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In vivo kinetics and displacement study of a carbon-11-labeled hallucinogen, N,N-[^{11}C]dimethyltryptamine

Kazuhiko Yanai[1], Tatsuo Ido[1], Kiichi Ishiwata[1], Jun Hatazawa[2], Toshihiro Takahashi[1], Ren Iwata[1], and Taiju Matsuzawa[2]

[1] Division of Radioisotope Research, Cyclotron and Radioisotope Center, and
[2] Department of Radiology and Nuclear Medicine, Research Institute for Tuberculosis and Cancer, Tohoku University, Sendai, 980, Japan

Abstract. The endogenous hallucinogen, N,N-dimethyltryptamine (DMT), was labeled with carbon-11 and its regional distribution in rat brain studied. [^{11}C]DMT showed higher accumulation in the cerebral cortex, caudate putamen, and amygdaloid nuclei. Studies of the subcellular distribution of [^{11}C]DMT revealed the specific localization in the fractions enriched with serotonin receptors only when a very low dose was injected into rats. The proportions of the radioactivity in receptor-rich fractions were greatly enhanced by pretreatment with the monoamine oxidase inhibitor, pargyline. Specific binding of [^{11}C]DMT to serotonin receptors in dog brain was demonstrated by a positron emission tomographic study in which 5-methoxy-N,N-dimethyltryptamine caused approximately 20% displacement of the radioligand from the receptors.

Key words: [^{11}C]Dimethyltryptamine – Serotonin receptors – Positron

N,N-Dimethyltryptamine (DMT), a methylated indolealkylamine having hallucinogenic properties, is an endogenous trace amine found in the serum or the urine of both normal subjects and psychotic patients (Narasimhachari and Himwich 1973a, b). Biosynthetic pathways of DMT exist in vivo. DMT has been implicated in the pathogenesis of schizophrenia, although the findings are controversial (Gillin et al. 1976; Checkley et al. 1980).

There are two distinct central serotonin receptors (serotonin$_1$, serotonin$_2$ receptors) with different physiological functions. Serotonin-elicited synaptic inhibition and excitation are mediated by serotonin$_1$ and serotonin$_2$ receptors, respectively (Peroutka et al. 1981). [^{11}C]Ketanserin and [^{11}C]mesulergin were developed as radiopharmaceuticals which bind to serotonin$_2$ receptors (Berridge et al. 1982; Kloster et al. 1984). Berger et al. (1978) also synthesized 5-methoxy-N,N-[^{11}C]dimethyltryptamine using carbon-11 formaldehyde. The use of 5-methoxy-N,N-[^{11}C]dimethyltryptamine for detection of serotonin receptors in vivo in mental disorders was also pointed out by these authors.

DMT is a serotonin analog and binds to the postsynaptic serotonin receptors. DMT binding is more selective toward 5-hydroxytryptamine binding sites (serotonin$_1$ receptors) than serotonin$_2$ receptors (Bennett and Snyder 1975; Fillion et al. 1977; Leysen et al. 1978; Glennon 1979; Peroutka and Snyder 1979). We have labeled indolealkylamines such as [^{11}C]DMT, 5-methoxy-N,N-[^{11}C]dimethyltryptamine (5-MeO DMT), N-[^{11}C]methyltryptamine (NMT) and [^{11}C]bufotenine using [^{11}C]methyliodide. [^{11}C]labeled indolealkylamines are expected to be serotonin receptor-mapping agents. Among these indolealkylamines [^{11}C]DMT was most easily transported through the blood-brain barrier and accumulated in the brain (Takahashi et al. 1985; Yanai et al. 1984). In the present study, we examined the tissue distribution of [^{11}C]DMT, regional distribution and subcellular distribution in the rat brain. Preliminary positron emission tomographic (PET) experiments with [^{11}C]DMT in the dog brain indicate the feasibility of examining serotonin receptor populations in the human brain in vivo.

Offprint requests to: Tatsuo Ido, Cyclotron and Radioisotope Center, Tohoku University, Aoba, Aramaki, Sendai 980, Japan

Materials and methods

Synthesis of [^{11}C]DMT and ^{45}Ti-DTPA

[^{11}C]CH_3I was prepared from [^{11}C]CO_2 using an automated synthesis system and trapped in 1.5 ml acetone containing 5 mg of N-methyltryptamine at −78° C (dry ice-acetone). The reaction mixture was heated to 50°–60° C for 5 min with stirring (Takahashi et al. 1985; Ishiwata et al. 1985). The labeled product was separated by high-performance liquid chromatography (HPLC) on a Radial-PAK silica column (Waters) with a solvent system of chloroform and methanol containing 1% dimethylamine (90:10). The [^{11}C]DMT fraction was collected and evaporated to dryness. [^{11}C]DMT was dissolved in saline and filtered through a membrane filter (0.22 μm). [^{11}C]DMT was obtained with a radiochemical purity greater than 99%. The entire production took 50 min and resulted in a radiochemical yield of 80%–90% (decay corrected). The specific activity was determined to be 10–76 Ci/mmol (average 33 Ci/mmol) at the time of use, the mass of DMT being determined by ultraviolet absorbance (254 nm) in HPLC. ^{45}Ti-DTPA was synthesized using the method of Ishiwata et al. (1981).

Tissue distribution and regional distribution in the rat brain

Male Wistar rats weighing 150–160 g were killed by cervical dislocation at 5, 10, 30, 60 min following the i.v. administration of about 100 μCi (3–4 μmol) of [^{11}C]DMT. Organ uptake of [^{11}C]DMT was expressed as the differential absorption ratio (DAR). DAR was defined as: (observed activity of tissue/weight of tissue) × (body weight of animal/total injected activity).

In the regional distribution study, male Wistar rats weighing 190–200 g were killed 20 min after the injection of 1–2 mCi of [^{11}C]DMT (30–60 nmol). The brains were frozen in crushed dry ice and coronal sections about 300 μm thick were cut on dry ice. Anatomical features were identified on the basis of the stereotaxic atlas of Konig and Klippel (1967). Specific nuclei and regions were removed with a hollow needle (inner diameter 1.2 mm) at −15° C and the radioactivity was counted (punch sampling procedure). The brain uptake is expressed as the percentage of administered dose per gram of tissue (%dose/g) after correction for radioactive decay.

Autoradiography was performed on rats which had been administered approximately 10 mCi of [^{11}C]DMT. The brain was removed 20 min after administration of radioactivity and frozen on crushed dry ice. The 30-μm sections of the brain were exposed to Kodak NMC-1 X-ray film at room temperature for 3 h.

Drug treatment

Reserpine was administered IP in a dose of 5 mg/kg body weight 6 h before. Pargyline (75 mg/kg) was injected IP 2 h before. Reserpine and pargyline were obtained from Sigma Chemical.

Subcellular fractionation of the brain tissue

Male Wistar rats (190–200 g) were killed by decapitation 20 min after the injection. All subsequent operations were conducted at 0°–4° C. Subellular fractionation was performed as follows: The brain homogenate in 0.32 *M* sucrose solution (10% wt/vol) was centrifuged at 800 *g* for 10 min. The pellet was resuspended and recentrifuged at 10 min to yield the nuclear (N) fraction. The pooled supernatant was centrifuged at 10,000 *g* for 12 min. The second pellet was crude mitochondrial (CM) fraction. The supernatant was recentrifuged at 100,000 *g* for 60 min, yielding the microsomal (MIC) fraction and soluble (S) supernatant. For rapid separation of S fraction from other fractions (N+CM+MIC), the brain homogenate was centrifuged at 100,000 *g* for 60 min.

Dynamic PET study

An adult dog weighing 12 kg was anesthetized with pentobarbital. The femoral artery and vein were cannulated and 11.9 mCi (1.34 μmol) of [^{11}C]DMT was injected IV. All images were obtained with the ECAT II (ORTEC). Continuous sequential scans were performed on the level of OM ± 0 cm from 2 to 62 min. The radioactivity in the plasma and dog brain was expressed as the percentage of administered dose per milliliter plasma volume and percentage dose per milliliter brain tissue volume, respectively. In the displacement study, 5-MeO DMT (4.59 μmol) was injected continuously from 42 min to 57 min after [^{11}C]DMT administration to displace the radioactive tracer from the specific serotonin receptors.

Table 1. Relative affinity of indole alkylamines toward serotonin receptors

	E.D.$_{50}$[a]	Affinity ratio 5-HT/D-LSD[b]	Brain uptake at 30 min[c]
Bufotenin	0.18	14	0.42
5-Methoxy-N,N-dimethyltryptamine	0.33	–	1.05
N,N-Dimethyltryptamine	0.8	4	1.85
Serotonin	1	40	–
D-LSD	0.003	0.9	–

[a] Binding capacity of indole alkylamines was measured with the displaceable potency of D-[^{3}H]LSD and was normalized as E.D.$_{50}$ of serotonin to be 1 (Fillion et al. 1977).

[b] Affinity ratios toward serotonin$_1$ receptors were expressed as the ratio: 50% displaceable concentration (E.D.$_{50}$) of D-[^{3}H]LSD binding to that of [^{3}H]5-hydroxytryptamine binding (Fillion et al. 1977)

[c] Brain uptakes was expressed as the differential absorption ratio at 30 min (Yanai et al. 1984).

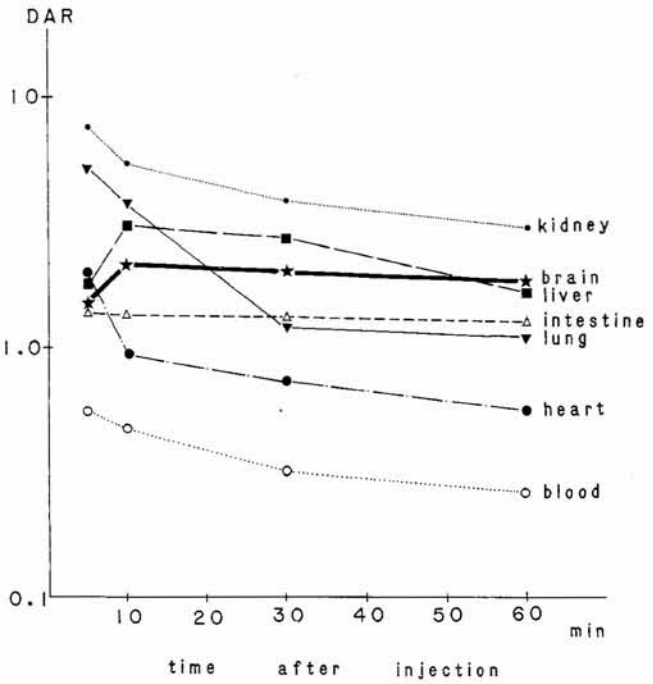

Fig. 1. Tissue distribution of [^{11}C]DMT in rats. *Each point* represents the mean value of three rats

Results and discussion

Table 1 shows the relative affinity of indolealkylamines toward serotonin receptors and the brain uptake of the 3 indolealkylamines. Indolealkylamine binding is more selective toward serotonin$_1$ receptors than serotonin$_2$ receptors. [^{11}C]DMT was obtained in high radiochemical yield and was transported easily through the blood-brain barrier,

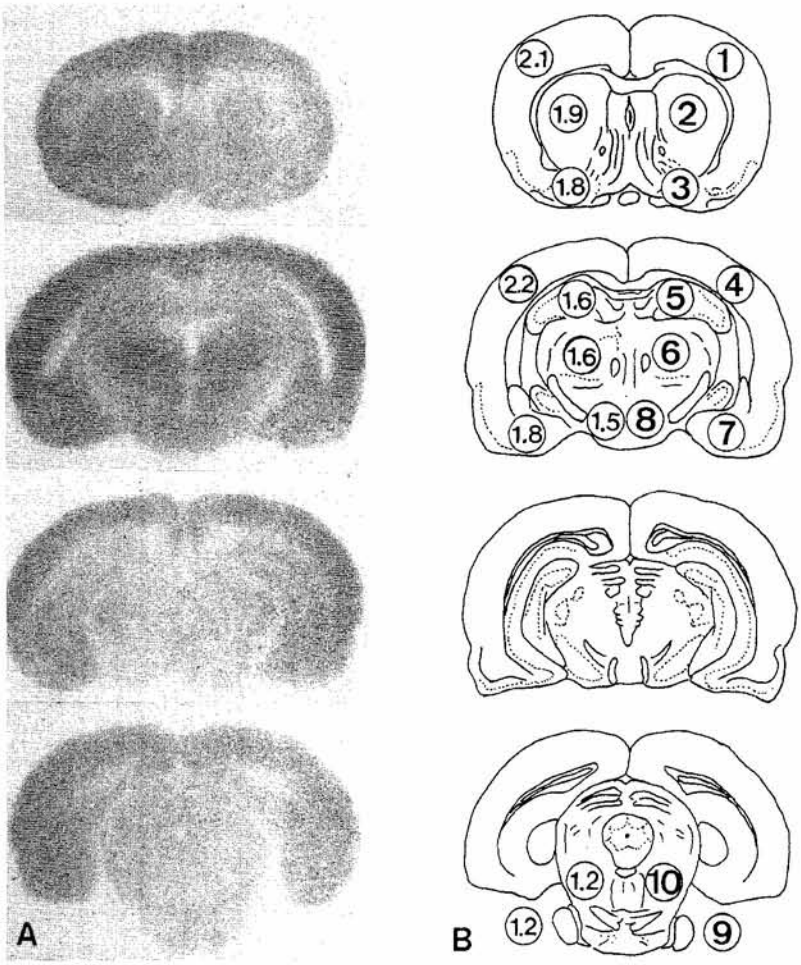

Fig. 2A, B. Regional distribution of [^{11}C]DMT in the rat brain:
A autoradiography (details are described in Materials and methods).
B Punch sampling procedure. Data are expressed as % administrated dose/g in the *left circles* of the schematic frontal sections of the rat brain. *Each point* represents the mean of four determinations. The *circles* on the right half indicate the location of tissue samples; 1 and 4 = cerebral cortex; 2 = caudate putamen; 3 = accubens nuclei, pallidum; 5 = hippocampus; 6 = thalamic nuclei; 7 = amygdaloid nuclei; 8 = hypothalamus; 9 = cerebellum; 10 = pons

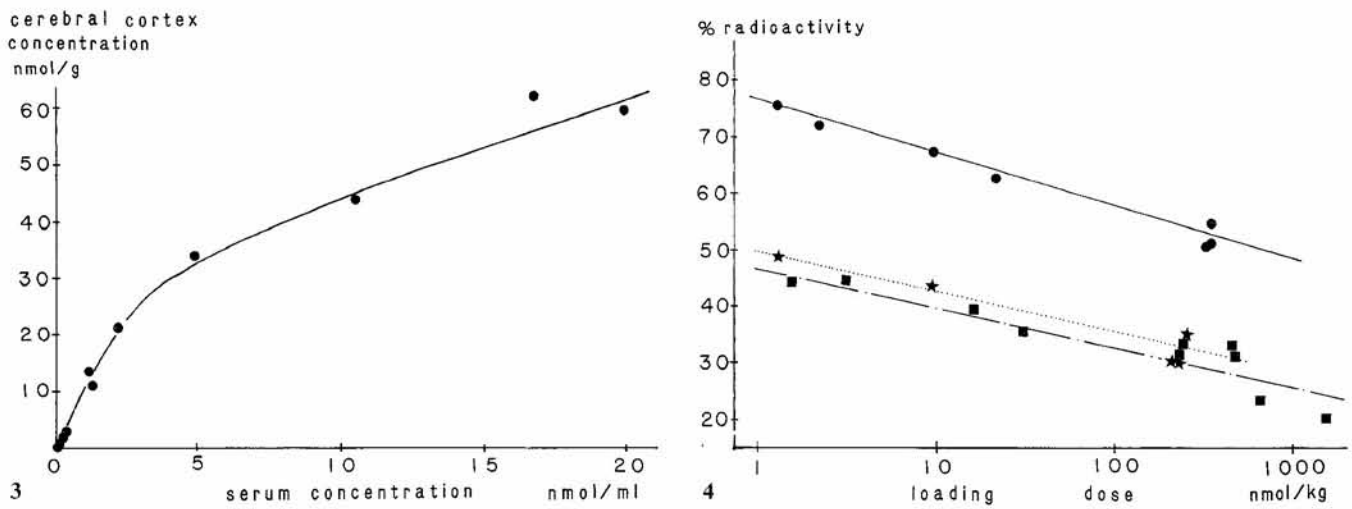

Fig. 3. Relationship between cerebral cortex and serum concentration of [^{11}C]DMT

Fig. 4. Effects of pretreatment with pargyline and reserpine on the subcellular distribution of [^{11}C]DMT. All data are expressed as percentage of radioactivity in N + CM + MIC fractions. N + CM + MIC represents the sediments at 100,000 *g*. Pargyline-treated rats = ●——●; Reserpine-treated rats = ★·····★; Control rats = ■—·—■

confirming that it was a favorable serotonin receptors mapping agent.

Figure 1 illustrates the tissue distribution of [^{11}C]DMT in rats. The uptake in the rat brain increased with time initially and attained equilibrium at 10 min. Blood radioactivity fell rapidly to a very low level within about 5 min after the administration. The process of DMT accumulation had the properties of an active uptake mechanism in the experiments using rat brain cortical slices. DMT accumulation is inhibited by many amines. Tertiary amines are more potent inhibitors than secondary or primary amines in rat brain cortical slices (Sangiah et al. 1979). Our data are consistent with the view that DMT is accumulated in the brain through an active transport system.

The regional distribution in the rat brain is summarized in Fig. 2. [^{11}C]DMT accumulated more in the cerebral cortex, caudate putamen, and amygdaloid nuclei. The uptakes in the cerebellum and medulla oblongata were the lowest in the brain. Figure 3 shows the relationship between the cerebral cortex and serum concentration of [^{11}C]DMT 20 min after the injection. A linear correlation was observed at lower serum concentrations, while the cerebral cortex concentration showed a tendency to be saturated at higher serum concentrations (over 5 nmol/ml).

The subcellular distribution of [^{11}C]DMT in the rat brain at various loading doses and the effects of reserpine and pargyline on the distribution are shown in Fig. 4. In control rats given 1100 nmol/kg of DMT, approximately 78%, 9.5%, 8.0%, and 4.5% of radioactivity were recovered in the supernatant (S), microsomal (MIC), crude mitochondrial (CM), and nuclear (N) fractions, respectively. The proportion of radioactivity in N, CM, and MIC was high in a low loading dose of DMT. Pargyline enhanced the proportions in the three fractions (N+CM+MIC). More than 75% of the radioactivity was recovered in the three fractions of pargyline-pretreated rats injected with about 1 nmol/kg. Pargyline inhibited the metabolite formation of [^{11}C]DMT in the rat brain homogenate (data not shown). Therefore, the effect of pargyline on the subcellular distribution is thought to be caused by the inhibition of metabolism of [^{11}C]DMT. One of the problems of in vivo receptor mapping studies is the metabolism of the radiopharmaceuticals used in the study. Barker et al. (1980) reported that DMT was metabolized in the in vitro experiment to give indoleacetic acid and N,N-dimethyltryptamine-N-oxide, and that the MAO inhibitor iproniazid inhibited the metabolism. [^{11}C]CO_2 could be detected at a rate of approximately 0.01% of injected activity per minute in our experiments. Reserpine, which inhibits re-uptake of neurotransmitters in the synaptic vesicles, had a negligible effect on the subcellular distribution. It was not expected that [^{11}C]DMT would accumulate in the synaptic vesicles of nerve endings in vivo. Serotonin$_1$ receptors are localized in the N (21%), CM (62%), and MIC (17%) fractions (Bennett and Snyder 1976). The subcellular distribution study indicated that much [^{11}C]activity was present in the S fraction, but that the proportion of [^{11}C]activity in the N, CM, and MIC fractions was greatly enhanced in the lower loading dose and/or in pretreatment with pargyline.

Data from the PET study of [^{11}C]DMT are given in Figs. 5 and 6. [^{11}C]DMT showed high accumulation in the brain in contrast with the image of ^{45}Ti-DTPA, which represented the regional cerebral blood volume (Fig. 5). In the displacement study, the calculated concentration in the posterior cerebral cortex was hardly altered by 5-MeO DMT injection (Fig. 6B). On the other hand, the radioactivity curve in the basal ganglia and frontal cortex showed a sharp decrease during 5-MeO DMT injection, followed by a lower constant level after the displacement. The concentration differences between pre-displacement and post-displacement were about 10–20 pmol/ml brain tissue volume in these regions. The concentration difference correlated well with the regional distribution and density of serotonin receptors in rats (Bennet and Snyder 1976; Biegon et al. 1982) and monkey brain homogenate (Bennett and Snyder 1975), although the species are different. These results demonstrated that serotonin receptors could be detected in the live dog with [^{11}C]DMT.

[^{11}C]DMT may be considered to be distributed into four kinetic pools; (1) a pool related to serotonin receptor populations; (2) a pool with an active transport system, which characterizes [^{11}C]DMT brain uptake; (3) a nonspecific binding pool; (4) a pool for free [^{11}C]DMT. More [^{11}C]DMT can be expected to be distributed in the serotonin receptor-related pool when the loading dose of DMT is low and/or metabolism of DMT is inhibited.

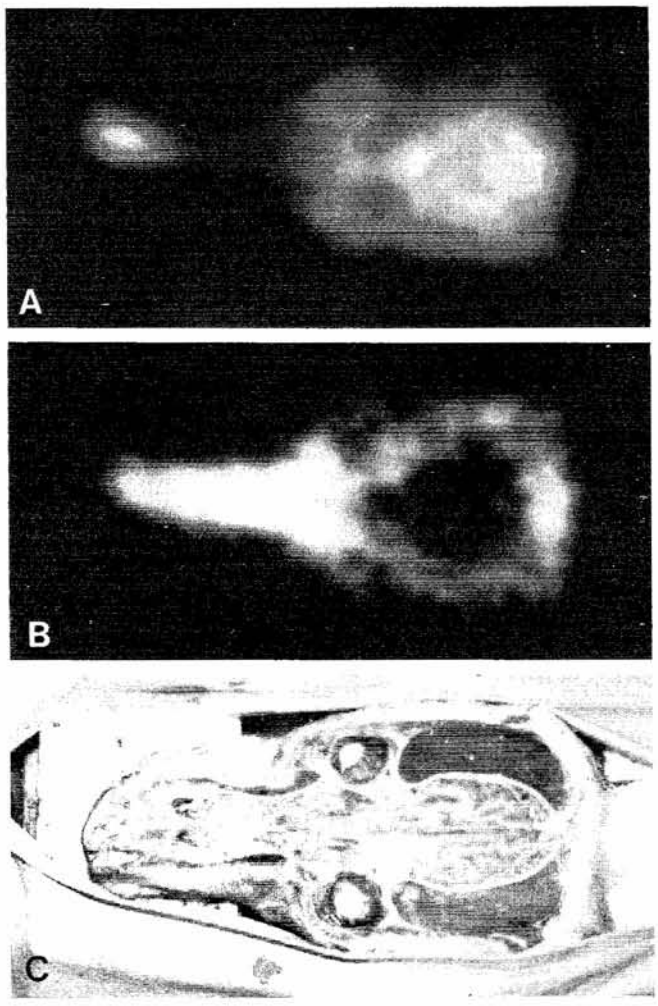

Fig. 5A–C. The PET images and anatomical transaxial section of the same plain in the dog brain. **A** [^{11}C]DMT. The PET image was obtained for 32–42 min after the injection. **B** ^{45}Ti-DTPA. **C** Anatomical section

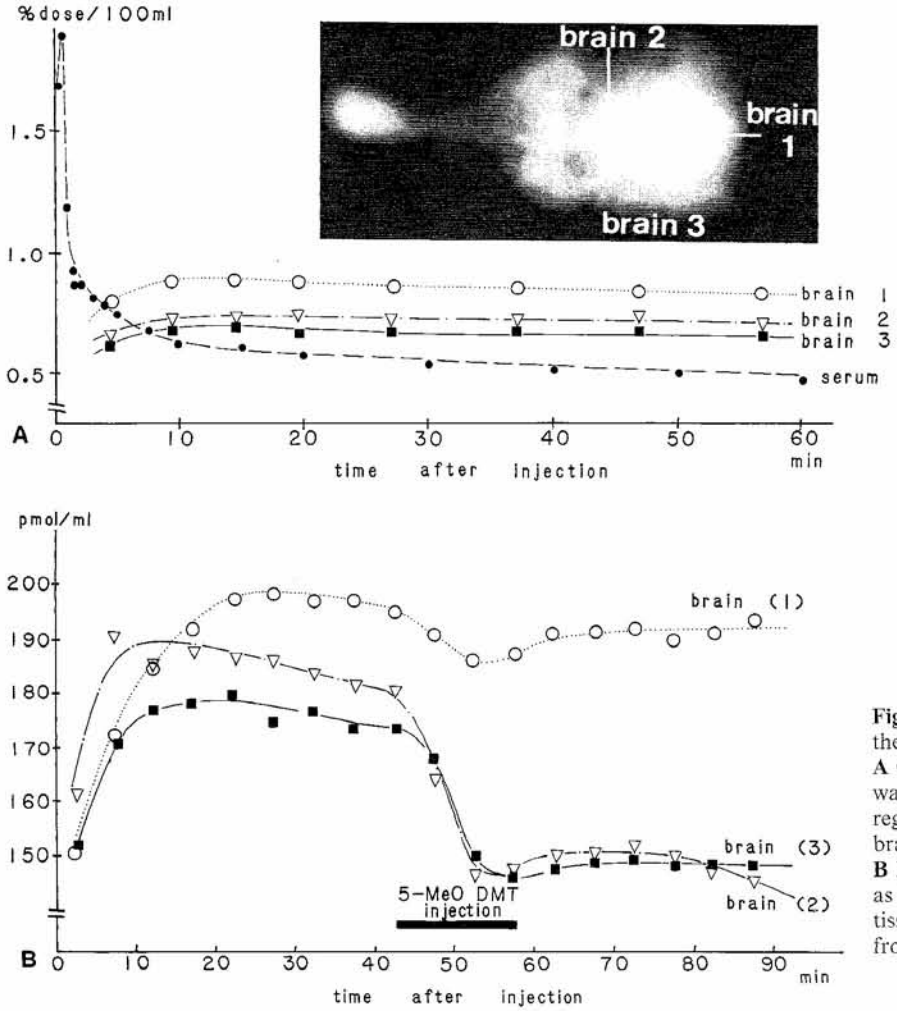

Fig. 6A, B. The time course of radioactivity in the dog brain.
A Control study. Plasma radioactivity curve was superimposed on this figure (●——●). The regions in this experiment (brain 1, brain 2, and brain 3) are shown in the PET image above.
B Displacement experiment. Data are expressed as the calculated concentration (pmol/ml brain tissue volume). (1) Posterior cerebral cortex; (2) frontal cortex; (3) basal ganglia

In conclusion, it is anticipated that [^{11}C]DMT will contribute toward the understanding of psychotic disorders such as schizophrenia and infantile autism because of its close association. Moreover, [^{11}C]DMT- and [^{11}C]-labeled related indolealkylamines are also expected to play an important role not only in serotonin receptor mapping studies but also in the study of the mechanism of action of hallucinogenic indolealkylamines in humans.

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