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Dose dependent occupancy of central dopamine D_2 receptors by the novel neuroleptic CP-88,059-01: a study using positron emission tomography and ^{11}C-raclopride

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Abstract. Positron emission tomography (PET) and ^{11}C-raclopride were used to measure the occupancy of central dopamine D_2 receptors by a new neuroleptic, CP-88,059-1. In a double blind dose escalation study, seven healthy male subjects received a predose of between 2 mg and 60 mg CP-88,059-1, 5 h before PET scanning. One additional subject was assigned to placebo predose. Receptor occupancy was defined as the percentage reduction in binding potential compared with that seen in the subject predosed with placebo and with that seen in seven unmedicated normal volunteers previously studied. Binding of ^{11}C-raclopride decreased in a dose dependent manner, and 85% dopamine D_2 receptor occupancy was achieved with the highest dose of CP-88,059-1. The findings confirm that brain dopamine D_2 receptors are blocked by CP-88,059-1 and suggest that an effective antipsychotic dose will be between 20 mg and 40 mg. The study highlights the potential of positron emission tomography in the preclinical evaluation of new drugs.

Key words: Positron emission tomography – Pharmacodynamics – ^{11}C-raclopride – Dopamine D_2 receptors – CP-88,059-1

It is generally considered that the antipsychotic effect of neuroleptics is mediated by blockade of central dopamine D_2 receptors. The strongest evidence for this hypothesis is the linear correlation between the affinity of drugs for D_2 receptors in animals and antipsychotic potency in man, a relationship that does not hold for any other neuroreceptor (Creese et al. 1976; Seeman et al. 1976; Peroutka and Snyder 1980). It is possible to study dopamine D_2 receptor binding of drugs in the living human brain using positron emission tomography (PET) and carbon-11 labelled raclopride, a highly specific antagonist of dopamine D_2 receptors (Farde et al. 1985). Using this approach, Farde et al. have demonstrated that clinically effective (antipsychotic) doses of a variety of neuroleptics result in at least 65% occupancy of central dopamine D_2 receptors (Farde et al. 1988b). This degree of occupancy can be demonstrated to occur as rapidly as 2 h after acute administration of neuroleptic (Farde et al. 1986).

There are three principal limitations to the clinical use of traditional neuroleptics. Firstly, between 20 and 40% of treated patients develop extrapyramidal side effects (EPSE) (Tarsey 1983). Secondly, the debilitating and often irreversible syndrome of tardive dyskinesia arises in up to 30% of patients on long term treatment (Baldessarini et al. 1980). Thirdly, there is a sub-group of about 20–30% of all schizophrenics who do not respond to conventional neuroleptics, in particular those patients who exhibit negative or deficit symptoms (Davis et al. 1980; Kane et al. 1988). Compounds which have broad antipsychotic efficacy in the absence of EPSE are therefore of great interest.

Recent work with the atypical dibenzodiazepine neuroleptic clozapine suggests that this compound is effective in the treatment of schizophrenia, including up to 30% of drug-refractory patients, while producing very few EPSE (Kane et al. 1988). It is hypothesised that the combined antagonist properties of clozapine at postsynaptic serotonergic $5HT_2$ *and* dopaminergic D_2 receptors are responsible for this clinical profile (Meltzer et al. 1989). It is possible that the likelihood of EPSE may relate to the relative occupancy of D_2 and $5HT_2$ receptors in the putamen by neuroleptics. The incidence of EPSE is also related to absolute levels of D_2 occupancy by neuroleptics. EPSE are more common when there is greater than 85% occupancy of D_2 receptors (Farde et al. 1992). Thus, in terms of striatal dopamine D_2 receptor occupancy by neuroleptics there appears to be an optimum level of between 65 and 85% which is antipsychotic and yet less likely to cause EPSE.

CP-88,059-1 is the hydrochloride salt of a benzisothiazoyl piperazine, related in structure to the experimental atypical antipsychotic drug tiosperone. It was developed in the search for a compound that potently

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blocks dopamine D_2 receptors but that binds with greater affinity to cerebral serotonin $5HT_2$ receptors. It has high affinity for both $5HT_2$ ($K_i = 0.42$ nM) and D_2 ($K_i = 4.8$ nM) receptors. The separation between these affinities ($5HT_2$: $D_2 = 11.4$) is greater than that observed for other antipsychotic drugs (e.g. for clozapine $5HT_2$: $D_2 = 4.5$, for chlorpromazine $5HT_2$: $D_2 = 0.3$) (data supplied by Pfizer Central Research; Investigator's Brochure 1991).

In this study PET and ^{11}C-raclopride were used to establish the minimum dose of CP-88,059-1 required to produce a level of striatal dopamine D_2 receptor occupancy between 65 and 85%. In addition, $C^{15}O_2$ inhalation was used to measure cerebral blood flow prior to measuring dopamine receptor occupancy. PET measurements of regional cerebral blood flow (rCBF) provide a non-invasive in vivo index of neuronal activity (Raichle 1987). These techniques can show discrete areas within the brain which are affected by the administration of psychotropic drugs, such as neuroleptics.

Materials and methods

Subjects. Eight healthy male volunteers between the ages of 20 and 34 were studied. Health was assessed by medical history, full clinical examination, haematological and biochemical profile and urinalysis. Magnetic resonance imaging (MRI) of the brain was performed on six of the subjects. Of the remaining two subjects one was unavailable for MRI and the other had metal implants following a limb fracture. All subjects were neuroleptic naive and had not taken any standard prescription drug therapy for at least 2 weeks prior to the study. Demographic details of the subjects are shown in Table 1. All subjects gave informed written consent and local ethics committee approval was obtained. Permission to administer radioisotopes was obtained from the Administration of Radioactive Substances Advisory Committee of the UK.

Synthesis of ^{11}C-raclopride. ^{11}C-raclopride was prepared at the Cyclotron Unit according to the method of Farde et al. (1988a). The mean specific activity of the eight preparations was 21315 MBQ/mmol (range 15772–30837) and the mean concentration of cold raclopride was 1.15 µg/ml (range 0.55–2.33). Raclopride tartrate was provided by Astra Research Centre, Sweden.

Drug administration and positron emission tomography. Using a double-blind dose escalation design, the eight subjects were allocated to receive one of seven different doses of CP-88,059-1 between 2 and 60 mg, or placebo. The placebo subject was randomly inserted into the dose escalation schedule. Medication was administered at 9.00 A.M. PET scans were performed on a CTI 931-08/12 (CTI Inc., Knoxville, Tennessee, USA) scanner (Spinks et al. 1988). Reconstructed images had an axial full width half maximum (FWHM) resolution of 7 mm and an in-plane FWHM resolution of 8.5 × 8.5 mm. All scans were performed between 14.30 and 15.30 P.M. Individual expanded polystyrene head supports were made to ensure comfort and relative immobility and a 22 g Teflon catheter was inserted into the left radial artery after establishing the presence of satisfactory collateral circulation and subcutaneous infiltration with 0.5% bupivicaine (Marcain©). Subjects were aligned in the scanner using a laser system so that the detectors were parallel to the orbito-meatal line. A 10-min transmission scan for correction of tissue attenuation of 511 keV gamma radiation was collected using a retractable ^{68}Ga/^{68}Ge ring source.

All eight subjects received bolus intravenous injections of ^{11}C-raclopride in saline (mean = 9.6 mCi, SD = 1.9; mean weight of cold raclopride injected was 5.96 µg, SD = 1.7). Dynamic scans were collected from the time of injection for a period of 60 min, divided into 25 time frames. Frame length was increased from 5 s initially to 10 min at the end of the study. Venous blood samples for estimation of serum concentrations of CP-88,059-1 were taken predose and at 1, 2, 4, 6, 8, 12, 24 and 36 h post-dose. Additional samples for prolactin assay were taken at the same time points up to 24 h post-dose. Measurement of CBF ws performed before ^{11}C-raclopride administration by inhalation of $C^{15}O_2$ and dynamic PET scanning. This method has been described in detail previously (Lammertsma et al. 1990) but in brief applies both dynamic and integral analyses to a dynamic sequence of 21 PET scans collected during and following inhalation of $C^{15}O_2$. The dynamic analysis is used to correct continuously monitored whole blood activity for delay and dispersion relative to tissue scans. An integral analysis including corrections for delay and dispersion is then used to calculate CBF on a pixel-by-pixel basis.

CP-88,059-1 pharmacokinetic analysis. For the estimation of serum concentrations of CP-88,059-1, the venous blood samples were kept at room temperature until clotted. Within 1 h of collection the serum was separated from coagulated blood using a refrigerated centrifuge. Serum samples were stored at −20° C before analysis with an high performance liquid chromatographic assay involving liquid-liquid extraction with detection by API-MS. The assay had a dynamic range of 0.5–50 ng/ml with a lower limit of quantitation

Table 1. Details of the 8 subjects studied: dose of CP-88,059-1 taken, serum levels of CP-88,059-1, striatal binding potential (BP), cerebral blood flow for three regions of interest, and prolactin response

Subject	Age (years)	Weight (kg)	Dose of CP-88,059 (mg)	CP-88,059 AUC {0–∞}[a] (ng · h/ml)	Binding potential (±SE[b])	Receptor occupancy (% reduction in BP)		Cerebral blood flow (ml/100 ml per min)			Prolactin response	
						vs Placebo	vs 8 Normals[c]	WB	CBL	STR[d]	AUC {0–12} mIU · h/l	Peak-baseline mIU
1	23	73	2	22.7	2.21 (±0.06)	2%	−0.4%	51.0	54.5	63.9	−20.4	126.5
2	29	65	5	59.4	1.74 (±0.04)	23%	21%	47.4	52.6	60.9	16.8	250.5
3	23	73	10	110.3	1.71 (±0.07)	24%	22%	43.1	45.9	60.7	58	513
4	23	54	Placebo	0	2.25 (±0.03)	0%	−2%	46.5	60.6	62.3	−132	−85
5	20	59	15	261.7	1.27 (±0.03)	44%	42%	49.9	60.6	73.3	203.3	558
6	29	79	20	239.5	0.94 (±0.03)	58%	57%	65.8	62.1	77.3	224.4	808
7	34	66	40	518.2	0.51 (±0.03)	77%	77%	49.7	50.1	58.6	267.3	767
8	24	87	60	638.8	0.39 (±0.02)	83%	82%	50.3	66.1	66.1	213	514

[a] AUC {0–∞}: area under the serum concentration-time curve from time 0 to infinity

[b] Standard errors of the estimate were calculated from the differences between predicted and measured data, as described by Carson (1986)

[c] Mean value of striatal binding potential (BP) from 8 normal male controls (2.20 ± 0.29)

[d] WB, whole brain; CBL, cerebellum; STR, striatum

of 0.5 ng/ml (Pfizer Central Research, Groton, CT, USA). The area under the serum CP-88,059-1 concentration-time curve from time 0 to the last time (t) with a measurable concentration (AUC{0–t}) was estimated using the trapezoid method. AUC from time t to infinity (AUC{t–∞}) was estimated as Cp_{est}/K_{el}, where Cp_{est} represents the estimated concentration at time t and K_{el} is the terminal phase rate constant, based on least squares regression analysis of the data obtained during the terminal log-linear phase. AUC from time 0 to infinity (AUC{0–∞}) was estimated as the sum of the AUC{0–t} and AUC{t–∞} values.

Prolactin assay. Plasma prolactin levels were determined by radioimmunoassay with intra and interassay coefficients of variation of 2.5% and 6.7%, respectively, and a lower limit of detection of 34 mIU/I (Maurer et al. 1986). The prolactin (PRL) response was analysed by two methods: (a) as the area under the response curve for 12 h post-dose, with subtraction of the pre-dosing baseline level using the trapezoid method; (b) as the peak-baseline value. The baseline PRL level was determined from a sample taken at 8 a.m., 1 h before pre-dosing.

Image analysis and modelling of data. The dynamic PET scans were analysed using image analysis software (Analyze version 3.0, Biodynamics Research Unit, Mayo Foundation, USA) on Sun 3/60 Workstations. Templates for striatal and cerebellar regions were defined on individual raclopride scans, summated to include activity between 30 and 60 min post-injection. These templates were then transferred automatically by computer onto the CBF scans. The positions of the regions were determined by inspection and, in six subjects, with reference to the appropriate transverse slices of their MRI scans. Regions of interest were defined for caudate (1 circular region each side, 4 pixels in diameter, 1 pixel = 2.05 × 2.05 mm), putamen (3 contiguous circular regions each side, 4 pixels diameter each) and cerebellum (1 circular region each side, 16 pixels diameter). All regions of interest were defined on two adjacent planes using regions of identical sizes. Average values for each anatomical structure were calculated (Sawle et al. 1990). The definition of striatal regions of interest in the subjects pretreated with 20, 40 or 60 mg CP-88,059-1 was more difficult in view of the extensive blockade of raclopride binding. For these scans the regions were positioned firstly by adjusting the threshold of the raclopride images to show the best possible definition between areas of differential ligand binding, and secondly with reference to the CBF images.

Time activity curves were plotted for each region in each subject and the kinetics of brain ^{11}C-raclopride was modelled using the cerebellum as a reference tissue (Hume et al. 1992). The model consists of two tissue compartments for the striatum that correspond to (a) free tracer and (b) receptor bound tracer. In contrast, the cerebellum, which is a region of the brain virtually devoid of dopamine D_2 receptors (Camps et al. 1989), consists of a free and non-specific compartment and total radioactivity in this region may be used as an estimate of free radioligand concentration in the brain. Four rate constants are used to describe tracer uptake in striatum. K_1 and k_2 are the constants for transport between plasma and tissue and k_3 and k_4 are the constants for binding to and dissociation from dopamine D_2 receptors, respectively. This model allows for the estimation of binding potential (BP) = k_3/k_4, assuming that the K_1/k_2 ratios for striatum and cerebellum are equal. Both K_1 and k_2 are blood flow dependent and are related to the blood-brain barrier permeability surface area product. Even if these entities differ between striatum and cerebellum it might be expected that K_1 and k_2 would be equally affected, maintaining the K_1/k_2 ratio. Another assumption in this model is that the levels of non-specific binding in the striatum and cerebellum are the same (Cunningham et al. 1991).

If a neuroleptic drug binds to the receptor population of interest, in this case dopamine receptors, and thereby occupies a certain proportion of the receptors, this will be reflected in a reduced number of receptors available for radioligand binding. The reduction in the number of available receptors will cause a proportional decrease in the $k_3 : k_4$ ratio (BP), assuming that k_4, i.e. the dissociation constant or k_D, remains constant. Dopamine D_2 receptor occupancy was calculated as the percentage reduction in BP for the predosed subject compared with the placebo predosed subject.

$$\text{Receptor occupancy} = \frac{(BP_{pl} - BP_x)}{(BP_{pl})} \times 100\%$$

where BP_{pl} = Binding potential for placebo predosed subject
BP_x = Binding potential for subject predosed with test drug

The main limitation of the present study is the use of a single baseline study to derive the reference value for BP (see Discussion). In view of this, the data were also analysed using the mean value for striatum binding potential obtained from seven additional healthy normal subjects as the reference value.

Results

Cerebral blood flow

There was no significant effect of CP-88,059-1 on striatal, cerebellar or global cerebral blood flow (CBF) (Table 1). CBF in these regions did not correlate significantly with the measure of ^{11}C-raclopride occupancy ($P < 0.37$).

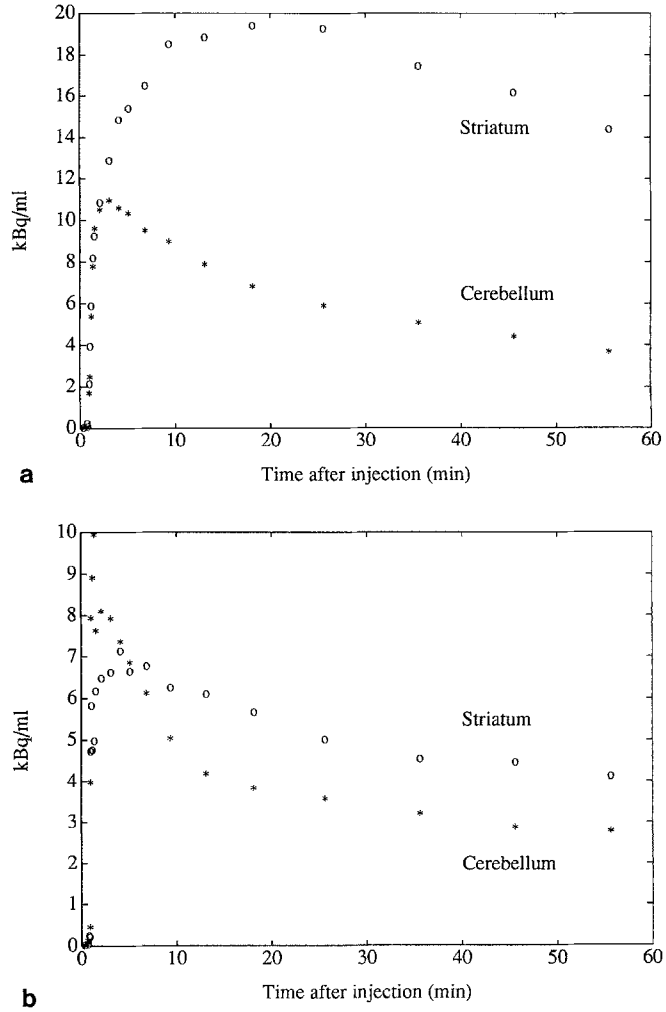

Fig. 1a, b. Time activity curves showing the level of radioactivity in the striatum (o) and cerebellum (x) for subject 4 (**a**) who received placebo predose and for subject 8 (**b**) who received predose of 60 mg CP-88,059-1

Binding potential

Following injection of ^{11}C-raclopride, radioactivity rapidly entered the brain in all subjects (Figs 1a, b). However, in the subjects who received CP-88,059-1 there was a progressive decrease in retention of ^{11}C-raclopride in the striatum with larger predoses, reflecting incremental central dopamine D_2 receptor blockade. Time activity curves are shown for striatum and cerebellum in subjects predosed with placebo (Fig. 1a) and 60 mg CP-88,059-1 (Fig. 1b). Values of k_3/k_4 (binding potential or BP) decreased with increasing predose of CP-88,059-1 (Fig. 2). A 20 mg predose of CP-88,059-1 decreased BP by 58% compared with the single placebo predose study whilst 60 mg predose decreased BP by 83%. The mean striatal BP including seven additional non-predosed normal male subjects (age 31–72) was 2.20 (SD 0.29). Using this mean value as the reference for BP, the 20 mg predose of CP-88,059-1 decreased BP by 57% whilst 60 mg predose decreased BP by 82%. Figure 3 shows PET images of the striata of each subject with brain activity normalised for integrated plasma activity at 45 min.

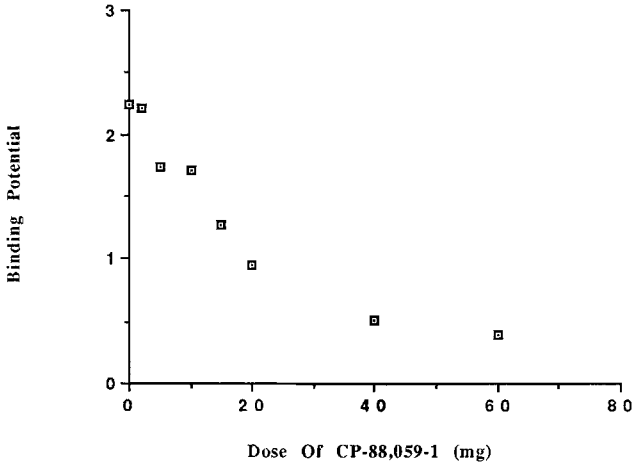

Fig. 2. Binding potential and dose of CP-88,059-1 in the 8 subjects studied

CP-88,059-1 pharmacokinetics

With increasing dose of CP-88,059-1 there was an increase in AUC{0–∞}, with the exception of the 20 mg dose where AUC{0–∞} was similar to that observed at the 15 mg dose level (Table 1). Similarly, serum CP-88,059-1 levels at the time of the PET scan tended to increase with increasing dose. There were strong correlations between AUC{0–∞} and binding potential for ^{11}C-raclopride ($r = -0.95$; *df* 6; $P < 0.0004$) and AUC{0–∞} and prolactin response ($r = 0.81$; *df* 6; $P < 0.01$).

Prolactin response

Placebo predose and the lowest (2 mg) dose of CP-88,059-1 failed to produce an increase in plasma PRL above baseline. The six subjects predosed with between 5 mg and 60 mg of CP-88,059-1 demonstrated elevated prolactin levels following medication (Fig. 4, Table 1).

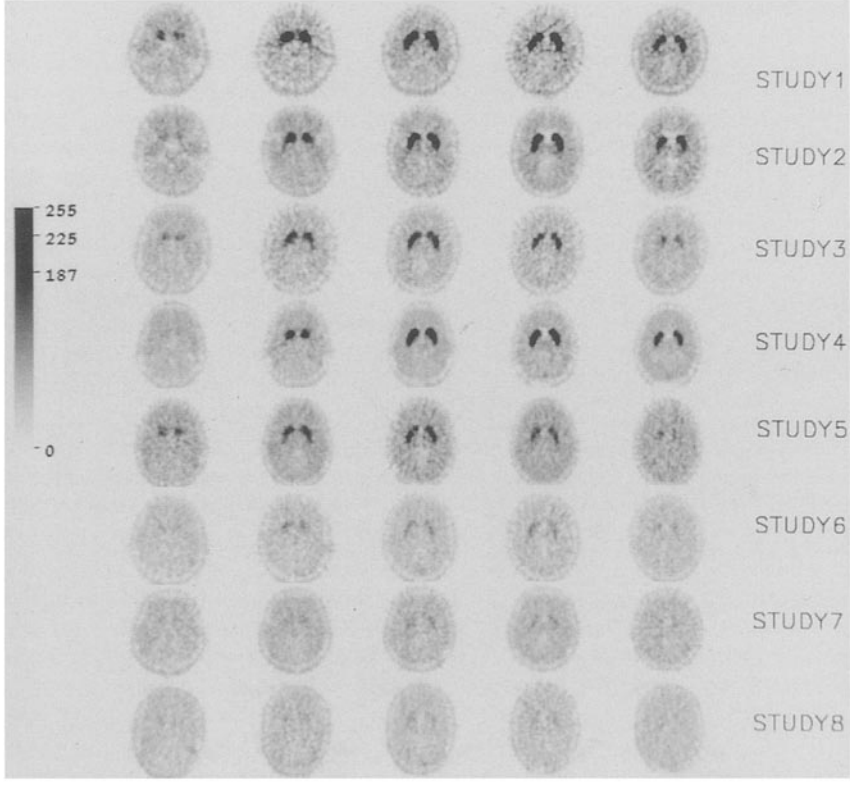

Fig. 3. PET images of the 8 subjects at the level of the striata. The images show summated activity for the last 30 min of the scans and are normalised for plasma activity at 45 min. The images are colour coded so that the regions of most intense ^{11}C-raclopride binding are darkest

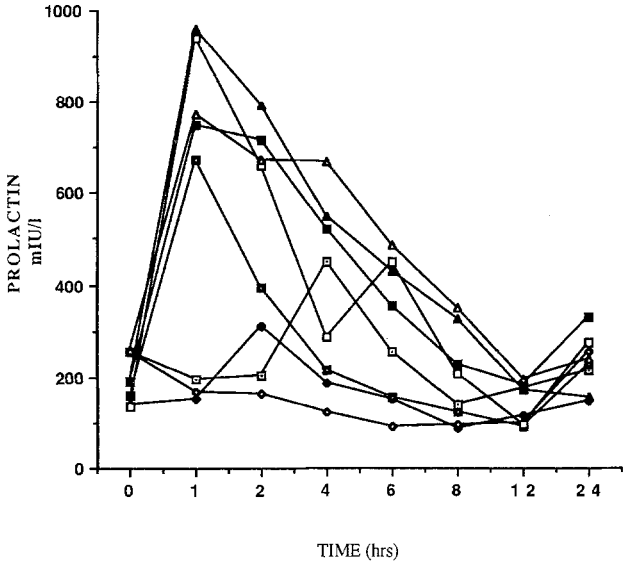

Fig. 4. Prolactin concentrations for all 8 subjects from 0 h (pre-dose) to 24 h post dose. —⊡— Subject 1; —◆— subject 2; —■— subject 3; —◆— subject 4; —■— subject 5; —□— subject 6; —▲— subject 7; —▲— subject 8

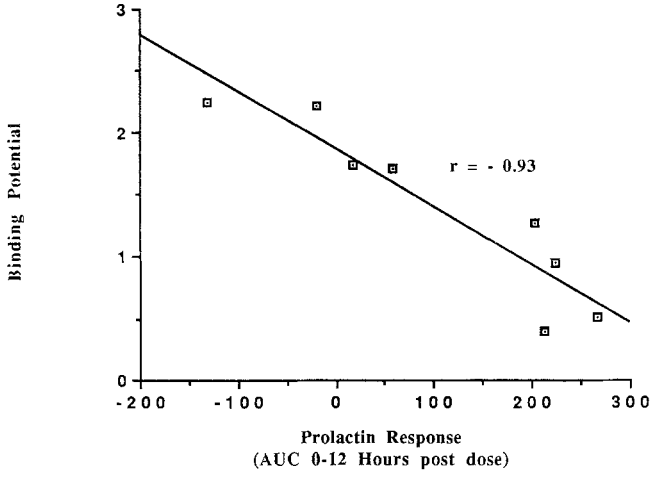

Fig. 5. Binding potential vs prolactin response for the 8 subjects. The correlation coefficient is −0.92

There was a significant negative correlation between the binding potential for ^{11}C-raclopride and prolactin response (Fig. 5).

Discussion

PET and ^{11}C-raclopride were used to examine brain dopamine D_2 receptor occupancy in normal subjects 5 h after taking a single oral dose of the new neuroleptic CP-88,059-1. With increasing dose of CP-88,059-1 there was a decrease in the binding potential (BP) of ^{11}C-raclopride, indicating increasing central dopamine D_2 receptor blockade. The results show that a single dose of between 20 and 40 mg CP-88,059-1 is likely to cause > 65% receptor occupancy – the degree associated with clinical doses of a variety of antipsychotic drugs (Cambon et al. 1987; Farde et al. 1988b). The correlation of the amount of CP-88,059-1 given or AUC{0–∞} of CP-88,059-1 with the PET derived measure of binding potential was greater than for the correlation with the prolactin response. This suggests that neuroleptic-induced prolactin increases, which are an index of dopamine D_2 antagonism at the pituitary level, may be a less precise measure of antagonism in the brain itself than the radioligand uptake method described here.

Chronic administration of neuroleptics to schizophrenics is reported to increase metabolic rate of glucose in the basal ganglia (DeLisi et al. 1985; Buchsbaum et al. 1987; Szechtman et al. 1988). However, our finding of no significant effect of neuroleptic dosage on cerebral blood flow is consistent with previous PET studies of acute neuroleptic administration (Volkow et al. 1986). More specifically, both striatal and whole brain blood flow showed no relationship with the level of D_2 antagonism.

In this study each subject had a single PET scan. Values for receptor occupancy for the predosed subjects were expressed as the percentage reduction in BP compared with the common baseline of the placebo predosed subject. This therefore assumes that the intersubject variation in baseline BP is much less than the changes in BP due to occupancy by neuroleptics and the use of such a single value, instead of a ratio measured in a separate PET experiment in each subject, introduces a systematic error in the calculation of BP for each individual. Previously, Farde et al. have described considerable variability in binding potential in normal volunteers, with ranges from 2.7 to 5.0 (Farde et al. 1988b). However, using averaged data for the reference value Farde et al. (1992) have also shown that the difference in the values obtained for D_2 occupancy varied by no more than 3% from values obtained in the same patients from paired studies. We therefore also calculated receptor occupancy in the predosed subjects using the average striatal BP from seven additional normal subjects as the reference value. The results obtained differed from the results calculated from the single baseline study by a maximum of 2.4%. Thus the dose-response profile remains unchanged. We suggest that this general approach, with a single "baseline" subject taking placebo, appears to be useful in preliminary dose finding studies. The extrapolation of the present results in normal subjects to guide clinical (phase II) studies in schizophrenics can be justified by recent studies (Farde et al. 1990; Martinot et al. 1990; Martinot et al. 1991) which have failed to confirm the earlier finding (Wong et al. 1986) of elevated D_2 receptors in drug naive schizophrenics.

It is uncertain whether the dose-response profile described would be different after chronic (weeks) administration of CP-88,059-1. Measurement of occupancy in the acute phase of medication could lead to overestimation of occupancy compared with that seen in the chronically medicated state (the usual clinical situation). Antagonism of D_2 autoreceptors in the acute phase of medication causes an increase in extracellular dopamine concentrations (Zetterstom et al. 1984). This increase is dose dependent and at most twofold after a single or repeated administration of neuroleptic. In theory this

could lead to decreased measurement of brain radioactivity due to competition between increased levels of endogenous dopamine and ^{11}C-raclopride at the D_2 receptor (Bunney and Grace 1978; Chiodo and Bunney 1985). Against this, Hume et al. (1992) have shown in the rat that dopamine levels need to be extensively raised by amphetamine to produce appreciable changes in raclopride binding and Farde et al. (1992) have suggested that amphetamine causes a maximum decrease in ^{11}C-raclopride binding of 16% in man.

In animal models, chronic neuroleptic administration leads to the upregulation of dopamine D_2 receptors (Burt et al. 1977; Clow et al. 1980). Earlier PET studies of the effects of haloperidol and sulpiride suggested that levels of occupancy do not change substantially from acute to chronic dosage (Farde et al. 1988b; 1989). However, Farde et al. (1990) have reported increased dopamine D_2 receptor density measured with PET in a patient 2 weeks after the withdrawal of sulpiride. This would suggest that in the chronically medicated state receptor occupancy is underestimated by PET due to upregulation of D_2 receptors. If this is a dose dependent effect then receptor occupancy will be more underestimated in patients with high occupancy. This makes it likely that the dose required for antipsychotic occupancy in the steady state will be less than that determined in a single dose study.

Dose finding studies using PET are now possible in the early preclinical development of drugs. We have previously described such a procedure for the evaluation of a novel, reversible MAO-B inhibitor, using PET and ^{11}C-deprenyl (Bench et al. 1991). Provided that there is an appropriate radioligand for the receptor or enzyme of interest and a quantitative model for the description of ligand binding then these studies can be performed quickly, in relatively few subjects. The present study confirms that the putative neuroleptic CP-88,059-1 occupies dopamine D_2 receptors in the brain. A single acute dose of between 20 and 40 mg CP-88,059-1 gives a level of occupancy commensurate with antipsychotic efficacy, though this is likely to overestimate the dose required in clinical, steady state conditions.

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