Visualization of Specific Binding Sites of Benzodiazepine in Human Brain

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Using ^{11}C-labeled Ro15-1788 and positron emission tomography, studies of benzodiazepine binding sites in the human brain were performed on four normal volunteers. Rapid and high accumulation of ^{11}C activity was observed in the brain after i.v. injection of [^{11}C]Ro15-1788, the maximum of which was within 12 min. Initial distribution of ^{11}C activity in the brain was similar to the distribution of the normal cerebral blood flow. Ten minutes after injection, however, a high uptake of ^{11}C activity was observed in the cerebral cortex and moderate uptake was seen in the cerebellar cortex, the basal ganglia, and the thalamus. The accumulation of ^{11}C activity was low in the brain stem. This distribution of ^{11}C activity was approximately parallel to the known distribution of benzodiazepine receptors. Saturation experiments were performed on four volunteers with oral administration of 0.3–1.8 mg/kg of cold Ro15-1788 prior to injection. Initial distribution of ^{11}C activity following injection peaked within 2 min and then the accumulation of ^{11}C activity decreased rapidly and remarkably throughout the brain. The results indicated that [^{11}C] Ro15-1788 associates and dissociates to specific and nonspecific binding sites rapidly and has a high ratio of specific receptor binding to nonspecific binding in vivo. Carbon-11 Ro15-1788 is a suitable radioligand for the study of benzodiazepine receptors in vivo in humans.

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Benzodiazepines are extensively prescribed for the treatment of insomnia, anxiety, convulsive disorders, and some kinds of spasticity. Their therapeutic effects are triggered by an interaction with specific benzodiazepine receptor, which have been demonstrated by radioligand technique (*1–3*). Acute and reversible changes of benzodiazepine receptors in the brain have been observed after acute stress and seizures in animal experiments (*4–8*). Alteration of benzodiazepine receptors have been reported in Huntington's disease (*9–12*) and Alzheimer's disease in human brain (*13*).

It should, therefore, be useful to measure benzodiazepine receptors in vivo in humans for the study of clinical pharmacology of benzodiazepines and also for elucidating the pathophysiological mechanism of some neuropsychiatric disorders, such as epilepsy, anxiety neurosis, Huntington's disease, and Alzheimer's disease.

Ro15-1788 (Flumazepil) is a benzodiazepine receptor antagonist and has a high affinity for the central type benzodiazepine receptor, irrespective of benzodiazepine subtype (*14–16*).

The synthesis and the kinetic studies of carbon-11-(^{11}C) labeled Ro15-1788 in baboons and the human brain have been reported by Mazière et al. (*17–20*).

In this report, we describe the kinetics of [^{11}C]Ro15-1788 in four volunteers and also those following pretreatment with cold Ro15-1788.

SUBJECTS

Four healthy men with age ranging from 25 to 53 yr took part in the study. The subjects gave informed written consent to participate in the investigation. No abnormalities were found either by physical examination or laboratory findings in any of the subjects. They did not take any medication within 1 mo of enrollment in the study.

MATERIALS AND METHODS

Carbon-11 Ro15-1788 was produced with a high specific activity of methylation of Ro15-5528 (nor-Ro15-1788) with $^{11}CH_3I$. All the procedures other than

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evaporation and filtration at the final stage were carried out with specifically designed equipment connected to a central control system (*21*). Radiochemical purity was >99%. The specific activity varied from 450 to 3,180 Ci/mmol at the end of synthesis.

Positron emission tomographic (PET) scanning was performed with a whole-body PET scanner* which has a three detector ring and can obtain five slices simultaneously with a center to center separation of 18 mm (*22*). The spatial resolution is 9.2 mm full width at half maximum (FWHM) in the center of the field-of-view and slice (axial) thickness is ~10 mm FWHM for cross slices and 13 mm FWHM for direct slices.

Subjects were asked to lie on the scanner bed with eyes closed but the ears were not occluded. The head was positioned with the aid of a laser line so that the center of the lowest slice corresponded to 10 mm above the subject's canthomeatal line. After the head was in place, a transmission scan was performed with a ring source of activity containing germanium-68–gallium-68 for attenuation correction. Intravenous needles were inserted in the arm for isotope injection and blood sampling.

Control Experiment

Serial emission scans (scan length 1 min) were performed for 20–30 min following an i.v. bolus injection of [^{11}C]Ro15-1788. The amount of injected radioactivity varied 4.9 to 9.5 mCi and the specific activity was between 230 and 1,460 mCi at the time of injection (Table 1).

Simultaneous venous blood samples were obtained serially, and the total radioactivity of 1 ml of blood was measured at the well counter. Two milliliters of methylene chloride were then added to 1 ml of the blood and the radioactivity of the extracted fraction of the blood was measured. Extraction efficiency determined by comparison with a standard was >95%.

At 20 min following injection, 20 ml of blood were sampled in two volunteers, 20 ml of methylene chloride were added into it, and a fraction of the blood extracted with methylene chloride was obtained. Thin layer chromatographic analyses were performed on silica gel with methylene chloride:methanol (9:1) and hexane: benzene: dioxane:ammonium hydroxide (70:50:45:5) as solvent.

TABLE 1
Subjects and Injected Dose of [^{11}C]Ro15-1788

		Control experiment		Saturation experiment			
		Injected Dose of [^{11}C]Ro15		Cold Ro15 (p.o.)		Injected dose of [^{11}C]Ro15	
Subject	Age/Sex	(mCi)	(μg)	(mg)	(mg/kg)	(mCi)	(μg)
1	49/M	9.5	12.6	20	0.3	3.0	64
2	50/M	5.0	3.0	30	0.5	6.1	0.7
3	53/M	6.1	1.9	50	1.1	6.1	1.9
4	25/M	4.9	1	150	1.8	4.9	6.7

Saturation Experiment

The saturation experiments were performed on the same day in Subject 1, and on another day within 1 mo after the control experiment in Subjects 2, 3, and 4. Subjects 1, 2, and 3 took 20, 30, and 50 mg, respectively, of cold Ro15-1788 orally 30 min prior to injection of [^{11}C]Ro15-1788. Subject 4 took 150 mg of cold Ro15-1788 divided into three at 45, 30, and 15 min prior to injection for maintaining the high blood level of Ro15-1788 concentration in the blood during the experiment. Serial emission scans were performed and venous blood samples were obtained in the same way as in the control experiments. The amount of injected radioactivity varied from 3.0 to 6.1 mCi in the saturation experiments.

Data Analysis

Regions of interest were in the frontal cortex, the temporal cortex, the medial occipital cortex, the basal ganglia (which includes the caudate, the putamen and the pallidum), the thalamus, the cerebellum, the brain stem, and the white matter (Fig. 1). The brain radioactivity was corrected for ^{11}C decay, and time-activity curves of ^{11}C activity in each of the brain regions were obtained. Partial volume effects were not corrected in these studies. Using a standard germanium-68–gallium-68 solution, a calibration factor was obtained, and the regional brain and the blood radioactivity were expressed as a percentage of the injected dose per ml of tissue (%dose/ml).

RESULTS

Initially, high uptake of ^{11}C activity was observed in the cerebral gray matter and the cerebellum. The radioactivity in the cerebral cortex then increased gradually and reached a maximum at 7–12 min following the injection, whereas the radioactivity in the basal ganglia, the thalamus, the cerebellum, the brain stem and the white matter reached a maximum at 2–7 min following injection and then decreased gradually. A high uptake of ^{11}C activity, therefore, was observed in the cerebral cortex, moderate uptake was observed in the basal ganglia, the thalamus, and the cerebellum, and low uptake was observed in the white matter and the brain stem after 10 min following injection (Figs. 2 and 4A, Table 2).

The radioactivity in the first sample of the blood, which was aspirated 1 min following injection, varied in each study. The peak of the radioactivity in the blood was probably missed. The radioactivity in the total blood after 5 min following injection remained stable

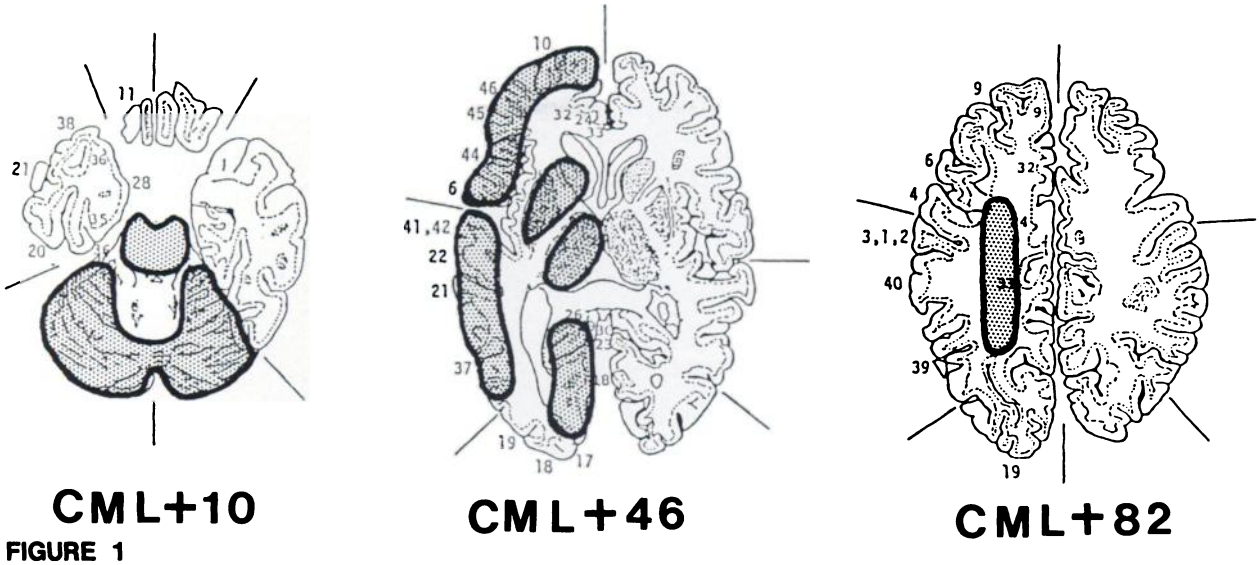

FIGURE 1
Regions of interest were in frontal cortex, temporal cortex, medial occipital cortex, basal ganglia, brain stem, and white matter. ROIs in cerebrum were in left hemisphere. Basal ganglia included caudate, putamen, and pallidum

to the end of the experiment or a transient and slight increase of the radioactivity at 5–10 min following injection was observed. The radioactivity of the extracted fraction decreased gradually, so the difference between the radioactivity in the total blood and the extracted fraction gradually increased.

The result of thin layer chromatographic analysis showed that >99% of the radioactivity in the extracted fraction of the blood was that of unmetabolized [^{11}C] Ro15-1788.

In the saturation experiments, the radioactivity in each of the brain regions including cerebral cortex reached a maximum within 2 min and decreased rapidly throughout the brain (Figs. 2 and 4B, Table 2). The radioactivity (%dose/ml) in the frontal cortex in the saturation experiments reduced to 48%, 22%, 31%, and 23% of those in the control experiments at 20 min following injection in four volunteers, respectively, (Fig. 5), whereas the blood activity kinetics were not significantly different between the two experiments.

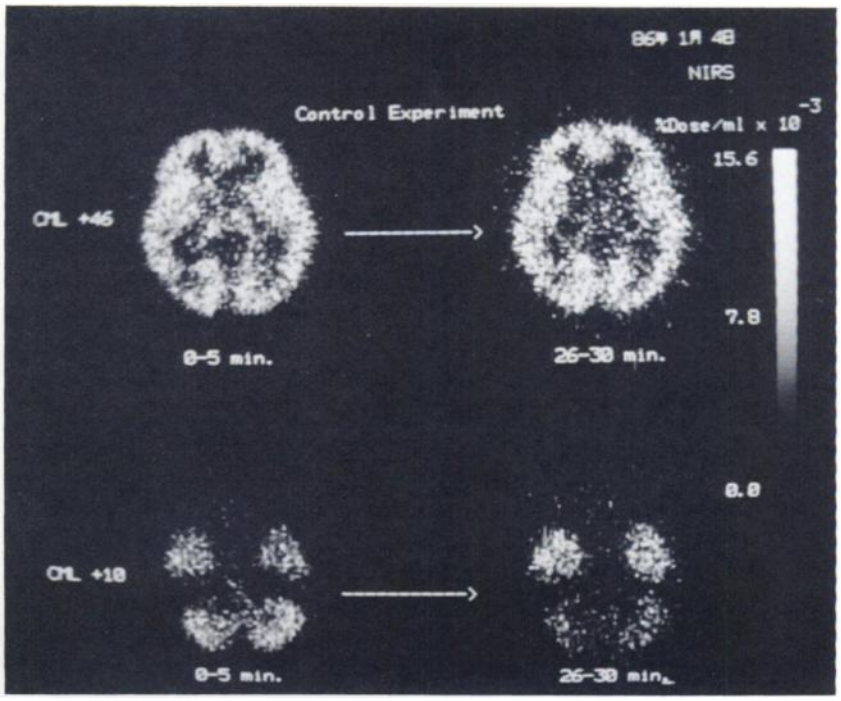

FIGURE 2
PET images of 25-yr-old, male volunteer (Subject 4) following i.v. injection of 4.9 mCi of [^{11}C]Ro15-1788. Initially (0–5 min following injection), high uptake of ^{11}C activity was observed in cerebral gray matter and cerebellum. Distribution of ^{11}C activity in brain changed with time, and at later time of study (26–30 min following injection), high accumulation of ^{11}C activity was observed in cerebral cortex and moderate uptake was seen in subcortical gray matter and cerebellum. Accumulation was low in brain stem and white matter. These images were cross-scaled

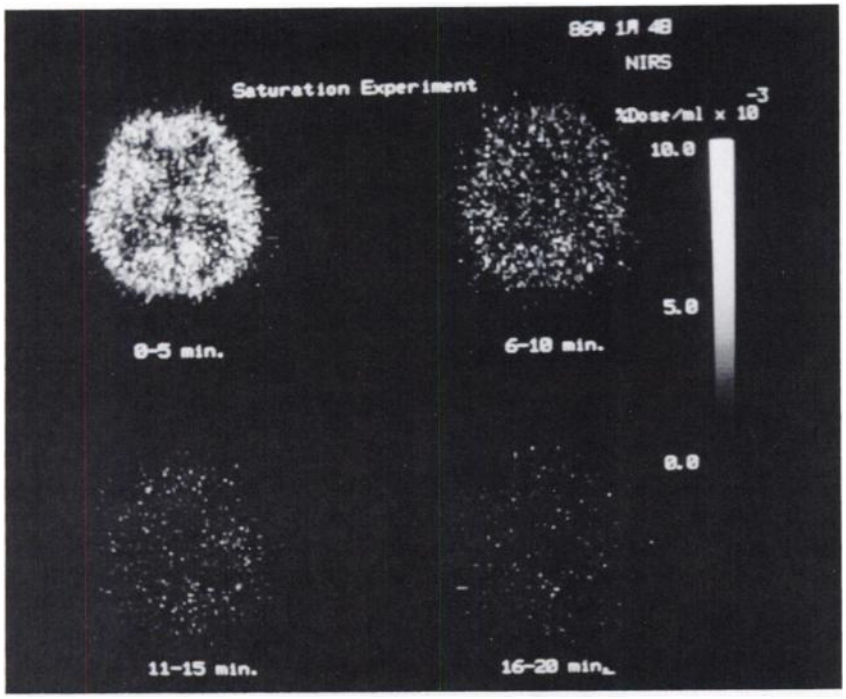

FIGURE 3
Serial PET images of Subject 4 in saturation experiment. Center of slice corresponded to 46 mm above canthomeatal line. Initially (0–5 min), high uptake of ^{11}C activity was observed in cerebral cortex and subcortical gray matter, but thereafter radioactivity decreased rapidly and remarkably throughout brain

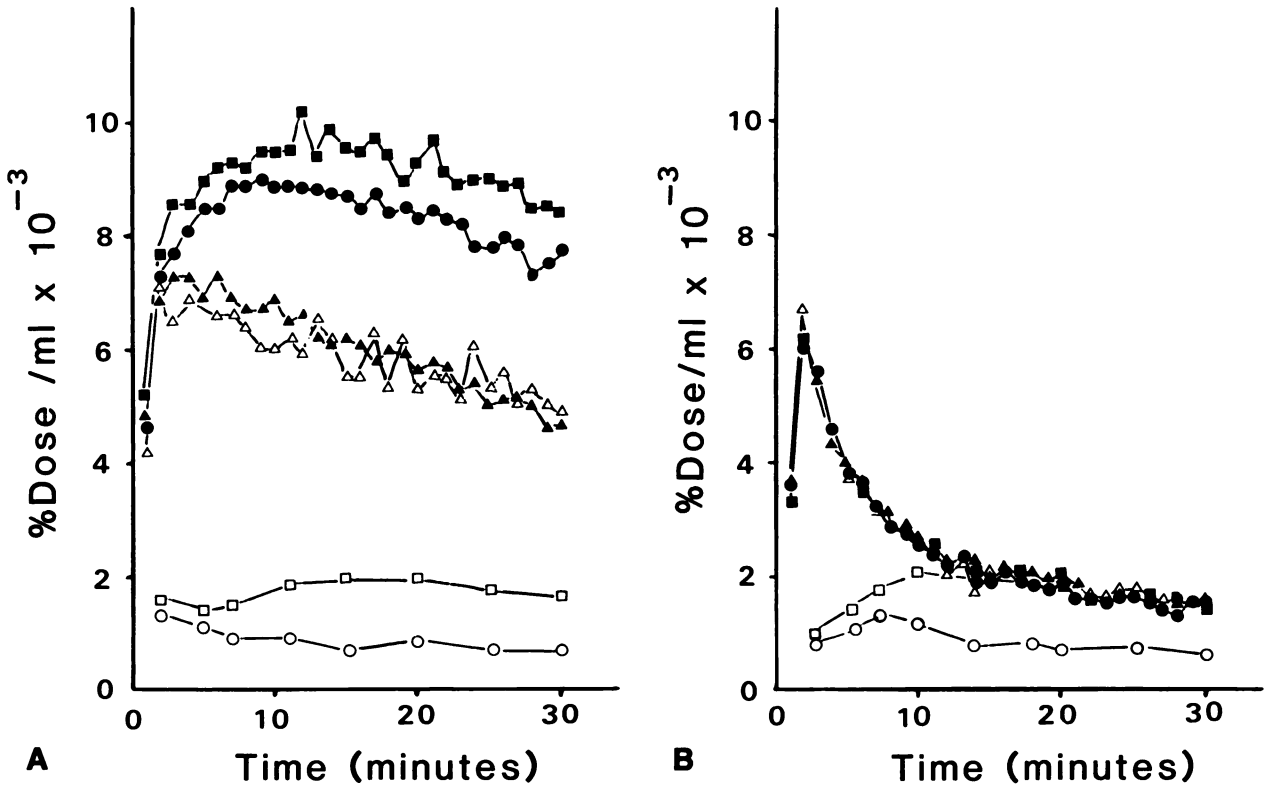

FIGURE 4
A: Time-activity curves in Subject 4 in control experiment. Radioactivity in frontal cortex (●—●), medial occipital cortex (■—■), basal ganglia (△—△), cerebellum (▲—▲), total blood (□—□) extracted fraction of blood with methylen chloride (○—○) was expressed as percentage of injected dose per ml of tissue. B: Time-activity curves in Subject 4 in saturation experiment. Radioactivity in each brain regions reached maximum within 2 min following injection and then decreased rapidly. No regional difference of radioactivity was observed

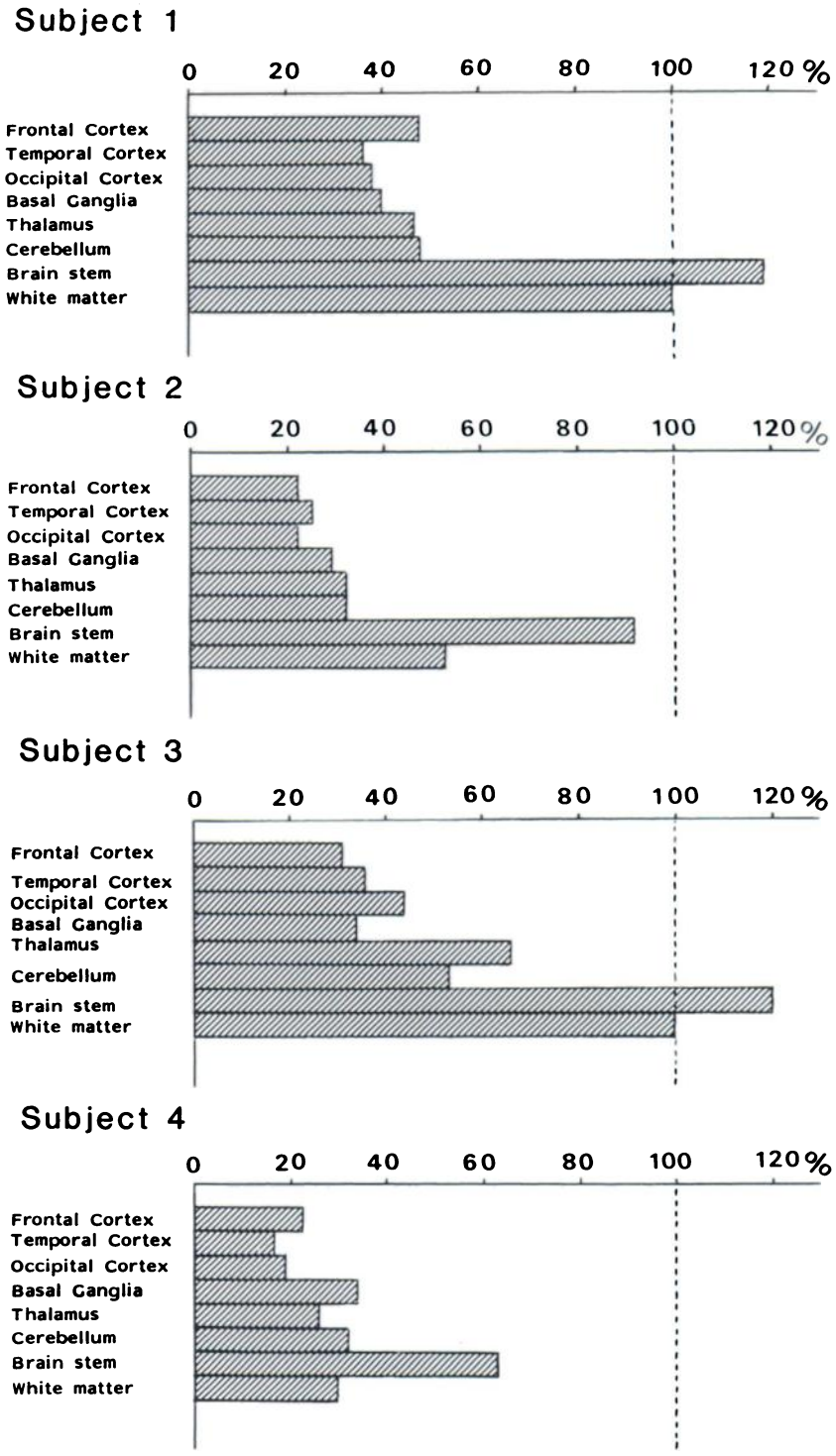

FIGURE 5
Ratio of radioactivity in each brain regions between control experiments and saturation experiments at 20 min following injection. Reduction rate was highest in cerebral cortex, which has highest density of benzodiazepine receptors. Subjects 1, 2, 3, and 4 took 20, 30, 50, and 150 mg, respectively, of cold Ro15-1788 prior to injection in saturation experiments. Reduction rates did not increase in dose dependent mode of pretreatment, possibly because of difference in subjects

DISCUSSION

Initial distribution of ^{11}C activity was similar to the distribution of the normal cerebral blood flow (*23*). After 10 min following injection, however, ^{11}C activity became high in the cerebral cortex, moderate in the subcortical gray matter and the cerebellum, and low in the brain stem. This distribution of ^{11}C activity was approximately parallel to the known distribution of benzodiazepine receptors in the human brain in vitro study (*3,15*). These kinetics of [^{11}C]Ro15-1788 in the brain agreed with the result of the previous report (*20*).

The blood analysis showed that injected [^{11}C]Ro15-1788 was metabolized rapidly to water soluble metabolites in the peripheral tissue during experiments. The transient increase of the total radioactivity in the blood may reflect the release of metabolized radioligand from the peripheral organs into the blood.

TABLE 2
Biodistribution of ^{11}C Activity Following Injection of [^{11}C]Ro15-1788

Brain region	Time after injection					
	2 min		10 min		20 min	
	Control	Saturation*	Control	Saturation*	Control	Saturation
Frontal cortex	6.7 ± 1.2	6.0 ± 1.6	8.4 ± 1.0	3.0 ± 1.0	7.8 ± 1.3	2.5 ± 1.3
Temporal cortex	7.0 ± 1.3	6.4 ± 1.4	8.9 ± 1.5	3.2 ± 1.1	8.3 ± 1.6	2.4 ± 0.9
Occipital cortex	7.6 ± 1.4	6.8 ± 1.4	9.0 ± 1.0	3.4 ± 1.1	8.0 ± 1.4	2.4 ± 0.8
Basal ganglia	7.0 ± 1.5	6.1 ± 1.6	6.5 ± 1.3	2.9 ± 1.0	5.2 ± 1.2	1.8 ± 0.6
Thalamus	6.3 ± 1.2	6.0 ± 1.4	6.0 ± 1.4	2.8 ± 1.9	4.8 ± 1.1	2.0 ± 0.6
Cerebellum	6.4 ± 0.7	5.9 ± 0.8	5.7 ± 1.3	2.8 ± 0.8	4.9 ± 0.7	2.0 ± 0.6
Brain stem	3.7 ± 1.1	3.3 ± 0.7	2.2 ± 0.6	2.1 ± 0.6	1.9 ± 0.6	1.8 ± 0.5
White matter	4.4 ± 0.8	4.2 ± 0.9	4.2 ± 1.1	2.6 ± 0.9	3.6 ± 1.6	2.1 ± 0.6
Blood (total)	8.3 ± 6.0	6.8 ± 6.4	3.7 ± 1.6	3.9 ± 1.2	3.5 ± 1.4	3.3 ± 1.1
Blood (extracted)	8.0 ± 7.1	6.2 ± 6.0	2.0 ± 0.7	2.1 ± 1.0	1.4 ± 0.3	1.6 ± 0.6

Mean % dose/ml × 10^{-3} (±s.d.) for four subjects or three subjects at each time.

However, [^{11}C]Ro15-1788 does not seem to be metabolized in the brain. Inoue et al. investigated the stability of [^{3}H]Ro15-1788 in the mouse brain (*8*). Male C3H mice were injected through the tail vein with 5 μCi of [^{3}H]Ro15-1788, and killed 30 min following injection. Radioactive materials in the brain homogenate were then extracted with methylene chloride, and thin layer chromatographic analysis of radioactive materials was performed. The results showed that almost all the radioactivity was that of unmetabolized [^{3}H] Ro15-1788. The result indicates that Ro15-1788 is not metabolized in the brain and the water soluble metabolites of Ro15-1788 in the blood do not pass through the blood-brain barrier.

Ro15-1788 has no major pharmacologic effect on its own when up to 1,000 mg is taken orally (*16*). It is, therefore, safe to perform the saturation experiment with this drug in humans.

In the saturation experiments, the kinetics of [^{11}C] Ro15-1788 in the brain were considerably different from those in the control experiments, and the radioactivity in the brain at the later time of the study was reduced significantly. The maximum reduction of the radioactivity was observed in the cerebral cortex, which has the highest density of benzodiazepine receptors in the brain (Fig. 5). The results indicate that [^{11}C]Ro15-1788 has a high ratio of specific binding to nonspecific binding in vivo.

While whether a larger amount of cold Ro15-1788 reduces the brain uptake of ^{11}C-Ro15-1788 further remains to be investigated, marked reduction of the radioactivity in our studies reaching to 22% in the saturation experiments suggests that most of benzodiazepine receptors in the brain had been occupied by pretreatment of cold Ro15-1788. The radioactivity in each of the brain regions in the saturation experiments can then be regarded as that of nonspecifically bound and free radioligand, and the difference of two time-activity curves in each of the brain regions between the two experiments represents the radioactivity of specifically bound radioligand. The specific binding of [^{11}C]Ro15-1788 reached a maximum at ~12–20 min following injection and declined slightly thereafter (Fig. 6). The decline of the specific binding reflected the decline of the unmetabolized [^{11}C]Ro15-1788 concentration in the blood.

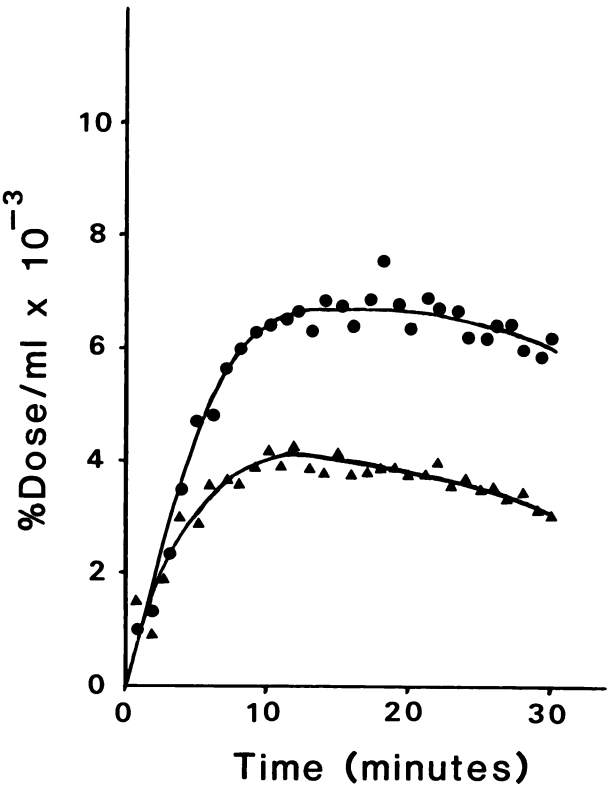

FIGURE 6
Difference of radioactivity in frontal cortex and cerebellum between two experiments in Subject 4. Difference reflects specific binding of [^{11}C]Ro15-1788 in brain

These kinetics indicate that [^{11}C]Ro15-1788 associates and dissociates to specific and nonspecific binding sites rapidly and has a high ratio of specific binding to nonspecific binding in vivo in the human brain. These characteristics are advantageous for quantitative analysis of receptor binding by PET (*24,25*).

Carbon-11 Ro15-1788 is a suitable radioligand for the study of the benzodiazepine receptor in humans in an atraumatic method by PET.

FOOTNOTES

* Positologica II, Hitachi Medical Corporation, Kashiwa, Chiba, Japan.

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REFERENCES

1. Möhler H, Okada T: Benzodiazepine receptor: Demonstration in the central nervous system. *Science* 198:849–851, 1977
2. Squires RF, Braestrup C: Benzodiazepine receptors in rat brain. *Nature* 266:732–734, 1977
3. Braestrup C, Albrechtsen R, Squires RF: High densities of benzodiazepine receptors in human cortical areas. *Nature* 269:702–704, 1977
4. Paul SM, Skolnick P: Rapid changes in brain benzodiazepine receptors after experimental seizures. *Science* 202:892–894, 1978
5. Robertson HA: Audiogenic seizures: Increased benzodiazepine receptor binding in a susceptible strain of mice. *Eur J Pharmacol* 66:249–252, 1980
6. Medina JH, Novas ML, Wolfman CNV, et al: Benzodiazepine receptors in rat cerebral cortex and hippocampus undergo rapid and reversible changes after acute stress. *Neuroscience* 9:331–335, 1983
7. Meidna JH, Novas ML, Robertis E: Changes in benzodiazepine receptors by acute stress: Different effect of chronic diazepam or Ro15-1788 treatment. *Eur J Pharmacol* 96:181–185, 1983
8. Inoue O, Akimoto Y, Hashimoto K, et al: Alterations in biodistribution of (^{3}H)Ro15-1788 in mice by acute stress: Possible changes in in vivo binding availability of brain benzodiazepine receptor. *Intl J Nucl Med Biol* 12:369–374, 1985
9. Möhler H, Okada T: The benzodiazepine receptor in normal and pathological human brain. *Br J Psychiat* 133:261–268, 1978
10. Reisine TD, Wastek GJ, Speth RC, et al: Alterations in the benzodiazepine receptor of Huntington's diseased human brain. *Brain Res* 165:183–187, 1979
11. Walker FO, Young AB, Penny JB, et al: Benzodiazepine and GABA receptors in early Huntington's disease. *Neurology* 34:1237–1240, 1984
12. Whitehouse PJ, Trifiletti RR, Jones BE, et al: Neurotransmitter receptor alterations in Huntington's disease: Autoradiographic and homogenate studies with special reference to benzodiazepine receptor complexes. *Ann Neurol* 18:202–202, 1985
13. Owen F, Poulter M, Waddington JL, et al: (^{3}H)Ro05-4864 and (^{3}H)Flunitrazepam binding in kanate-lesioned rat striatum and in temporal cortex of brains from patients with senile dementia of the Alzheimer type. *Brain Res* 278:373–375, 1983
14. Hunkeler W, Möhler H, Pieri L, et al: Selective antagonist of benzodiazepines. *Nature* 290:514–516, 1981
15. Richards JG, Möhler H: Benzodiazepine receptors. *Neuropharmacol* 23:233–242, 1984
16. Haefely S, W, Bonetti EP, Burkard WP, et al: Benzodiazepine antagonists. In *The Benzodiazepines: From Molecular Biology to Clinical Practice*, Costa E, ed. New York, Raven Press, 1983, pp 137–146
17. Mazière M, Hantraye P, Prenant C, et al: Synthesis of Ethyl 8-Fuoro-5,6-dihydro-5-(^{11}C)methyl-6-oxo-4H-imidazo(1,5a) (1,4)benzodiazepine-3-carboxylate (Ro15-1788-^{11}C) specific radioligand for the in vivo study of central benzodiazepine receptors by positron emission tomography. *Intl J Appl Radiat Isot* 35:973–976, 1984
18. Mazière M, Prenant C, Sastre J, et al: ^{11}C-Ro15-1788 et ^{11}C-Flunitrazepam, deux coordinats pur l'étude par tomographie par positions des sites de liaison des benzodiazépines. *C R Acad Sci III*, 296:871–876, 1983
19. Hantraye P, Kaijima M, Prenant C, et al: Central type benzodiazepine binding sites: A positron emission tomography study in the baboon's brain. *Neurosci Lett* 48:115–120, 1984
20. Samson Y, Hantraye P, Baron JC, et al: Kinetics and displacement of (^{11}C)Ro15-1788, A benzodiazepine antagonist, studied in human brain in vivo by positron tomography. *Eur J Pharmacol* 110:247–251, 1985
21. Suzuki K, Inoue O, Hashimoto K, et al: Computer-controlled large scale production of high specific activity (^{11}C)Ro15-1788 for PET studies of benzodiazepine receptors. *Intl J Appl Radiat Isot* 36:971–976, 1985
22. Takami K, Ueda K, Okajima K, et al: Performance study of whole-body, multislice positron computed tomograph -Positologica II. *IEEE Trans Nucl Sci* NS-30:734–738, 1983
23. Yamamoto YL, Meyer E, Menon D, et al: Regional cerebral blood measurement and dynamic positron emission tomography. In *Positron Emission Tomography of the Brain*, Heiss WD, Phelps ME, eds. Berlin, Heidelberg, New York, Springer-Verlag, 1983, pp 78–84
24. Huang S, Phelps ME: Principles of tracer kinetic modeling in positron emission tomography and autoradiography. In *Positron Emission Tomography and Autoradiography: Principles and Application for the Brain and Heart*, Phelps ME, Mazziotta J, Schelbert H, eds. New York, Raven Press, 1986, pp 287–346
25. Farde L, Hall H, Ehrin E, et al: Quantitative analysis of D_2 dopamine receptor binding in the living human brain by PET. *Science* 231:258–261, 1986