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Central benzodiazepine receptor occupancy by zolpidem in the human brain as assessed by positron emission tomography

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Abstract

The central benzodiazepine receptor occupancy by zolpidem in man is unknown. The present study used positron emission tomography (PET) and [^{11}C]flumazenil to assess in five healthy volunteers, central benzodiazepine receptor occupancy in brain regions with high receptor densities 1 h following an acute oral administration of twice the usual hypnotic dose of zolpidem (20 mg). Receptor occupancy was measured in five discrete structures (middle frontal gyrus, middle temporal gyrus, posterior occipital cortex, lateral parietal cortex, and cerebellar cortex) and in a large neocortical area as the fractional change in the [^{11}C]flumazenil bound/free ratio for the interval 15–40 min post-administration of the radiotracer. The free-radioligand concentration was estimated from the pons, a reference structure virtually devoid of central benzodiazepine receptor. With individual pons values, mean occupancy was about 21% but with spurious inter-subject variability. With pons values averaged across the five subjects and separately for control and treated condition, the occupancy was (mean $\pm$ S.D.) $27 \pm 11\%$ for the whole neocortex, and ranged from 26 to 29% in the five discrete structures ($P < 0.01$). By showing hypnotic effect at moderate occupancies, this study directly provides evidence for the full-agonist properties of zolpidem in human.

Keywords: PET (positron emission tomography); [^{11}C]Flumazenil; Imidazopyridine; Benzodiazepine receptor, central

1. Introduction

Zolpidem is an imidazopyridine hypnotic with rapid onset and short duration of action after oral administration (Depoortere et al., 1986; Zivkovic et al., 1988) which acts as a full agonist at central benzodiazepine receptor (Arbilla et al., 1986; Zivkovic et al., 1992). The pharmacological profile of zolpidem in rodents differs from that of the conventional benzodiazepine hypnotics in that it produces sedative/hypnotic effects at doses much lower than those active against convulsions, and is virtually devoid of myorelaxant activity (Depoortere et al., 1986; Zivkovic et al., 1988). Displacement studies of [^{3}H]flumazenil binding to rodent and primate brain, have demonstrated that zolpidem distinguishes between three central benzodiazepine binding site populations which display high, intermediate and low affinity for this compound (Benavides et al., 1993). Studies with recombinant type A γ-aminobutyric acid ($GABA_A$) receptor suggest that these sites may be associated to $GABA_A$ receptors respectively containing α_1 subunits (type I benzodiazepine binding sites, also called ω_1 sites), α_2 or α_3 subunits (type II benzodiazepine binding sites, also called ω_2) or α_5 subunits (ω_5). The selectivity of zolpidem for type I central benzodiazepine binding sites is also observed in the rodent brain in vivo, where after i.p. administration this compound preferentially inhibits [^{3}H]flumazenil binding to brain regions enriched in type I central benzodiazepine binding sites such as the brain cortex and cerebellum (Benavides et al., 1992).

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Zolpidem possesses a very high intrinsic activity at the central benzodiazepine receptor in a number of functional models: thus it produces a large GABA shift in vitro (Arbilla et al., 1986) and is more efficacious than the benzodiazepines in enhancing dorsal root potentials in cats, in increasing the latency to onset of isoniazid-induced convulsions in the mouse (Zivkovic et al., 1988) and in potentiating GABA-induced currents in rat cerebellar Purkinje cells (V. Itier, in preparation). This high intrinsic activity may explain why the hypnotic activity of zolpidem in rats appears at doses producing a very low occupancy ($\sim 5\%$) of central benzodiazepine receptor, as assessed by the in vivo inhibition of [^{3}H]flumazenil binding (Benavides et al., 1992). The high intrinsic activity of zolpidem at the central benzodiazepine receptor and its selectivity for the type I central benzodiazepine binding site (Benavides et al., 1988, 1992) may contribute to its preferential hypnotic profile. However, the level of central benzodiazepine receptor occupancy necessary for the manifestation of the hypnotic activity of zolpidem in man is as yet unknown.

Positron emission tomography (PET) is a suitable tool to investigate in vivo central benzodiazepine receptor occupancy, because it can be performed directly in humans in a non-invasive way and under controlled conditions. Extensive PET studies of [^{11}C]flumazenil in both non-human primates and humans have documented that this radioligand has ideal characteristics to assess the central benzodiazepine receptor in vivo (Hantraye et al., 1984; Samson et al., 1985; Pappata et al., 1988; for review: Abadie and Baron, 1990) and thus should permit to investigate directly in humans the interaction between zolpidem and the central benzodiazepine binding site. Clearly, the central benzodiazepine receptor occupancy by zolpidem might be a more suitable indicator of its pharmacological effects as an agonist than its concentration time-course in plasma or tissue (Ito et al., 1993).

Only two previous [^{11}C]flumazenil PET studies regarding the central benzodiazepine receptor occupancy by agonists have been reported in the literature. Shinotoh et al. (1989) studied six subjects 1.5 h after administration of anticonvulsant doses (30 μg/kg p.o.) of clonazepam; they found a percentage inhibition of total neocortical [^{11}C]flumazenil uptake at pharmacological equilibrium, ranging from 15.3% to 23.5%. However, because in this calculation free and non-specifically bound ligand are not subtracted from total uptake, the resulting percentage inhibition underestimates the real receptor occupancy. Pauli et al. (1991), by evaluating the fractional decrease in specifically bound-free (B/F) ratio in neocortex after administration of a high dose of diazepam (30 mg p.o.) (F was estimated in a reference region, the pons, which is virtually devoid of central benzodiazepine receptors, Maloteaux et al., 1988), reported receptor occupancies of 24% and 14%, 2 and 23 h after diazepam administration, respectively. Thus, despite differences in methodology, both studies suggested moderate central benzodiazepine receptor occupancy by these two different benzodiazepines given at relatively high doses, consistent with earlier in vivo [^{3}H]flumazenil binding studies in rats (Miller et al., 1987; Facklam et al., 1992; Benavides et al., 1992). The central benzodiazepine receptor occupancy by non-benzodiazepine compounds used for the treatment of sleep disorders has however not been previously determined.

The objectives of this study were 2-fold: (i) to characterize further the methodological procedure for the determination in humans of in vivo central benzodiazepine binding site occupancy by an agonist, not only in a large neocortical, but also in discrete, receptor high-density areas; (ii) to estimate in healthy volunteers, the neocortical central benzodiazepine receptor occupancy by clinically relevant doses of the hypnotic compound, zolpidem, following an acute oral (single-dose) administration. The results (i) suggest the feasibility of estimating central benzodiazepine receptor occupancies with PET both in clinical conditions of treatment and on a small sample of subjects; and (ii) provide evidence for the full-agonist properties of zolpidem at central benzodiazepine receptors in vivo, in humans.

2. Materials and methods

2.1. Subjects

The study protocol was approved by the regional Ethics Committee of Caen, France. After giving their written informed consent, five young unmedicated, physically and mentally healthy caucasian males were included into the study. Their mean ($\pm$S.D.) age was 24.2 ± 2.49 years and their mean ($\pm$S.D.) weight was: 69 ± 2.65 kg.

Inclusion criteria were the following: normality of the physical examination, laboratory blood tests (especially liver and kidney functions), electrocardiography, and lack of past or present mental disease (based on the criteria of the diagnostic and statistical manual of mental disorders, third edition, revised, American Psychiatric Association, 1987). Furthermore, the scores on the auto-evaluation anxiety scale of Spielberger (Trait-X form) (Spielberger, 1983) were below the accepted threshold of 40 in all subjects (mean score: 34.8). Brain X-ray computed topography scan was normal in each subjects. None of them had been treated with psychotropic drugs for at least 2 months before the PET study.

Exclusion criteria were: (1) participation to a clinical trial within the 3 months preceding the study; (2) excessive use of coffee, tea (> 6 cups per day), alcohol (> 150 g per week) or tobacco (> 15 cigarettes per day).

Once included in the protocol, the subject was asked to avoid any medication until the PET study. Alcohol was not permitted during this period. After the PET study, car-driving was not permitted for the remaining of the day.

2.2. Pharmacological paradigm

On the morning of the PET study, in fasting conditions, urinary screening for several drugs (benzodiazepines, barbiturates, phenothiazines, tricyclics, opioids, cocaine, cannabis, amphetamines) was carried out and reported as negative in all subjects. There was no trace of alcohol in urine. A breakfast (without methylxanthine supply) was allowed before the PET session.

Each PET study consisted of two successive injections of trace amounts of [^{11}C]flumazenil at high specific radioactivity, one before (control condition) and one after (treatment condition) oral zolpidem administration (see below).

For each subject, a catheter was inserted into the brachial vein on each arm and connected to glucose 5% or saline. The radioligand was injected as a bolus through one catheter and venous blood samples were collected through the opposite catheter (see below).

PET data acquisition started at the end of the injection of the radioligand and lasted for 60 min. During the first PET session, the subjects were lying, at rest and with ears unblocked. At the end of the first study, a clinician gave them orally two 10 mg tablets of zolpidem (i.e. twice the usual therapeutic dose).

Zolpidem has a mean plasma elimination half-life of 2.4 h (range: 0.7–3.5 h) with a T_{max} ranging between 0.5 and 3 h (Langtry and Benfield, 1990; Durand et al., 1992). According to these pharmacokinetic parameters and with the aim to determine optimal central benzodiazepine receptor occupancy, the second PET study was performed about 1 h after the administration of zolpidem. In order to assess the bio-availability of zolpidem after its oral administration, two venous blood samples were collected during the second PET study to measure zolpidem concentration: a first dosage was contemporaneous with the radioligand injection and a second one was performed at the end of the 60 min scanning period (i.e. at $t = 60$ min and $t = 120$ min post-ingestion referred to as T_1 and T_2, respectively, in what follow).

None of the identified zolpidem metabolites possess pharmacological activity and, moreover, they are present in the brain only in trace amounts (Langtry and Benfield, 1990; Durand et al., 1992). To quantify zolpidem plasma concentrations, a high pressure layer chromatography (HPLC) procedure with fluorimetric detection (quantitation threshold: 0.5 ng/ml) was used (Guinebault et al., 1986).

Subjects were allowed to stand up and walk around between the two studies and, as far as possible, they were prevented from falling asleep during the study. All subjects were aware of the pharmacological effects of zolpidem.

Pulse rate and arterial blood pressure were measured at 10, 30 and 60 min within each session and were stable in all cases.

2.3. [^{11}C]Flumazenil PET procedure

In this work, we used a strictly standardized and controlled protocol.

2.3.1. Radiochemistry

[^{11}C]Flumazenil was prepared by methylation of the desmethyl-precursor with [^{11}C]methyl iodide (Mazière et al., 1984). The radiochemical yield was around 55% and the duration of the synthesis never exceeded 60 min. [^{11}C]Flumazenil was isolated on a semi-preparative HPLC column (μ-porasil, 7.8 mm * 30 cm, Waters, France) with a mobile phase consisting of dichloromethane : ethanol : water : ethylamine (99 : 0.96 : 0.02 : 0.02 v/v/v/v) and detection took place at 254 nm. The collected fraction was evaporated to dryness, dissolved in physiological saline and run through a Millipore filter.

The average specific radioactivity obtained at the time of first and second injections (mean ± S.D.) was 12.1 ± 4.5 and 19 ± 10.9 GBq/μmol, respectively (non-significant difference by paired t-test). The injected doses were (mean ± S.D.) 306.7 ± 101.4 MBq for the first injection and 365.6 ± 48.1 MBq for the second one.

2.3.2. PET camera procedure

In order to allow subsequent determination of the brain structures of interest in relation to Talairach's Stereotaxic Atlas (see below), we used a similar positioning for both PET and X-ray computed tomography-scan, derived from that of Fox et al. (1985) and which has been described elsewhere (Abadie et al., 1992). This procedure is based on external osseous landmarks (identified on a lateral skull X-ray), the glabella-inion line, chosen by reference to the anterior commissure-posterior commissure bi-commissural line of Talairach. This positioning procedure was repeated for each of the two PET scanning procedures, thus ensuring optimal head repositioning, as well as for the X-ray computed tomography scan.

Seven brain slices were acquired simultaneously with a time-of-flight TTV 03 PET camera (LETI, France).

The intrinsic axial resolution of the camera is 9 mm and the intrinsic physical in plane resolution is 5.5 mm with 3 mm undetected void between slices. Before each emission data acquisition, a transmission scan for attenuation correction was carried out with an external source ^{68}Ge-^{68}Ga. The coregistred PET and X-ray computed tomography slices were made to parallel the corresponding glabella-inion line at the following levels: −4, +8, +20, +32, +44, +56, and +68 mm, from this line, respectively.

2.3.3. PET image

From the 60 min data acquired in list-mode after the radioligand injection, several image frames were reconstructed as a standardized scheme (Abadie et al., 1992) as follows: (1) 15–40 min 'equilibrium' frame; (2) 'kinetic' 5 min frames from 0 to 60 min ($n = 12$); (3) 8–60 min; 'late' frame, this cumulated image permitting adequate placing of regions of interest (see below).

2.3.4. Definition of structures of interest

According to the main aim of our study (see Introduction), a large neocortical area was defined as the weighted mean of 84 circular regions of interest (regions of interest diameter: 7 mm), sampling across the entire neocortex over both hemispheres. Regarding the exploratory analysis on individual brain regions, five distinct central benzodiazepine receptor-rich structures (averaged over both hemispheres) were sampled: (1) middle frontal gyrus; (2) middle temporal gyrus; (3) posterior occipital cortex, (4) lateral parietal cortex and (5) cerebellar cortex (computed as weighted means of ten, eight, ten, eight, and five regions of interest, respectively). All regions of interest were defined on the 8–60 min PET frames based on the coregistered X-ray computed tomography scan cuts and according to the Stereotaxic Atlas of the Human Brain by Talairach and Tournoux (1988). All data were expressed in percentage of injected dose/liter of tissue (% ID/L).

The pons was defined using a 13 mm circular region of interest according to the procedure described previously (Abadie et al., 1992).

2.3.5. [^{11}C]Flumazenil plasma pharmacokinetics

During each PET scanning study, six venous blood samples were collected at 5, 10, 20, 30, 40, 60 min after radioligand injection and the concentration of unmetabolized [^{11}C]flumazenil was measured on each sample using a previously validated extraction procedure (Barré et al., 1991; Debruyne et al., 1991); for each sample, the percentage of metabolization was determined with this procedure. The values for unmetabolized plasma [^{11}C]flumazenil were also expressed in % ID/L.

2.4. Data analysis

2.4.1. Plasma [^{11}C]flumazenil pharmacokinetics

For each time post-radioligand injection, a paired t-test was conducted to assess [^{11}C]flumazenil changes from the control to the treatment condition. In addition, the area-under-the-curve (AUC) for the unchanged [^{11}C]flumazenil plasma data was determined by means of the trapezoidal rule for the 'equilibrium' interval between 15 and 40 min after injection.

2.4.2. PET data analysis

2.4.2.1. Brain [^{11}C]flumazenil pharmacokinetics. The 0–60 min brain pharmacokinetics of [^{11}C]flumazenil before and after zolpidem administration were analysed through the use of the set of 12∗5 min 'kinetic frames'. To this end, we performed a monoexponential fitting for each anatomical structure (excluding all values before the peak), using the following conventional equation:

$$C(\%\mathrm{ID}/L) = C_0 \cdot e^{-kt} \quad (1)$$

where C_0 represents the intercept of the fitted curve with the ordinate axis (i.e., the 'extrapolated peak'), and k the elimination constant. Fitting was considered good in all instances ($P < 0.05$ by χ^2-test between experimental and fitted data).

2.4.2.2. Central benzodiazepine receptor occupancy in vivo

Theory. According to classical basic equations, the law of mass action allows to describe the equilibrium state of two different ligands (i.e. a radioligand and a cold ligand) competing for the same receptor site (Lassen, 1992); when a cold ligand (L), with no active metabolite, is competing with a radioligand for the central benzodiazepine binding sites, the fractional receptor occupancy ($O(L)$) can be expressed as follows (Lassen, 1992; Ito et al., 1993):

$$O(L) = 1 - [B/F]_l/[B/F]_o \quad (2)$$

where F represents the free concentration of the radioligand in brain tissue, and B the specifically bound radioligand; the l and o subscripts refer to the cold ligand and the control conditions, respectively. This approach rests on several assumptions (Lassen, 1992), which, however, are all granted as valid in the case of [^{11}C]flumazenil, as follows: (i) the plasma [^{11}C]-flumazenil is the genuine receptor ligand and the blood-brain barrier has a high permeability for this tracer (Samson et al., 1985); (ii) there is negligible brain entry of [^{11}C]labeled metabolites (which are formed in liver) (Swahn et al., 1989; Persson et al.,

1989); (iii) non-specific [^{11}C]flumazenil binding in brain is negligible (Samson et al., 1985; Pappata et al., 1988).

In addition, to be valid, this measurement requires that F be negligible relative to K_d for flumazenil, i.e. that PET studies are carried out at truly tracer amounts of the radioligand.

Procedure. The specifically bound radioligand (B) was estimated in each structure of interest from the simple equation:

$$B = T - F \quad (3)$$

where T represents the total, and F the free, [^{11}C]flumazenil brain concentrations, respectively. In the present study, F was estimated by the pons radioligand concentration, with the assumption that non-specific binding is negligible (see above) and that the pons value represents F in all brain structures (Abadie et al., 1992), either in the control or the treatment conditions.

The values for both T and F were estimated during the pharmacological pseudoequilibrium interval (i.e., 15–40 min) (Savic et al., 1988; Abadie et al., 1992). Practically, the values for T in gray matter structures of interest were obtained by averaging the region of interest data from five successive 5 min frames (i.e., images four to nine). Regarding F, it was estimated from a region of interest defined on the 15–40 min PET frame; the use of this cumulated frame rather than the 5 min frames was to maximize the measurement accuracy of the low levels of radioactivity recorded in the pons (Abadie et al., 1992). Thus, F was estimated as the individual pons value in each condition. In a further step, we estimated F as the average value across all five subjects for each condition, because F may vary within each subject from the control to the treatment condition (Pauli et al., 1991).

2.4.3. Statistical analyses

2.4.3.1. [^{11}C]Flumazenil plasma pharmacokinetics. The values for each time of sampling were compared from the control to the treatment condition with paired t-tests.

Table 1
Plasma zolpidem concentration at 60 min (T_1) and 120 min (T_2) after zolpidem administration (20 mg p.o.)

Subject	T_1 (ng/ml)	T_2 (ng/ml)
1	81.8	152.1
2	92.0	68.2
3	98.5	52.0
4	192.4	123.1
5	22.7	140.4
Mean ± S.D.	97.5 ± 61.0	107.0 ± 44.6

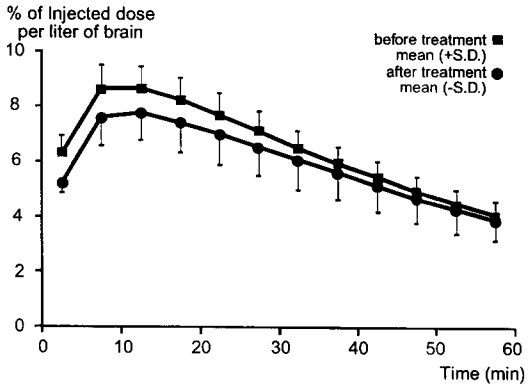

Fig. 1. Time course of [^{11}C]flumazenil whole neocortex uptake (mean ± S.D.) before and after zolpidem administration (P = 0.05, MANOVA).

2.4.3.2. PET data. According to our main objectives, the statistical analysis was first conducted on the whole neocortex data from the control to the treated condition. In a second step, we analyzed the five discrete neocortical and cerebellar areas.

[^{11}C]Flumazenil brain pharmacokinetics. The analysis of brain pharmacokinetic parameters took into account the individual plasma [^{11}C]flumazenil values, because the latter must influence the former. A set of analysis of covariance (ANCOVAs) with repeated measures were carried out to investigate the significance of changes in the parameters C_0 and k across the five subjects, with adjustment for individual plasma pharmacokinetics.

Receptor occupancy. Changes in B/F ratio values from the control to the treatment condition (i.e., receptor occupancies) across the five subjects were statistically assessed by repeated measures analysis of variance (ANOVAs). Since the expression B/F includes a within-study normalization for ligand availability, it was not necessary here to take into account the plasma values.

3. Results

3.1. Plasma concentrations of zolpidem (Table 1) and clinical effects

Measurable concentrations of zolpidem in plasma were found in all the subjects. There was a slight increase from T_1 (t = 60 min) to T_2 (t = 120 min) in subjects 1 and 5; and a decrease in subjects 2, 3 and 4. On average, however, there was no significant change from T_1 to T_2. All subjects fell asleep or were drowsy at the time of the second PET session.

Table 2
Results of [^{11}C]flumazenil brain pharmacokinetic analysis (C_0 values) (%ID/L) according to a monoexponential model (see Methods)

Subject	1		2		3		4		5	
Brain area	C	T	C	T	C	T	C	T	C	T
Whole neocortex	9.6	8.6	10	8.2	10.5	10.5	11.3	9.20	12.5	10.5
Mid. frontal gyrus	10	9.2	11.2	8.9	11	11	11.2	11.3	13.8	12.3
Post. occip. cortex	10.5	8.2	8.9	7.6	12.5	11.5	12.1	9.7	11.9	11.2
Parietal cortex	9.5	8.1	9.7	8.1	10.6	10.5	10.7	9.2	11.3	10.5
Mid. temp. gyrus	9.9	8.6	9.4	8	10.3	10.6	11.4	9.2	12.7	10.1
Cerebellum	9.6	7.8	9.7	8.5	9.3	9.5	12.5	9.6	11.3	9.9

C = control; T = treatment.

3.2. Plasma pharmacokinetics of [^{11}C]flumazenil

In the control condition, the values for [^{11}C]-flumazenil venous plasma data (corrected for metabolization) were $1.46 \pm 0.8\%$ ID/L, and $0.79 \pm 0.39\%$ ID/L at 5 and 60 min post-injection respectively, with marked inter-subject variability (data not shown). There was a trend for an increase in mean values in the treatment condition as compared to the control, from 10 min onwards, which did not reach statistical significance for any time. There was no significant effect of zolpidem on [^{11}C]flumazenil metabolization (data not shown).

3.3. Brain pharmacokinetics

As an illustration of [^{11}C] time-course in the brain, Fig. 1 shows the mean ($\pm$S.D.) values for the entire neocortex across the five subjects before and after zolpidem administration. There was a trend for decline in mean [^{11}C] uptake values across all times in the treated as compared to control condition, which reached statistical significance ($P = 0.05$ by repeated mesure MANOVA)

3.3.1. Monoexponential analysis

Table 2 shows the individual C_0 values for the entire neocortex and five discrete brain areas. The ANCOVA, taking into account the [^{11}C]flumazenil plasma values (15–40 min interval) showed a decrease in C_0 values in the whole neocortex from control to treated conditions which was at the limit of statistical significance ($P = 0.052$). For the five discrete brain areas, a statistically significant global decrease was found (treatment effect: $P = 0.034$) with a significant region effect ($P = 0.032$) but no significant interaction, indicating that this effect of zolpidem was essentially uniform across regions.

The ANCOVAs on the elimination constant (k) showed no statistically significant differences (data not shown).

Table 3
Pons ^{11}Cflumazenil uptake values (expressed in % ID/L) in control (C) and treatment (T) conditions, 15–40 min after radioligand injection

Subject	Before zolpidem (C)	After zolpidem (T)	Difference ($T - C$)
1	1.44	2.50	1.06
2	1.23	0.98	− 0.25
3	1.02	1.29	0.27
4	1.57	1.87	0.30
5	1.39	1.22	−0.17
Mean $\pm$ S.D.	1.33 ± 0.21	1.57 ± 0.61	0.24 ± 0.52 [a]

[a] No statistically significant effect of zolpidem (paired t-test).

3.4. Pons data (Table 3)

There was no statistically significant effect of zolpidem on pons % injected dose/liter of brain values.

3.5. Receptor occupancy (Table 4A,B)

The individual and mean fractional occupancies are shown for the five discrete regions and the whole neocortex, calculated with the individual and the averaged pons values in each condition (Table 4A and 4B, respectively). The mean fractional occupancies estimated with the individual pons values ranged from 20% to 22% with spurious negative values for subject 5 and a high coefficient of variation (Table 4A). However, the analysis with averaged pons values provided more stable results which were amenable to statistical treatment (Table 4B). For the whole neocortex, a statistically significant decrease after zolpidem administration, as compared to the control condition ($P < 0.01$) was found. The global ANOVA for the five discrete areas showed a significant region effect ($P < 0.001$) and a statistically significant treatment effect in the form of a decrease in B/F values ($P < 0.01$), with a significant interaction. According to the post-hoc analysis, this effect of zolpidem was statistically significant for each of these five discrete brain regions, but the calculated probability was not uniform among them.

4. Discussion

The present paper documents in humans the feasibility of measuring with PET the fractional central benzodiazepine receptor occupancy by zolpidem at twice the dose recommended for the treatment of sleep disturbances, and shows that this dose results in only moderate central benzodiazepine binding site occupancies.

4.1. Plasma levels of zolpidem

In each subject, we found a measurable concentration of zolpidem in the plasma with values consistent with published data obtained after oral administration of 20 mg of this drug (Langtry and Benfield, 1990; Durand et al., 1992). This indicates that the absorption of zolpidem was adequate in each subject. The zolpidem plasma level across the five subjects did not show statistically significant changes from 1 to 2 h after drug administration. The intersubject variability (increase for subjects 1 and 5; decrease for subjects 2, 3 and 4) is not surprising in view of the known variability in T_{max} values after oral administration of this drug (Langtry and Benfield, 1990; Durand et al., 1992). Overall, these data retrospectively justify our choice of timing for the second administration of [^{11}C]flumazenil.

4.2. [^{11}C]Flumazenil pharmacokinetics

The pharmacokinetic analysis of brain and plasma [^{11}C]flumazenil performed in this study is only descriptive but allows an objective characterization of the tracer time-course in the treatment as compared to the control condition.

In agreement with previous PET studies with benzodiazepines (Shinotoh et al., 1989; Pauli et al., 1991), we found that the oral administration of zolpidem influences significantly the [^{11}C]flumazenil regional brain pharmacokinetics, but was without significant effect on the plasma pharmacokinetics of the radioligand. There was however a marked inter- an intra-individual variability in plasma [^{11}C]flumazenil pharmacokinetics.

With regard to brain [^{11}C]flumazenil pharmacokinetics, zolpidem caused a decrease in C_0 values, that was marginally significant for the whole neocortex and clearly significant for the five discrete brain areas; C_0 is the intercept of the elimination slope with the y-axis and therefore represents the 'extrapolated peak' objectively estimated from the experimental PET data. This effect of zolpidem on C_0 was not accompanied by any change in the elimination constant (k) which suggests that zolpidem reduces the brain uptake, but not the clearance of [^{11}C]flumazenil. The zolpidem-induced decrease in C_0 values is likely to reflect the expected pharmacological competition between [^{11}C]flumazenil and zolpidem for a common binding site (i.e., occupancy) (Benavides et al., 1988), although a non-specific effect on cerebral blood flow, which is known to decrease after i.v. administration of high doses of zolpidem in baboons (Cudennec et al., 1988), cannot be entirely ruled out. Nevertheless, these results are in line with previous human PET studies with [^{11}C]-flumazenil in which both clonazepam administration and partial saturation by unlabeled flumazenil reduced [^{11}C]flumazenil peak values without obvious change in elimination rate (Shinotoh et al., 1986; Pappata et al., 1988); of note, flumazenil is known not to affect cerebral blood flow in man, Forster et al., 1987).

Consistent with earlier studies with benzodi-

Table 4
Fractional receptor occupancy after zolpidem administration (20 mg p.o.)

Subject	1	2	3	4	5	Mean ± SD	P
(A) F determined as the individual pons value							
Whole neocortex	62%	5%	19%	27%	−11%	20% ± 27	
Middle frontal gyrus	61%	6%	18%	26%	−13%	20% ± 27	
Posterior occipital cortex	66%	12%	18%	27%	−10%	22% ± 28	
Parietal cortex	65%	5%	18%	26%	− 4%	22% ± 27	
Middle temporal gyrus	60%	11%	17%	26%	− 6%	22% ± 24	
Cerebellum	61%	12%	17%	30%	−10%	22% ± 26	
(B) F determined as the average pons value in each condition							
Whole neocortex	28%	44%	14%	25%	23%	27% ± 11	0.009
Middle frontal gyrus	28%	45%	11%	24%	21%	26% ± 12	0.008 [a]
Posterior occipital cortex	35%	49%	12%	25%	24%	29% ± 14	0.006 [a]
Parietal cortex	33%	43%	12%	25%	28%	28% ± 11	0.004 [a]
Middle temporal gyrus	25%	48%	11%	24%	26%	27% ± 13	0.010 [a]
Cerebellum	25%	54%	10%	28%	26%	29% ± 16	0.011 [a]

[a] The global ANOVA for the five discrete areas showed a significant region effect ($P < 0.001$) and a significant treatment effect in the form of a decrease in B/F ($P < 0.01$) with a significant interaction. P values for each discrete area by post-hoc analysis are also shown.

azepines, the apparent C_{max} of flumazenil was significantly reduced by zolpidem in our study while the apparent T_{max} was not affected (see Fig. 1).

4.3. Central benzodiazepine receptor occupancy by zolpidem

4.3.1. Methodological issues

This study is the first to estimate central benzodiazepine receptor occupancy by zolpidem in humans. We assessed the free radioligand concentration by measuring pons [^{11}C]flumazenil concentration. Although this structure is not totally devoid of central benzodiazepine receptors (central benzodiazepine receptor density in the pons represents about 2% of that present in the frontal cortex, Litton et al., 1994), this method is convenient because the region of interest for pons is readily defined (Abadie et al., 1992). A second assumption relates to the non-specific (NS) binding, which may affect the estimation of F from pons values. However, previous studies have shown that this fraction can be considered as negligible (see Abadie and Baron, 1990, for review). Furthermore, any error in estimation of F due to non-specific binding would cancel in the calculation of occupancies as the free fraction of tracer in the brain (designated as $f_2 = F/[F + NS]$) is a constant which does not show saturability and which appears in both terms of Equation 2 (Mazoyer, 1991). The use of individual pons values to estimate fractional central benzodiazepine receptor occupancy resulted in spurious results (with e.g. 'negative' occupancies in one subject, Table 4A). This problem most likely stems from the variability in [^{11}C]flumazenil uptake in the pons, which itself reflects low count-rates in this small structure as well as differences in PET study conditions from the control to the treated state (possibly including small differences in head positioning). This issue was previously addressed by us (Abadie et al., 1992). Furthermore, under conditions of a weak central benzodiazepine receptor occupancy such as those encountered in the present investigation, even small errors in the determination of F will lead to large errors in calculated occupancies, because the measurement of occupancy is the result of two successive subtractions (see Equations 2 and 3). To deal with this problem, we therefore performed inter-subject averaging of F across all subjects for each condition, as described by Pauli et al. (1991) (see below). Although obviously inapplicable to single-subject investigations, this method appears suitable to estimate an average receptor occupancy in small samples of subjects, especially if modest occupancy is expected, as supported by our results. A different issue regarding the estimation of receptor occupancy based on B/F (Equation 2) relates to the assumption that F be much lower than the K_d (which in turn implies adequate specific radioactivity) (Lassen, 1992). This assumption was fulfilled here, as the individually estimated F values ranged from 0.12 to 0.98 nM (data not shown), i.e. 10–80-fold lower than the K_d for flumazenil estimated in humans by PET (Pappata et al., 1988; Price et al., 1991; Abadie et al., 1992; Delforge et al., 1993), and these values did not change significantly from the control to the treatment condition.

4.3.2. Pharmacological issues

Based on the data shown in Table 4B, and assuming that central benzodiazepine occupancy is a linear function of the dose, we can infer that at the usual posology of zolpidem (10 mg), central benzodiazepine receptor occupancy in neocortical structures should lie around 15%. This receptor occupancy is lower than that which would correspond to the 15–23% inhibition of total [^{11}C]flumazenil binding by a dose of clonazepam (30 μg/kg p.o.) inducing sleep and ataxia (Shinotoh et al., 1989), and than that reported for a sedative dose (30 mg p.o.) of diazepam (24%) by Pauli et al. (1991). Similarly, using [^{123}I]Iomazenil, single photon emission computed tomography and B/F ratios, Videbaek et al. (1993) have recently reported a central benzodiazepine receptor occupancy ranging from 20 to 30% in the neocortex during steady-state midazolam i.v. infusion at doses inducing sleep (6 mg $\cdot$ h^{-1}). These studies would be consistent with previous in vitro and in vivo investigations in rodents demonstrating that zolpidem possesses an intrinsic activity greater than other benzodiazepine receptor agonists such as diazepam, clonazepam and midazolam (see Introduction).

In vitro and in vivo investigations in rodents have demonstrated that zolpidem displays selectivity for type I central benzodiazepine receptors. Moreover, autoradiographic studies in human and non-human primate brain sections have demonstrated a similar selectivity (Dennis et al., 1988). The data from the present investigation do not show a regional selectivity of zolpidem at displacing [^{11}C]flumazenil binding, in the human brain. A possible explanation for this finding is that the regions of interest selected include those preferentially enriched in type I central benzodiazepine binding sites but did not include those enriched in central benzodiazepine receptor with low affinity for zolpidem such as the hippocampus, striatum and spinal cord, because low receptor densities in these small areas may result in spurious [^{11}C]flumazenil specific binding measurements (Abadie et al., 1992). Moreover, studies in rodents have shown that the regional selectivity of zolpidem is best evidenced at high doses of this compound (Benavides et al., 1992), perhaps due to the inherent variability of studies carried out at low receptor occupancy. However, for obvious ethical reasons, doses higher than the one used in the present study would be difficult to evaluate in humans.

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