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ACCELERATED COMMUNICATION

Quantitative Evaluation of Benzodiazepine Receptors in Live *Papio papio* Baboons Using Positron Emission Tomography

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SUMMARY

The binding of the ^{11}C-labeled benzodiazepine antagonist Ro 15-1788 (flumazenil) was measured in the neocortex of live *Papio papio* baboons by positron emission tomography. This allowed us to calculate *in vivo* (i.e., at physiological temperature, neurotransmitters concentrations, and ionic environment) the apparent density of available benzodiazepine receptors (B'_{max}) and the dissociation constant of Ro 15-1788 (K_d). By coadministering increasing doses of unlabeled Ro 15-1788 with [^{11}C]Ro 15-1788 and assuming that nonsaturable radioactivity indicated the free ligand concentration, we were able to obtain saturation isotherms. We showed that a state of quasiequilibrium was reached 50 min after the administration of the radioligand. Linear Scatchard plots allowed us to calculate B'_{max} at 78 and 50 pmol/ml of cerebral tissue in the occipital and frontal cortices, respectively. In both these areas, K_d is on the order of 6 nM, with a Hill number very close to unity. This indicates that Ro 15-1788 binds *in vivo* with high affinity to an homogeneous population of saturable sites. A similar measurement was carried out on a naturally photosensitive *P. papio* baboon. Absolute values of B'_{max}, K_d, and Hill number were similar to those of the control baboons. Although results concerning this baboon can only be considered as a case report, this similarity may suggest that its epileptic syndrome is not related to a large change in B'_{max} or K_d, at least in occipital and frontal cortices. Our results showed that quantitative estimation by positron emission tomography of some characteristics of benzodiazepine receptors is possible in live baboons and may represent a supplementary tool for investigating further the molecular mechanisms of benzodiazepine receptor function in physiological and physiopathological conditions. We suggest that a similar method of quantification of classic *in vivo* [^{3}H]Ro 15-1788 binding could be usefully adapted when studying rodent models of epilepsy, stress, and other neuropsychological disorders. On the other hand, the similarity between the B'_{max} and K_d values we obtained in baboons and those recently reported in humans using similar methods emphasizes that most of the *in vivo* characteristics of the benzodiazepine receptors of baboons are very close to those of human benzodiazepine receptors. This confirms that *P. papio* baboons are a suitable animal model for studying the pharmacology of benzodiazepine receptor ligands before clinical applications in humans.

Since the existence of specific binding sites for [^{3}H]diazepam was shown in the brain of rats (1, 2) and humans (3), a large number of biochemical and behavioral experiments have led to rapid advances in the understanding of the pharmacology of central type benzodiazepine receptors. However, *in vitro* binding studies revealed that interactions between ^{3}H-ligand and benzodiazepine receptors are particularly dependent on temperature, ionic environment, and γ-aminobutyric acid concentrations (4). This raises the question of to what extent the *in vitro* binding characteristics of a drug reflect the behavior of the drug *in vivo*. The development of *post mortem* methods to measure radiolabeled ligands that were bound in brains of living rodents (5–7) offered a new way of investigating the interactions between central type benzodiazepine receptors and their ligands in living organisms.

Noninvasive binding studies of benzodiazepine receptors have been carried out more recently in live subhuman primates (8–11) and humans (12–14) by using PET for external detection and the selective benzodiazepine receptor antagonist [^{11}C]Ro 15-1788 as an *in vivo* positron-emitter radioligand.

Measurement of the specific binding of radiolabeled Ro 15-1788 can be obtained *in vivo* by PET or *"ex vivo"* by classic methods after the intravenous administration of a tracer dose of the radioligand (labeled with ^{11}C or ^{3}H). Unfortunately, values describing the *in vivo* characteristics of the receptors, such as the apparent density (B'_{max}) and the dissociation constant for Ro 15-1788 (K_d), cannot be directly measured. Thus,

ABBREVIATIONS: PET, positron emission tomography; [^{11}C]Ro 15-1788, ethyl 8-fluoro-5,6-dihydro-5-[^{11}C]methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate; nor-Ro 15-1788, desmethyl Ro 15-1788, i.e., ethyl 8-fluoro-5,6-dihydro-6-oxo-4H-imadazo[1,5-a][1,4]bendodiazepine-3-carboxylate.

after a tracer dose was administered, the changes in the specific binding of the radioligand observed under the experimental conditions did not show conclusively that the basic characteristics of receptors had been altered. Indeed, the concentration of available free radioligand could be influenced by changes in some parameters of the radioligand biodistribution (such as cerebral blood flow, blood-brain barrier permeability, and metabolism). Consequently, the specific binding of the radioligand after bolus administration can be altered by changes in these parameters. Recent studies in humans were carried out using tracer doses of [^{11}C]Ro 15-1788, which were coadministered with increasing saturating doses of unlabeled Ro 15-1788. The PET measurement of the Ro 15-1788 bound in brain also led to an estimation of the B'_{max} and K_d of human benzodiazepine receptors (15, 16), using a simple calculation method at quasiequilibrium. This calculation method could be more widely used as a supplementary tool to quantitatively investigate the characteristics of the central type benzodiazepine receptors in animals under physiological, pathological, or pharmacological conditions.

During the last decade, numerous studies of the pharmacology of the benzodiazepine receptors have been carried out in the subhuman primates *Papio papio* baboons (17). Some of these baboons display a light-sensitive epilepsy syndrome (18), which can be decreased (19, 20) or enhanced (21, 22) by the administration of benzodiazepine receptor ligands. For this reason, nonphotosensitive and photosensitive baboons are very interesting animal models to use when investigating the pharmacological effects produced by benzodiazepine receptor ligands. Moreover, these effects observed in *P. papio* baboons could be, to a certain extent, indicative of those that may be produced in humans, if the benzodiazepine receptors of live baboons possess characteristics closely related to those of human receptors. Consequently, functional similarity between benzodiazepine receptors in humans and in subhuman primates has to be investigated further *in vivo*.

For this purpose, an approach to *in vivo* quantification of the B'_{max} of benzodiazepine receptors and their K_d for [^{11}C]Ro 15-1788 in the brain of both nonphotosensitive and photosensitive *P. papio* baboons was carried out with PET data, using a simple calculation method at quasiequilibrium.

Materials and Methods

Biological model

Nine PET experiments were carried out on six *P. papio* baboons (control group) weighing 11.2 ± 2.9 kg (mean ± SD). The same number of experiments were also performed on a *P. papio* baboon weighing 11.8 ± 1.1 kg (mean ± SD) that displayed a natural light-sensitive epilepsy syndrome. Photosensitivity was tested by intermittent light stimulation (25 Hz) and concomittant electroencephalographic recording (18) 1 week before each PET experiment, with the animal in a primate chair that confined the hips and neck. Under light stimulation, the photosensitive baboon generally displayed photosensitivity characterized by rapid cloni of the eyes (+1) and sometimes by hindlimb and forelimb cloni (+3).

Four hours before the beginning of the PET experiment, the animals were anaesthetized by intramuscular administration of ketamine (10 mg/kg) and prepared as previously described (9). Regular cardiac frequency and basal electroencephalographic activity allowed monitoring of the normal physiological state of the animals during the experiments. Each animal was rested for a period of at least 2 weeks between PET studies.

Drugs

Unlabeled Ro 15-1788 and nor-Ro 15-1788 were supplied by the Hoffman-La Roche Laboratory (Basel, Switzerland). [^{11}C]Ro 15-1788 was synthesized in our laboratory by a process of rapid methylation of nor-Ro 15-1788 with $^{11}CH_3I$ (23). Specific radioactivity of the radioligand at the time of the injection was 400–1200 mCi/μmol.

[^{11}C]Ro 15-1788 and unlabeled Ro 15-1788 were administered intravenously. The unlabeled antagonist was administered in a galenic preparation used for humans (Hoffman-La Roche laboratory), with the exception of the 2 mg/kg dose, where Ro 15-1788 was dissolved in ethanol (300 μl) and propyleneglycol (700 μl) and diluted to 2 ml with saline. Previous separate PET experiments showed that, at the doses used, these latter solvents did not modify the binding of [^{11}C]Ro 15-1788.

PET Experimental Procedure

The experimental procedure was the same as in previous experiments (9). External detection of the radioactivity was performed with the one-slice ECAT II ORTEC PET device. Only the four full-saturation experiments (where detectable radioactivity was very low) were performed with a seven-slice time-of-flight camera (TTVO1; LETI, Grenoble, France).

Image acquisition started immediately after the administration of about 10 mCi of the tracer and the repeated scanning lasted for 90 min after the injection. The scanning time was progressively expanded from 1 to 2, 5, and 10 min in order to maintain a similar total count rate/scan, despite the ^{11}C radioactive decay. Thirty sequential scans were performed at the same brain level (OM+10 mm) throughout the experiment. On the image obtained 20 min after the injection of the radioligand, circular regions of interest were placed in the frontal and occipital cortices. These were based on the histological cross-section of the baboon brain with the orbito-meatal plane as reference (24). ^{11}C decay was automatically corrected for in the acquisition data. The concentration of radioactivity in each regional area of the brain was measured during each sequential scan and expressed as the percentage × 10^{-2} of injected radioactive dose/ml of cerebral tissue (or as pmol of Ro 15-1788/ml of tissue when necessary). This was plotted versus time.

In vivo binding studies

Quantification of the characteristic parameters of the [^{11}C]Ro 15-1788 binding in baboon brain was made using a method previously reported (15). This method of analysis is relevant if three assumptions are taken into account. 1) The main metabolite of [^{11}C]Ro 15-1788, [^{11}C]Ro 15-3890, which cannot cross the blood-brain barrier, does not significantly contribute to cerebral radioactivity values after intravenous administration of [^{11}C]Ro 15-1788 (25) and, thus, the cerebral radioactivity measured by PET represents unchanged [^{11}C]Ro 15-1788. 2) Nonsaturable [^{11}C]Ro 15-1788 radioactivity measured by PET in full-saturation studies represents the free fraction of the radioligand, because of very low nonspecific binding of Ro 15-1788 (7). So, free [^{11}C] Ro 15-1788 (F) can be estimated by measuring the nonsaturable [^{11}C] Ro 15-1788 binding. Regional F (in pmol/ml Ro 15-1788) is calculated as the product of the percentage of the injected [^{11}C]Ro 15-1788 dose/ml measured during full-saturation experiments multiplied by the total dose of Ro 15-1788 (nmol) injected in control and partial-saturation experiments (15). 3) Estimated free ligand (F) and the specifically bound fraction of the radioligand (B) reach a quasiequilibrium state soon after systemic administration of the tracer (15, 16). In a state of equilibrium, B is expressed by the following equation (26):

$$B = B'_{max} \cdot F/(K_d + F)$$

where B'_{max} is the maximum number of available binding sites and K_d is the dissociation constant.

Brain regional bound fraction as a function of brain regional free fraction was determined in separate experiments by coadministration of increasing doses of unlabeled Ro 15-1788 with a tracer dose of [^{11}C] Ro 15-1788.

Control Experiments. Two normal baboons were injected with radioactive tracer doses of 522 and 377 MBq (i.e., 14.2 and 10.3 mCi) [^{11}C] Ro 15-1788, which were equivalent to 24.1 and 14.8 nmol, respectively. The photosensitive baboon was injected with tracer doses of 394 and 388 MBq (i.e., 10.8 and 10.6 mCi) [^{11}C] Ro 15-1788, which were equivalent to 33.7 and 10.4 nmol of Ro 15-1788, respectively.

Partial-Saturation Experiments. The saturation studies were performed by coadministration of [^{11}C]Ro 15-1788 with increasing doses of unlabeled Ro 15-1788. Five normal baboons were injected in five separate experiments with a total mass of Ro 15-1788 of 159, 173, 261, 273, and 429 nmol, representing 0.003, 0.005, 0.0075, 0.010, and 0.0150 mg/kg, respectively. The photosensitive baboon received 126, 229, 306, 408, and 662 nmol of Ro 15-1788, representing 0.003, 0.004, 0.005, 0.0075, and 0.010 mg/kg.

Full-Saturation Experiments. In order to measure free ligand, full-saturation experiments were carried out twice in two normal baboons and one photosensitive baboon, by administration of a large dose of the unlabeled antagonist (2 mg/kg) 5 min before an injection of [^{11}C] Ro 15-1788. The two averaged regional free ligand kinetic curves from each baboon were used for further calculation.

Regional B was calculated each 10 min after the injection of the radioligand by subtraction of the regional mean of nonsaturable binding (NS) from total regional binding (B_t), following the simple equation:

$$B = B_t - NS$$

In order to determine the time where a state of quasiequilibrium between free and bound ligand was reached, the regional B/F ratios were plotted against time. The regional B (expressed in pmol/ml of tissue) calculated from the different partial-saturation experiments were plotted against the corresponding estimated F (expressed in nM). This was plotted every 10 min after the administration of the radioligand. Linearization of these latter plots by Scatchard transformation allowed calculation of the regional apparent B'_{max} and K_d values every 10 min by linear regression. The corresponding Hill number (n_H) was calculated by regression analysis of log ($B/B'_{max} - B$) versus log(F) every 10 min after equilibrium was reached. Regional values obtained at equilibrium were averaged to calculate mean parameters, which were compared by the Student t test.

Results

To make easier reading, all the absolute values of the regional [^{11}C]Ro 15-1788 brain concentrations measured by PET are not shown in detail in this paper. Only typical values or patterns of the cerebral kinetics of the radioligand are shown.

When a tracer dose of [^{11}C]Ro 15-1788 was injected intravenously alone, total radioactivity rapidly entered the brain compartment. During these control experiments, a peak of maximal radioactivity was measured after 20 min (B_t = 2–4 × 10^{-2}% of the total injected dose of tracer/ml of cerebral tissue). In contrast, when animals were pretreated with a large dose of unlabeled Ro 15-1788, the concentration of radioactivity in the brain reached a maximum (B_t = 1–2 × 10^{-2}% of injected dose/ml of tissue) in less than 1 min after administration of the radioligand and then decreased gradually until the end of the experiment. During these full-saturation experiments, regional values of the radioactive concentration were very similar in all animals studied. Twenty minutes after the injection of [^{11}C]Ro 15-1788, nonsaturable binding was only 0.2–0.4 × 10^{-2}% of injected dose/ml of cerebral tissue, which represents about 10% of B_t measured during control experiments.

Fig. 1 shows that bound ligand reached a peak after about 20 min in both control and photosensitive baboons. A slow washout of bound ligand was observed until the end of the experiments. Bound ligand was always higher in the occipital cortex

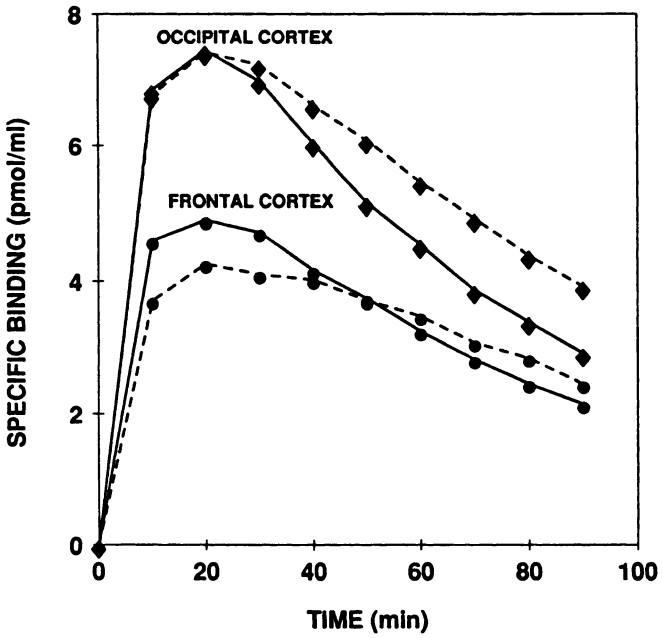

Fig. 1. Kinetics of the specifically bound [^{11}C]Ro 15-1788 in the neocortex of nonphotosensitive (——) and photosensitive (– – –) baboons.

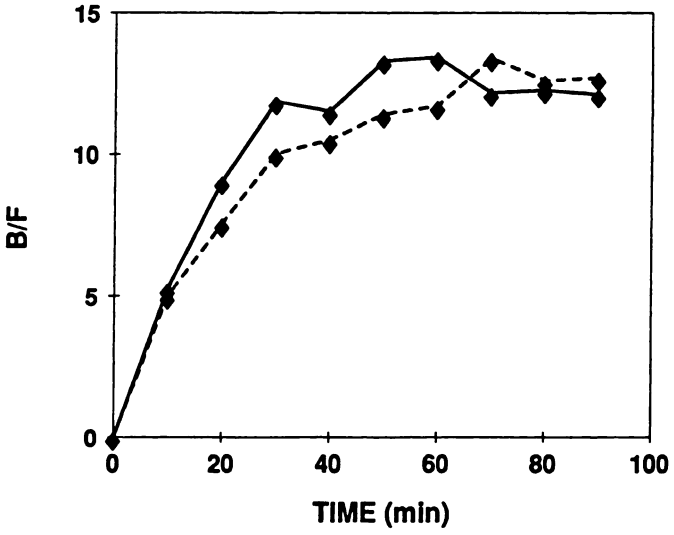

Fig. 2. Kinetics of the B/F ratio after the administration of a tracer dose of [^{11}C]Ro 15-1788 in the occipital cortex of nonphotosensitive (——) and photosensitive (– – –) baboons.

than in the frontal cortex in all animals studied (Fig. 1). In the nonphotosensitive baboons, the maximal value of B measured at 20 min was 7.45 and 4.9 pmol/ml (representing 3.4 and 2.5 × 10^{-2}% of injected dose/ml of tissue) in occipital and frontal cortices, respectively. The B values measured in the photosensitive baboon were 7.38 and 4.1 pmol/ml (representing 3.0 and 1.63 × 10^{-2}% of injected dose/ml of tissue) in frontal and occipital cortices, respectively.

Fig. 2 illustrates the kinetics of the mean regional B/F ratio in the occipital cortex during control experiments. After a slow rise, lasting 40–45 min, this ratio reached a constant value. This suggests that a state of equilibrium was reached between free and bound ligand at 50 min, lasting to the end of the experiments. Whatever the photosensitivity of the animals, an 8–10 and a 12–14 B/F ratio was obtained in frontal and occipital cortices, respectively.

When increasing doses of unlabeled Ro 15-1788 were coadministered with the radioligand, kinetic values of regional radioactivity were decreased, when compared with the values when the radioligand was injected alone (Fig. 3). This decrease in [^{11}C]Ro 15-1788 concentration (in percentage) is dose dependent, with the lowest value leading to detection of only nonsaturable binding (Fig. 3).

B values plotted against the corresponding F values every 10 min during the state of equilibrium displayed the beginning of a typical hyperbolar pattern of saturation isotherm curves. Fig. 4 illustrates this phenomenon (*inset*) 60 min after administration of the radioligand in nonphotosensitive baboons and shows that the corresponding Scatchard plots displayed a typical linear pattern (correlation coefficients > 0.9; $n = 7$). Corresponding Hill plots were also linear (correlation coefficient > 0.95; $n = 7$) and Hill numbers were close to unity. Similar results were obtained in the photosensitive baboon (data not shown).

Apparent K_d values calculated from Scatchard plots were of the same order of magnitude (4–9 nM) whatever the time (from 50 to 90 min after radioligand injection), the region, and the animal considered. B'_{max} calculated every 10 min in nonphotosensitive and photosensitive baboons was higher in the occipital cortex (62–94 pmol/ml) than in the frontal cortex (29–57 pmol/ml). Table 1 shows that no interanimal or interregional differences were observed between mean regional K_d (5.8 nM) values and between Hill numbers calculated during quasiequilibrium ($p < 0.01$, $n = 5$). Furthermore, mean Hill numbers were not significantly different from 1 (Table 1). Mean B'_{max} was higher in the occipital cortex (79 pmol/ml) than in frontal cortex (49 pmol/ml) ($p < 0.01$; $n = 5$) (Table 1). No significant interanimal differences were observed between nonphotosensitive and photosensitive regional B'_{max} values.

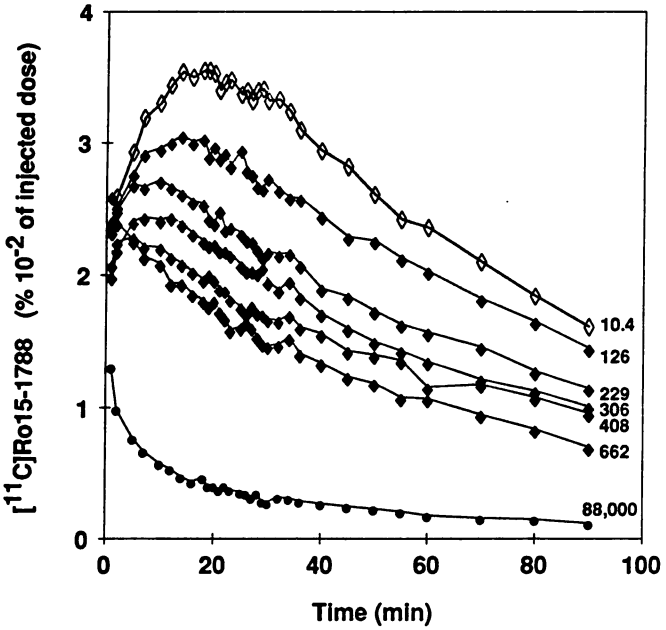

Fig. 3. Dose-dependent decrease of the total radioactivity kinetics in the occipital cortex of a photosensitive baboon produced by coadministration of unlabeled Ro 15-1788. ◇, Control; ♦, partial saturation; ●, full-saturation. *Numbers at the right*, total mass (nmol) of injected Ro 15-1788.

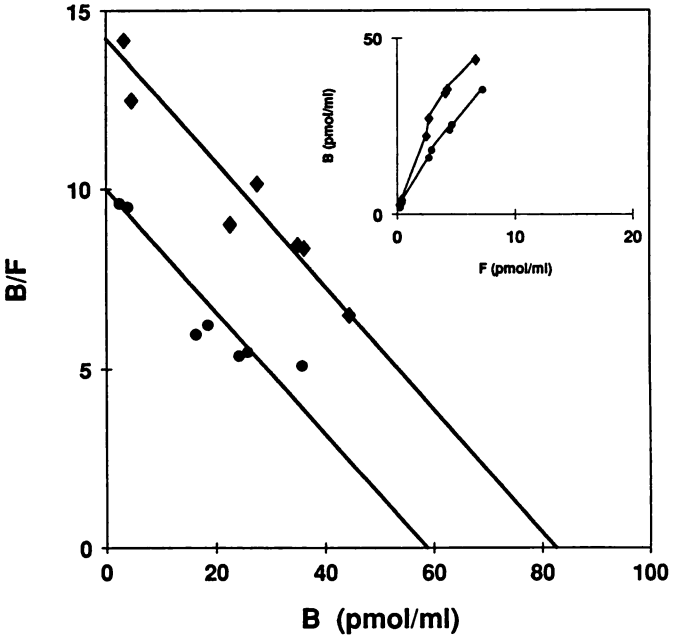

Fig. 4. Typical saturation isotherms (*inset*) and Scatchard plots in the frontal (●) and occipital (♦) cortices of nonphotosensitive baboons 60 min after injection of [^{11}C]Ro 15-1788. Each of the seven *points* resulted from one PET measurement in a different animal of the control group, except that one baboon was used for one control PET binding experiment and one partial-saturation experiment. Frontal cortex: B'_{max} = 59.1 pmol/ml; K_d = 5.8 nM; regression coefficient = 0.91; $n = 7$. Occipital cortex: B'_{max} = 82.5 pmol/ml; K_d = 5.8 nM; regression coefficient = 0.97; $n = 7$.

Discussion

Relevance and interest of this method of quantification. Our study showed that [^{11}C]Ro 15-1788 is a suitable radioligand for quantitative *in vivo* binding studies of central type benzodiazepine receptors in the brains of nonhuman primates. A large quantity of [^{11}C]Ro 15-1788 rapidly enters the brain after intravenous administration. Full-saturation experiments performed on baboons also clearly showed that, compared with B_t, corresponding nonsaturable radioactivity used to estimate F was very low. This allowed an accurate measurement of the specific binding of Ro 15-1788, not only as a function of time but also as a function of the mass of radioligand injected. The present results showed that in baboons a state of equilibrium between bound and free ligand was attained 50 min after the injection of [^{11}C]Ro 15-1788. This and the extremely rapid rate of displacement of bound [^{11}C]Ro 15-1788 (10, 11) indicate that the rate of decrease of free ligand is slow, considering the rate constants of association and dissociation of the radioligand to benzodiazepine receptors. As in human studies (15, 16), equilibrium mass action law can be applied to the study of the binding of Ro 15-1788 in a live *P. papio* baboon brain. In addition, when equilibrium was reached, we observed a dose-dependent effect of increasing doses of unlabeled Ro 15-1788 on the bound ligand and also a highly significant linearity of the corresponding Scatchard plots in control and photosensitive baboons. These two latter observations strongly suggest that typical ligand-receptor interactions were measured.

This clearly demonstrates that *in vivo* binding of [^{11}C]Ro 15-1788 can be quantitatively studied using an *in vivo* equilibrium method similar to the classic ligand-receptor interaction model used *in vitro*. In addition, our PET results suggest that this method of quantification may be usefully adapted to classic *in*

TABLE 1

Mean B'_{max}, K_d, and n_H values calculated *in vivo* in cerebral cortex of living baboons at equilibrium after intravenous administration of [^{11}C] Ro 15-1788

B'_{max}, K_d, and n_H are the average of five values, which were calculated by Scatchard analysis every 10 min from t_o + 50 min to t_o + 90 min. In the study of the nonphotosensitive baboons, each point of the Scatchard and Hill plots represents a PET measurement at a given time in one of the six animals of the control group (one baboon was examined twice). One animal was used for determination of the photosensitive baboon's parameters. Values are mean ± standard deviation.

Baboons	Occipital cortex			Frontal cortex		
	B'_{max}	K_d	n_H	B'_{max}	K_d	n_H
	pmol/ml	nM		pmol/ml	nM	
Nonphotosensitive	77.5 ± 7.3	5.8 ± 0.3	0.99 ± 0.04	49.9 ± 5.3	4.9 ± 0.7	1.01 ± 0.06
Photosensitive	78.7 ± 11.5	6.0 ± 1.2	0.96 ± 0.04	48.8 ± 7.0	5.8 ± 1.6	1.06 ± 0.05

vivo studies of [^{3}H]Ro 15-1788 binding to benzodiazepine receptors in rodents. This approach could lead to the investigation of the K_d and B'_{max} of Ro 15-1788 in rodent models of physiopathological conditions such as stress, benzodiazepine withdrawal state, or epilepsy.

***In vivo* B'_{max} and K_d values: comparison with previous reports in animals and humans.** The pattern of [^{11}C]Ro 15-1788 brain kinetics measured in subhuman primates by PET is very similar to the human pattern previously observed with the same method (12–14). The 0.6 frontal/occipital cortex B_t ratio measured in baboons is very close to the ratio reported in humans (15). The 6 nM K_d value calculated in baboons was strikingly similar to those measured in humans (15, 16). Furthermore, the 6 nM K_d of [^{11}C]Ro 15-1788 measured by PET in baboons is very close to the 8 nM K_d obtained in rats at 37° *in vitro* with [^{3}H]Ro 15-1788 (27). Regional B'_{max} values obtained in occipital and frontal cortices by PET in baboons (79 and 49 pmol/ml, respectively) were of the same order of magnitude as those reported in humans (90 pmol/ml) by Persson *et al.* (16) and slightly higher than those reported by Pappatà *et al.* (15), which were 42 and 34 pmol/ml in human frontal and occipital cortices, respectively. Regional Hill numbers calculated in baboons were close to unity. This shows that Ro 15-1788 binds *in vivo* to an homogeneous population of saturable sites, as in humans (16). Compared with regional B_{max} values measured by *in vitro* binding assays on membrane homogenates of animal or human cortices, the occipital and frontal B'_{max} values measured *in vivo* using our techniques are of the same order of magnitude. Considering that 1 ml of cerebral tissue contains approximately 100 mg of protein, the 79 pmol/ml B'_{max} measured in occipital cortex of baboon in the present study can be estimated as approximately 790 fmol/mg of protein. This value, although slightly lower, agrees with the density calculated *in vitro* in subhuman primates (28) and in humans (3, 28, 29). However, the same estimation of 50 pmol/ml for B'_{max} measured by PET in frontal cortex of live baboons seems lower than the B_{max} measured in the same cortical region *in vitro* (28). So, it may be suggested that the possible overestimation of free ligand by nonsaturable binding approximation could lead to an underestimation of B/F ratios and, consequently, B'_{max}. Furthermore, measurement of the absolute value of radioactivity/ml of tissue ratio may be underestimated *in vivo* by the partial volume effect inherent in PET (30), especially in the frontal cortex, which is small compared with other cortical structures in baboons.

Thus, these results may suggest that benzodiazepine receptors in the live *P. papio* baboon are functionally close to human receptors. Therefore, the *in vivo* binding and the pharmacological and physiopathological studies of benzodiazepine receptor ligands in baboons could be preliminary steps to clinical investigations in humans.

Physiopathological study: a case report of a photosensitive baboon. No difference was observed in either the regional K_d or B'_{max} values between the nonphotosensitive baboons and the photosensitive baboon. Although it can be only considered as a case report, there is similarity to the observations made in a previous *in vitro* case report (31). However, we observed in the present study that, before equilibrium, the mean regional values of the specifically bound [^{11}C]Ro 15-1788 measured after administration of similar tracer doses are slightly lower in the frontal cortex of the photosensitive baboon than in nonphotosensitive baboons. This phenomenon suggests that differences in the *in vivo* binding kinetics of Ro 15-1788, after administration of a tracer dose of [^{11}C]Ro 15-1788, could be separated from K_d and B_{max} modulation and may even depend on differences in the biodistribution parameters. We feel that this simple method of quantification could be very useful in evaluating *in vivo* the alteration of the central type benzodiazepine receptors in certain physiopathological conditions.

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