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Striatal and frontal cortex binding of 11-C-labelled clozapine visualized by positron emission tomography (PET) in drug-free schizophrenics and healthy volunteers

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Abstract. The binding of 11C-labelled clozapine in the brain was studied in three drug-free schizophrenic patients and in three healthy volunteers. High radioactivities were found in the striatum and in the frontal cortex. The rate constant k_3, which is proportional to receptor association rate and the number of receptors, was lower in the frontal cortex compared to the striatum. No obvious difference between the two brain areas was seen for the dissociation rate constant from the receptors (k_4). Two schizophrenic patients were reexamined after pretreatment with haloperidol, one after 6 weeks of treatment with a low oral dose, the other one after an IV injection 1 h before 11C-clozapine was given. After haloperidol pretreatment, the binding of 11C-clozapine in striatum and frontal cortex was reduced, more pronounced in the striatum, indicating competition for D-2 dopamine binding sites. Our finding indicates that clozapine has an affinity for a receptor population in the frontal cortex that is predominantly not of the dopamine-D2 type. This feature might be of importance for the unique clinical profile of the drug.

Key words: Positron emission tomography – Schizophrenia – Frontal cortex – Clozapine – Dopamine receptors – Second messenger

Clozapine, a dibensodiazepine derivative, has a clinical and pharmacological profile that differs from classical neuroleptics (Stille et al. 1971; Simpson and Varga 1974). It has a clearcut antipsychotic effect without inducing extrapyramidal side effects (EPS) (Angst 1971; Ekblom and Hâggström 1974; Gerlach et al. 1974). In the rat, clozapine does not induce catalepsy and has no or only a weak effect on amphetamine-induced stereotypies (Stille et al. 1971; Fog 1972). These differences when compared to other neuroleptic drugs have led various investigators to propose that clozapine may exert its antipsychotic effect by means of other mechanisms than dopamine receptor blockade (Hyttel 1974; Burki et al. 1975). Others have explained the lack of EPS by a more selective affinity of clozapine for mesolimbic relative to striatal dopamine receptors (Anden and Stock 1973; Sawyers et al. 1975).

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In contrast a close correlation has been demonstrated between the affinity for central dopamine receptors in vitro and the antipsychotic potency in man for a variety of neuroleptics (Seeman et al. 1976). Further, a close relationship between antipsychotic efficacy of neuroleptics and affinity for the dopamine-D2 receptor subtype has been demonstrated in animal studies (Peroutka and Snyder 1980).

In the last few years the possibility has emerged to study receptor binding properties of psychoactive drugs by use of positron emission tomography (PET). In studies on central nervous dopamine receptors, 11C-methyl-spiperon, 11C-raclopride, 11C-SCH23390 and 11C-bromospiperone among others, have been used as ligands (Wagner et al. 1983; Farde et al. 1985, 1987, 1988a, b; Maziere et al. 1985; Sedvall et al. 1986).

Previous studies in monkeys using the PET technique have shown 11C-labelled clozapine affinity for dopamine receptors in the striatum (Hartvig et al. 1986). When compared to two other dopamine receptor antagonists, 11C-methyl-spiperon and 11C-raclopride, the affinity k_d for the dopamine receptors was lower for clozapine (Hartvig et al. 1988). Because of the relatively small size of the monkey brain, binding to other brain areas was difficult to evaluate although some binding to serotoninergic receptors in the frontal cortex was suggested (Hartvig et al. 1988). Occupation of central nervous dopamine receptors by clozapine has also been demonstrated in humans (Farde et al. 1988b). They found that a patient on clozapine treatment had a 65% occupancy of dopamine D2-receptors in putamen using 11C-labelled raclopride as the ligand.

The present study is a part of investigations intended to provide knowledge of the properties of an atypical neuroleptic, clozapine. Due to its unique clinical profile, strong antipsychotic action and lack of EPS, we postulate that clozapine differs from classical neuroleptics with respect to pharmacological (Bondesson and Lindström 1988; Cheng et al. 1988), receptor binding properties and anatomical distribution within the brain.

Methods

Subjects. Three healthy male volunteers (age: 33, 35 and 41 years) and three male schizophrenic patients (age: 24, 34 and 39 years) participated in the study, which was ap-

proved by the Ethics Committee of the Medical Faculty, University of Uppsala and the local Isotope Committee of the University Hospital in Uppsala. All participants gave their informed consent. One patient had never received neuroleptics and the other two had been without medication for more than 5 years. Computed tomography of their brains showed no abnormalities. One patients was re-examined with PET after 6 weeks of treatment with a low dose of haloperidol (1 mg b.i.d.) and another patient was given 1.0 mg haloperidol IV 1 h before a new investigation using 11C-clozapine.

Synthesis of N-(methyl-11C)clozapin. The radionuclide, 11C, was obtained at the Tandem Accelerator Laboratory at the The Svedberg Laboratory, University of Uppsala after bombardment of a target with nitrogen and trace amounts of oxygen using a 10 MeV proton beam. The 11C atoms produced in the nuclear reaction rapidly formed 11C-carbon dioxide in the target. The 11C-carbon dioxide was in a series of steps converted to 11C-methyl iodide and used in the N-alkylation of the demethyl analogue of clozapine (Långström et al. 1982, 1984). The product was analyzed with respect to identity, radiochemical and chemical purity by reversed-phase liquid chromatography. The radioactivity given, after filtration through a 0.22 μm filter, was 40–100 MBq. The specific radioactivity of the radiolabelled drug injected intravenously to the subjects was 100–200 MBq/μmol.

Positron emission tomography. The subject was placed in a helmet in a fixed position in the tomograph so that the lowest transection of the head included cerebellum, thalamus, part of the brainstem and the basal section of the temporal lobes. A PC 384-3B positron emission tomograph (AB Scanditronix, Uppsala, Sweden) equipped with two detector rings was used, allowing radioactive distribution in three transaxial slices interspaced with 14 mm to be recorded. Images were obtained from the time of injection of the radioactive dose and for 12, 40 and 300 s at predetermined intervals. Data collection and analysis of images from the positron emission tomograph were done as described previously (Eriksson et al. 1982). The regions of interest from CT-scan and PET images were outlined simultaneously. The following regions of interest were analyzed: areas corresponding to striatum, mesencephalon, thalamus, frontal cortex, white substance of the temporal lobes, occipital cortex and cerebellum. Extracranial soft tissue and eyes were also analyzed. The measured activity with PET in different regions was corrected for physical decay of radioactivity to the time of administration of the radioactive dose.

Calculation and kinetic analysis. The relative distribution of 11C-clozapine derived radioactivity in the brain as measured with PET as "uptake" was calculated from the corrected radioactivity per cm^3 and divided by the radioactive dose/g body weight. Thus a value of 1.0 corresponds to the radioactivity uptake that would have been obtained with an even distribution of the dose in the body. No correction was made for blood-borne radioactivity in the brain. The blood volume is only 6–8% of the total volume of the tissue measured in the regions of interest and the reference tissue. The lipophilic profile of clozapine also contributes to a proportional higher activity in the tissue compared to the blood. Only during the first minutes after administration will the blood-borne radioactivity affect the total brain activity more than a few per cent (Eckernâs et al. 1987).

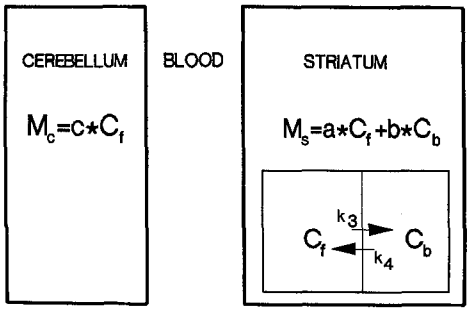

Fig. 1. Part of three compartment model used in the PET calculations

A three-compartment model has been used in the calculation of receptor kinetics with PET (Fig. 1), comprising plasma, free and bound ligand in the tissue (Wong et al. 1974). Under certain conditions, such as presence of a receptor-free region, i.e. cerebellum and poor penetration of radiolabelled metabolites across the blood-brain barrier, the model can be reduced to two compartments including only free and bound ligand concentrations (Wong et al. 1984; Eckernâs et al. 1986; Lundqvist 1986; Hartvig et al. 1988). The two-compartment model, using high specific radioactivity, can be presented by the following equation:

$$dC_b/ds = k_3 * C_f - k_4 * C_b \quad (1)$$

where C_f and C_b are the free and bound ligand concentrations, respectively. The kinetic analysis of this reduced model has been discussed recently (Eckernâs et al. 1986).

Assuming tracer conditions, k_3 is a constant proportional to the association rate k_{on} and numbers of receptors, B_{max}, whereas k_4 represents the rate of dissociation from the receptor. The ratio k_3/k_4 is, assuming tracer conditions, also equal to B_{max}/k_D. k_D is the ratio of the molecular dissociation and association rates.

The data were analysed in the following way. The measured radioactivity in striatum (M_s) is assumed to be a sum of free and bound ligand concentration, while the measured radioactivity in cerebellum (M_c) is proportional to the free ligand concentration, i.e.

$$M_s = a * C_f + b * C_b \quad (2)$$

$$M_c = c * C_f. \quad (3)$$

The proportional constants a, b and c are dependent on unspecific binding, volume effects and calibration factors of the PET system. If the ratio M_s/M_c is formed

$$M_s/M_c = a/c + b/c * C_b/C_f \quad (4)$$

and plotted as a function of time a measure of a/c can be obtained at time = 0. We can get a measure of the bound ligand concentration as

$$b * C_b = M_s - a/c * (c * C_f) = M_s - a/c * M_c. \quad (5)$$

Equation (1) in its integrated form is then used

$$C_b = k_3 * C_f \, ds - k_4 * C_b \, ds \quad (6)$$

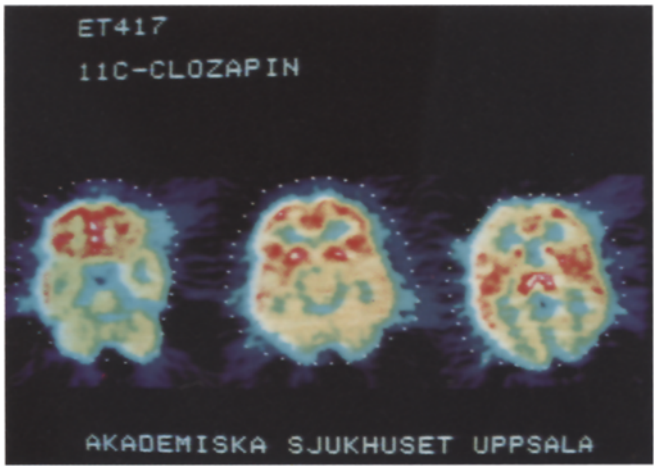

Fig. 2. Distribution of radioactivity derived from 11C-clozapine in the human brain visualized by positron emission tomography

Integrated images in the interval 20–40 min
Highest radioactivity is indicated by white, violet and red colours, yellow is medium activity, green and blue means low activity. The regions of interest were defined through simultaneous comparison to CT-scan images. Middle image represents: occipital cortex, temporal cortex, frontal cortex and striatum. Left image represents the region 14 mm below and the right image 14 mm above the middle transection

or

$$\frac{C_b}{\int_0^t C_f \, d_s} = k_3 - k_4 \frac{\int_0^t C_b \, ds}{\int_0^t C_f \, ds} \tag{7}$$

and C_f and C_b are replaced by their proportional measured quantities in eqns. (3) and (5) in order to obtain

$$\frac{(M_s - a/c * M_c)}{\int_0^t M_s \, ds} = \frac{b}{c} * k_3 - k_4 * \frac{\int_0^t (M_s - a/c * M_s) \, ds}{\int_0^t M_s \, ds}. \tag{8}$$

Equation (8) can be plotted and $b/c * k_3$ determined as the intercept and k_4 as the slope of a linear relation.

Results

The highest radioactivity in the brain derived from 11C-clozapine was seen in the striatum, but high amounts were also seen in the temporal lobes and in the frontal cortex for all subjects examined (Fig. 1). The amount of radioactivity in the frontal cortex was less than in the striatum but higher than the radioactivity measured in the cerebellum (Fig. 2). The values for the receptor binding rates k_3, k_4 and k_3/k_4 for 11C-clozapine were calculated for striatum and frontal cortex for each subject and the data are presented in Table 1. Some differences in the k_3 and k_4-values can be settled between the six individuals examined, with an extreme for control no. 2, who had high values for both k_3 and k_4 in the frontal cortex. The k_3 values were higher in the striatal areas compared to the frontal cortex for all but control no. 2. In patient no. 3 and control no. 3 a substantial difference between k_4 values in striatum and frontal cortex was demonstrated. Due to the low number of subjects and the interindividual variation no difference between schizophrenics and controls can with certainty be found.

In patient no. 2, 6 weeks of treatment with haloperidol 2 mg/day resulted in a decreased binding of 11C-clozapine, more pronounced in the striatum than in the frontal cortex. The same results were seen in patient no. 3, who had an intravenous injection of 1.0 mg haloperidol 1 h before 11C-clozapine was given. The change in k_3 values is almost entirely due to the haloperidol pretreatment, because under physiological conditions k_3 is independent of changes in the cerebral blood flow. To our experience in other PET investigations the difference in the same subject between two following investigations without treatment is only a few percent. The dissociation constant k_4 for clozapine was slightly affected by haloperidol pretreatment in patient no. 2. In striatum no affection at all was seen and the reduction in frontal cortex was only 5%. In patient no. 3 the difference was more pronounced. A reduction with 17% after haloperidol pretreatment in striatum and in frontal cortex k_4 was roughly 28% higher after haloperidol treatment. The changes in k_4 after haloperidol pretreatment probably depend on the fact that the model originally is

Table 1. Receptor kinetic parameters k_3, k_4 and k_3/k_4 for 11C-clozapine in striatum and frontal cortex in healthy controls and schizophrenic patients

Subject	k_3		k_4		k_3/k_4	
	Striatum	Frontal CTX	Striatum	Frontal CTX	Striatum	Frontal CTX
Control 1	0.045	0.022	0.055	0.051	0.83	0.43
Control 2	0.038	0.072	0.069	0.110	0.60	0.60
Control 3	0.026	0.012	0.050	0.015	0.50	0.80
Patient 1, drug-free	0.031	0.010	0.095	0.076	0.35	0.14
Patient 2:						
a) drug-free	0.045	0.029	0.058	0.056	0.80	0.50
b) haloperidol PO[a]	0.029	0.021	0.058	0.053	0.50	0.40
Patient 3:						
a) drug-free	0.022	0.012	0.048	0.018	0.40	0.70
b) haloperidol IV[b]	0.014	0.009	0.040	0.023	0.30	0.36

[a] A daily dose of 2 mg orally for 6 weeks before retest
[b] The dose was 1 mg IV 1 h before retest

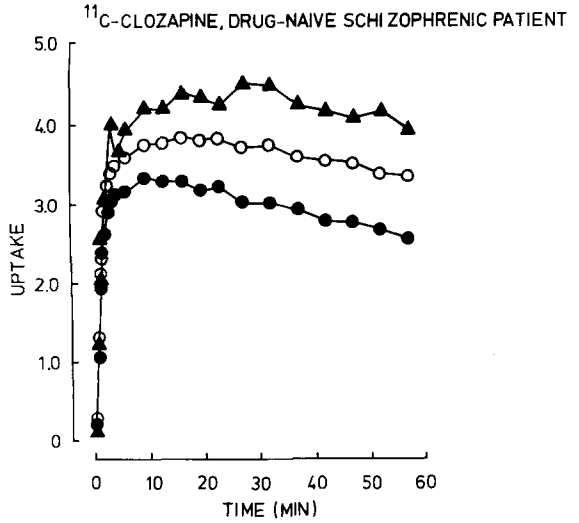

Fig. 3. 11C-clozapine derived radioactivity measured in the striatum, the frontal cortex and the cerebellum over time in the human brain using positron emission tomography. ● Cerebellum; ○ frontal cortex; ▲ striatum

designed for measuring a specific receptor population. Clozapine is acting on a mixed population of receptors and therefore the blockade by haloperidol alters the mean value of k_4.

Discussion

By using the PET technique it has recently been demonstrated, that a large number of classical and atypical neuroleptics all have dopamine-D2 binding properties to striatal structures when using a specific 11-C-labelled ligand (Farde et al. 1988a). This is in agreement with the present study of clozapine an atypic neuroleptic. Our study includes few individuals but verifies the previous observations in the monkey, namely that clozapine has affinity for the striatal structures, the brain area with the highest density of dopamine receptors. The reduction of 11C-clozapine binding in the striatum by pretreatment with haloperidol suggests that clozapine binds to dopamine receptors also in man. In the monkey, pretreatment with clozapine (Hartvig et al. 1988) decreased the striatal binding of 11C-raclopride, a selective D2-dopamine receptor antagonist, indicating specific affinity of clozapine to the dopamine receptors. However, the 11C-clozapine binding in the frontal cortex as well as in the striatum was reduced after spiperone treatment in the monkey, which indicates a competition for dopamine and serotonin receptors (Hartvig et al. 1988).

The hypothesis that the low incidence of EPS seen in humans treated with clozapine would depend on a low affinity for the dopamine receptors in the striatum (Anden and Stock 1973) thus seems less probable. It is more likely that this is due to receptor kinetic properties, as, i.e. a high dissociation rate (k_4) from the receptor compared to 11C-N-methylspiperone and 11C-raclopride was found in the monkey studies (Hartvig et al. 1986, 1988). These studies also revealed that the elimination half-life of clozapine from plasma was of the same order as that from the striatal area. Clozapine has a strong antipsychotic effect and binds to the dopamine D2-receptors. However, the dissociation rate (k_4) of the compound is high (Hartvig et al. 1988), indicating a blockade only for a short period of time. Despite of this clozapine can be administered once a day with excellent antipsychotic effect, indicating the possibility of other mechanisms of action than dopamine-D2 receptor kinetics. Therefore, the suggested relationship between persisting blockade of the dopamine-D2 receptors and the antipsychotic effect of neuroleptics (Peroutka and Snyder 1980) is not so obvious.

The binding of 11C-clozapine to frontal cortex not displaced by haloperidol is the most interesting finding of this study. Dopamine-D2 receptor density in the frontal cortex is probably not of such a magnitude that they are detected by the present PET technique. In a study using the specific dopamine-D2 receptor antagonist raclopride, the difference in distribution ratios (radioactivity in a brain region/"free", not protein-bound, radioactivity in plasma) were only a few per cent when compared to its inactive (R)-enantiomer (Farde et al. 1988a). The present data raise questions about the significance of frontal cortex in the schizophrenic symptomatology, which have come into focus of interest during the last few years (Muller 1985; Andreasen et al. 1986).

The role of the D1-dopamine receptor in schizophrenia has been disputed, but several findings during the last years point to the importance of the D1 receptor as a target of antipsychotic drugs (Andersen and Braestrup 1986; Kebabian et al. 1986; Hall et al. 1987; Seeman and Grigoriadis 1987; Andersen 1988). There are some evidence of two different states of D1-dopamine receptors, adenylate cyclase-coupled and -uncoupled. Atypical neuroleptics like clozapine are in an animal in vitro study found to be acting on adenylate acyclase-coupled dopamine-D1 receptors (Andersen and Braestrup 1986), while classical neuroleptics have the adenylate cyclase-uncoupled D1 receptor as target. Some investigators have proposed a linkage between D1 and D2 receptors with a modulation of the receptor signal via second messengers (Worley et al. 1986; Chipkin and Latranyi 1987). In accordance with this the influence on the D1 receptor could be stronger via, for example, the D2 receptor than at a direct action on the D1 receptor. In a study mapping second messenger systems it is stated that the number of second messenger binding sites exceed typical transmitter receptor proteins 10–100 fold (Worley et al. 1986). The influence on the cellular function could therefore be determined more by signal amplification produced by second messenger systems than by the number of membrane receptor molecules.

The radioactivity in the frontal cortex not displaced by haloperidol in the present study might represent binding to D1 receptors, alternatively second messenger proteins. It is also possible that the unique properties of clozapine in schizophrenia are due to its effect on non-dopaminergic receptors in the frontal cortex in contrast to some investigators proposing selectivity for dopamine receptors in limbic structures (Anden and Stock 1973; Saywers et al. 1975).

Our future studies will be focused on the question what kind of receptor or receptors, that clozapine binds to in the frontal cortex. Hopefully these studies, including the PET technique, will increase our understanding of the antipsychotic properties of clozapine and thereby also the underlying mechanisms of schizophrenia.

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