969; De Maio, 1972). Also in animal experiments, this drug has been observed to differ from the classical neuroleptic drugs in the sense that it produces no catalepsy and only a weak antagonism of the stereotypes induced by apomorphine and amphetamine (Stille, Lauener & Eichenberger, 1971; Stille & Hippius, 1971). The most characteristic biochemical change produced by neuroleptic drugs is an increase in the turnover of the brain dopamine (Bobon & others, 1970). In the present work, the clozapine-induced influence on the turnover of dopamine has been studied in determining its principal metabolite, homovanillic acid (HVA), in the rabbit brain. Since dopamine occurs both in the corpus striatum and in some nuclei of the limbic system (about 75 and 25%, respectively, of the total dopamine in the rabbit brain; Andén, 1972), these two regions have been analysed separately.

Adult, white rabbits, 1·2 – 1·9 kg, were injected intravenously with 5 mg kg^{-1} clozapine at different time intervals before death or with different doses of clozapine, or the classical neuroleptic agent haloperidol, 4 h before death by intravenous injection of air. The brains were rapidly dissected on ice-cold Petri dishes.The brain parts from two animals were pooled. First, the corpus striatum was removed. Then, the greater part of the limbic system including all the dopamine nuclei (amygdala, preoptic area, olfactory tubercle, part of the nucleus accumbens, nucleus interstitialis striae terminalis, and septum, but not hippocampus or gyrus cinguli) was removed by incisions just in front of the optic chiasma and through the rhinal fissures. Finally, pieces of the occipital cortex, whose weights were between those of the corpus striatum and the limbic system, were cut out. The HVA was determined spectrofluorimetrically after freezing the tissue on dry ice, extraction with 0·1 N HCl, anionic exchange chromatography and oxidation (Andén, Roos & Werdinius, 1963; Korf, Roos & Werdinius, 1971). The homogenate of the corpus striatum was divided into two aliquots and 3 μg HVA were added to one of them. The recovery through the whole procedure was 90 $\pm$ 1·1% (mean $\pm$ s.e., n = 67). No correction for recovery was made.

Clozapine (5 mg kg,$^{-1}$ i.v.) produced an increase in the concentration of HVA both

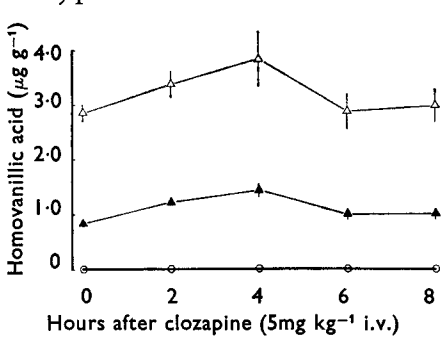

FIG. 1. Concentrations of homovanillic acid in the corpus striatum (△-△), the limbic system (▲-▲) and the occipital cortex (○-○) at different times after intravenous injection of clozapine (5 mg kg^{-1}) to rabbits. The values are means $\pm$ s.e. from 5 experiments at each interval.

* (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine).

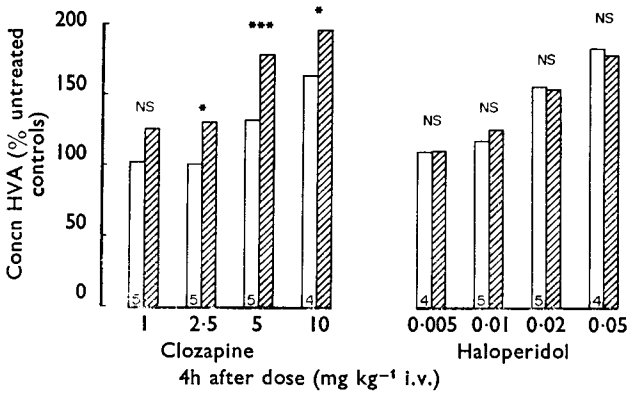

FIG. 2. Concentrations of homovanillic acid (HVA) in the corpus striatum (open columns) and in the limbic system (hatched columns) of rabbits 4 h after intravenous injections of clozapine or haloperidol in different doses (mg kg^{-1}). The values are expressed in per cent of untreated controls run in parallel (see text). They are means of 4–5 experiments as indicated in the figure. The whole material was treated by one-way analysis of variance followed by *t*-test (degrees of freedom within groups 58, variance within groups 447·379, F = 9.880). The statistical significances of the differences between the corpus striatum and the limbic system are indicated above the corresponding columns ($P < 0{\cdot}001$: ***, $P < 0{\cdot}05$: *, $P > 0{\cdot}05$: NS = not significant).

in the corpus striatum and in the limbic system. In both samples, the maximal effect was seen after 4 h and the values had returned to normal after about 6 h (Fig. 1). No significant amounts of HVA were detected in the occipital cortex at any time interval. This dose of clozapine did not induce any obvious change in the concentration of dopamine in the corpus striatum or in the limbic system of rabbits (data not shown). Previously, the peak effect of haloperidol on the HVA in the rabbit corpus striatum was observed between 3 and 6 h after i.v. injection of 0·5 mg kg^{-1} (Andén, Roos & Werdinius, 1964). Hence, the concentrations of HVA in the corpus striatum and in the limbic system were compared 4 h after intravenous injections of different doses of clozapine or haloperidol (Fig. 2). In each experiment, the pooled corpora striata or limbic systems of two untreated rabbits were analysed as a control and the value set to 100%. Each individual value in the experimental groups was calculated as per cent of the concentration found in the control, run the same day. In Fig. 2, each column represents the means of 4–5 such values. All the doses of clozapine tested induced a greater percentage rise of HVA in the limbic system than in the corpus striatum. The difference was highly significant ($P < 0{\cdot}001$) after 5 mg kg^{-1} and probably significant ($P < 0{\cdot}05$) after 2·5 and 10 mg kg^{-1}. On the other hand, all doses of haloperidol elevated the HVA to about the same extent in the corpus striatum and in the limbic system when tested in the same response range.

About the same difference between the limbic system and the corpus striatum, as described here after treatment with clozapine, was obtained when haloperidol was given in combination with an anti-acetylcholine drug (Andén, 1972). Therefore, it is of interest that the acetylcholine receptors have been reported to be blocked by clozapine but not to any noticeable extent by other neuroleptic drugs such as haloperidol (Stille & others, 1971). This anti-acetylcholine property could explain why clozapine fails to produce catalepsy and to antagonize apomorphine and amphetamine stereotypies in animals, since the same effects are seen when other neuroleptic drugs are given together with anti-acetylcholine agents (Morpurgo & Theobald, 1964). Anti-acetylcholine drugs are also well-known to reduce the extrapyramidal manifestations without interfering with the antipsychotic action in man treated with neuroleptic drugs. The biochemical and functional findings after treatment with clozapine can, thus, be due to a combined neuroleptic and anti-acetylcholine effect.

There is, of course, also the possibility that the dopamine receptors in the limbic system are blocked to a greater extent than those in the corpus striatum by clozapine but not by haloperidol. Finally, the results indicate that determinations of HVA in the corpus striatum and in the limbic system of rabbits may be of value in predicting the ability of neuroleptic drugs to induce in man extrapyramidal and antipsychotic actions, respectively.

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The effect of trivastal, haloperidol and dibutyryl cyclic AMP on [^{14}C]dopamine synthesis in rat striatum

We have previously reported that apomorphine inhibits the biosynthesis of [^{14}C]-dopamine from [^{14}C] tyrosine in striatal slices more effectively than it inhibits tyrosine hydroxylase activity *in vitro* (Goldstein, Freedman & Backstrom, 1970). The effective inhibition of [^{14}C] dopamine biosynthesis in striatal slices by apomorphine could either be due to accumulation of the drug in the dopamine-containing neurons and its subsequent inhibition of tyrosine hydroxylase or to the drug's stimulation of the dopamine receptor resulting in a feed-back control of dopamine biosynthesis. We have now further investigated the effects of activation and blockade of dopamine receptors on [^{14}C] dopamine biosynthesis in striatal slices. For the activation of the dopamine receptors animals were treated with trivastal (1, 2″-pyrimidyl)-4-piperonyl-piperazine: (T495) a recently described dopamine receptor stimulating agent (Corrodi, Fuxe & Ungerstedt, 1971) and for blockade haloperidol was used. Since adenylate cyclase may be the receptor for dopamine in the striatum (Kebabian, Petzold & Greengard, 1972) we have also investigated the effects of dibutyryl cyclic AMP (dB-cAMP) on [^{14}C] dopamine biosynthesis in striatal slices.

Male Sprague-Dawley rats, 250–300 g, were decapitated and the striata immediately dissected, sliced and incubated at 37° in Krebs Henseleit medium. The incubation