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Abstracts

In vivo binding of 3-N-[^{11}C]methylspiperone([^{11}C]NMSP) was saturable in the rat forebrain, but not in the cerebellum. Nonspecific binding was almost equivalent in all brain regions except for white matter. [^{11}C]NMSP binding was localized to receptor-rich fractions in low administrated doses(less than 20 nmol/kg body weight). Striatum-to-cerebellum ratio was a function of time after injection and administrated dose. This ratio remained constant in low doses of under 30 nmol/kg. The radioactivity curve of cerebellum in control positron emission tomographic study almost equalled that of striatum in the dog pretreated with spiperone(2 mg), indicating that the amount of binding in the cerebellum might be considered as a nonspecific binding and unbound pool. The data obtained by the pretreatment study was quite different from that of displacement, which suggested that [^{11}C]NMSP in the striatum was not completely displaced from the neruleptic binding sites.

Introduction

Abnormality of dopamine receptors has been implicated in the pathogenesis of several neuropsychiatric disorders such as Parkinsonism, tardive dyskinesia, and schizophrenia. Evidence to support this hypothesis is based on the modulation of dopamine receptors in the animal model or postmortem brain specimens.[1-3)]

Neurotransmitter receptors are usually measured *in vitro* with ligand-binding assay of synaptic membrane preparation.[4,5)] Dopamine or Neuroleptic receptors also have been demonstrated under *in vivo* conditions after injection of [^{3}H]spiperone[6-8)], [^{3}H]pimozide[9)], [^{3}H]reserpine[10)], or [^{14}C]tiapride.[11)] Among the drugs, spiperone appears to be a very appropriate ligand for *in vivo* experiments.

The mapping of neurotransmitter receptors with positron emission tomography(PET) is the current topics in the nuclear medicine and may be of enormous clinical use in the future.[12-17)] Wagner et al have synthesized 3-N-[^{11}C]methylspiperone([^{11}C]-NMSP) and shown by PET that [^{11}C]NMSP accumulates selectively in striatum of baboon and man.[18)] But fundamental aspects of

in vivo neuroleptic binding with [^{11}C]NMSP are still unclear.

In this article we report some characteristics of *in vivo* [^{11}C]NMSP binding, i.e, saturability, nonspecific binding, subcellular distribution and reversibility of the specific binding.

Materials and Methods

Preparation of [^{11}C]NMSP.

[^{11}C]NMSP was prepared by the methylation of spiperone with [^{11}C]methyliodide in a biphasic reaction mixture.[19] At first aqueous tetrabutylammonium hydroxide(10 µmol/20 µmol) was cooled in a reaction vessel at -78°C(dry ice-acetone). Methylene chloride solution(1.5 ml) containing 3-4 mg of spiperone was added to the reaction vessel and [^{11}C]CH_3I was bubbled through the solution. The reaction mixture was mixed by sonication for 5 min at 40°C. The labeled product was isolated by high performance liquid chromatography(HPLC) on a Radial-PAK silica column(Waters) with a solvent system of chloroform/methanol/dimethylamine(99.75:0.25:0.0025). The specific activity and radiochemical purity were verified by HPLC on a µ-Porasil column in the same solvent system. The specific activity was approximately 30-60 Ci/mmol at the time of use and the radiochemical purity was over 99%.

Tissue distribution in rats.

Male Wistar rats weighing 160-180g were used in the distribution study. The administrated doses were calculated with specific activity and injected radioactivities. All data are expressed as the percentage of injected dose per gram of wet tissue(%dose/g). The injected dose given to each rat was about 40 nmol/kg body weight for [^{11}C]NMSP.

Regional distribution in the rat brain.

Male Wistar rats(160-180g) were killed at various time after the injection of [^{11}C]NMSP(less than 10 nmol/kg). The striatum, frontparietal cortex, posterior cortex, cerebellum, hippocampus, medulla oblongata, hypothalamus and thalamus were excised according to the methods of Glowinski and Iversen.[20]

Autoradiography.

Male Wistar rat was injected with approximately [^{11}C]NMSP(10 mCi) through a dorsal tail vein. The brain was removed at 20 min after the administration and frozen in crushed dry ice. The 30 µm coronal sections of the brain were exposed to Kodak X ray film(XAR-5) at room temperature for 3 h.

Subcellular distribution of the brain tissue.

Male Wistar rats(160-180g) were killed by decapitation at 45 min after the injection. All subsequent operations were conducted at 0-4°C. Subcellular fractionation was performed as described by Whittaker and Barker.

For the rapid separation of soluble supernatant(S) from nuclear(N), crude mitochondrial(CM), and microsomal(MIC) fractions, the brain homogenate was centrifuged at 100,000×g for 60 min.

The detection of [^{11}C]CO_2 in expired gas.

Male Wistar rats were placed in the modified metabolic cage for the detection of [^{11}C]CO_2 in expired gas. Expired [^{11}C]CO_2 was trapped with Soda Lime and counted with autowell γ-counter.

Dynamic PET study.

An adult dog weighing 13 kg was anesthetized with pentobarbital. [^{11}C]NMSP(approximately 5 mCi) was injected intravenously and continuous sequential scans were performed on the level of OM+0 and OM-10 mm from 2 to 72 min after the administration. About 1 ml of the arterial blood was collected at the indicated time interval and then centrifuged to separate the plasma for the measurement of carbon-11 radioactivity. The radioactivity in the plasma and the dog brain was expressed as percent of administrated dose/ml plasma volume and percent dose/ml brain tissue volume, respectively. In the displacement study, spiperone(2 mg) was injected continuously from 37 to 52 min after [^{11}C]NMSP administration. In the pretreatment experiment the dog was pretreated with spiperone(2 mg) 15 min before [^{11}C]NMSP administration.

Results

Organ distribution and regional distribution in the brain.

Organ distribution of [^{11}C]NMSP and regional distribution of [^{11}C]NMSP in the rat brain are shown in Figure 1 and Table 1. [^{11}C]NMSP accumulated highly in the liver, lung, and kidney(Fig. 1) although the brain uptake was not so high. Blood radioactivity fell to very low level within 5 min after the injection.

Time-activity curve was quite different in each brain region. The radioactivity in the striatum increased with time and reached 1.9 %dose/g at 60 min. On the other hand time-activity curve in the cerebellum showed a rapid elimination. Striatum-to-cerebellum ratio increased from 3.4 at 10 min to 10.5 at 60 min after injection.

[^{11}C]NMSP concentration in the forebrain and cerebellum as a function of N-methylspiperone dose.

Fig. 2 shows the concentration of carbon-11 radioactivity in the forebrain and cerebellum of rats as a function of dose at 45 min after the administration of [^{11}C]NMSP. As the dose of N-methylspiperone is increased, the concentration of carbon-11 radioactivity in the cerebellum was increased in a linear manner. In contrast to this, the radioactivity was found to increase much more rapidly in the forebrain at low doses. Thereafter increase of binding in this region paralleled that in the cerebellum. These data

indicate that the radioactivity curve in the forebrain may adequately be decomposed into two components: 1)A linear element equal to that found in the cerebellum. 2)a saturable element obtained by subtracting the concentration in the cerebellum from that in the forebrain. This saturability allowed the determination of a maximum binding capacity(Bmax) of [^{11}C]NMSP and the administrated dose corresponding to 50% maximum binding capacity(Kd), which were 10-14 pmol/kg of tissue and 10-20 nmol/kg of body weight, respectively.

Imaging of nonspecific binding pool.

Fig. 3 shows the ARG images obtained from rat administrated a large amount of N-methylspiperone(3300 nmol/kg). These images are expected to demonstrate the nonspecific binding pool of [^{11}C]NMSP in the brain. Regional difference in the brain was not observed except that the distribution in the white matter was lower than gray matter.

Striatum-to-cerebellum and frontparietal cortex-to-cerebellum ratios as a function of administrated dose.

Fig. 4 shows striatum-to-cerebellum and frontparietal cortex-to-cerebellum ratios of rats 45 min after the injection of [^{11}C]NMSP as a function of log dose of N-methylspiperone. The striatum-to-cerebellum carbon-11 radioactivity ratio as a function of log dose was decreased in sigmoid fashion. The ratio remained constant at a value of approximately 8 in low doses(less than 30 nmol/kg). Likewise, the frontparietal cortex-to-cerebellum ratio kept constant at 4.5 in low doses(less than 30 nmol/kg) and decreased with the injected dose of N-methylspiperone.

[^{11}C]CO_2 in expired gas.

As shown in Fig. 5, expired [^{11}C]CO_2 was increased rapidly up to a rate of 0.015-0.016 % of injected activity per minute and diminished gradually.

Subcellular distribution of [^{11}C]NMSP in the rat brain.

Fig. 6 shows the effects of administrated dose on the subcellular distribution of [^{11}C]NMSP. As the log dose of N-methylspiperone was decreased, the relative radioactivity of carbon-11 in the N+CM+MIC fractions was increased. Carbon-11 radioactivity in the three fractions(N+CM+MIC) of forebrain and striatum were higher than that of cerebellum and medulla oblongata. More than 95% of radioactivity was recovered in the three fractions(N+CM+MIC) of forebrain in rats injected with 1-10 nmol/kg of [^{11}C]NMSP. Therefore most of [^{11}C]NMSP was expected to bind to postsynaptic neuroleptic binding sites in a low loading dose.

The effects of displacement and pretreatment on the regional distribution of [^{11}C]NMSP in the rat brain.

The regional distribution of [^{11}C]NMSP in control, displacement, and pretreatment study was illustrated in Fig. 7. The carbon-11 radioactivity in the striatum and cerebral cortex was displaced by spiperone(5 mg/kg) although

the radioactivity in other regions was not altered by spiperone displacement. On the other hand [^{11}C]NMSP was less accumulated in the striatum and cortex of spiperone-pretreated rats and the regional difference was very slight in the brain. The concentration displaceable by a higher amount of spiperone was lower in the striatum than the concentration difference between control study and pretreatment experiment. These data indicated that [^{11}C]NMSP in the striatum was not completely displaced from the specific binding sites of neuroleptics by a large amount of unlabeled spiperone. [^{11}C]NMSP distribution in other organs were not changed by spiperone pretreatment and displacement.

Dynamic PET study.

In control study [^{11}C]NMSP was accumulated in the striatum as shown in Fig. 8. [^{11}C]NMSP accumulation was reversible by spiperone(2 mg). The displaceable concentration by spiperone was not as high as the difference between control PET study and pretreatment experiment. Time activity curve of the cerebellum in control study was similar to that of striatum in the pretreatment. Plasma radioactivity was rapidly decreased and kept constant after 15 min. The plasma radioactivity curve was not altered by spiperone pretreatment and displacement.

Discussion

[^{11}C]NMSP is a analog of spiperone, which is labeled with carbon-11 by methyl-for-hydrogen substitution. The Ki for 3-N-methylspiperone was found to be approximately 250 pM against [^{3}H]spiperone binding sites and striatum/cerebellum ratio was 20 at 60 min after injection.[22] But fundamental aspect of [^{11}C]NMSP in *in vivo* binding condition is not clearly investigated.

Laduran et al pointed out that *in vivo* binding is specific if the drug used fulfills the three criterias; 1) a specific retention of the drug must appear in receptor-rich regions. 2) the binding must be reversible i.e. displaceable by a higher amount of unlabeled antagonist or agonist. 3) the subcellular distribution must show the specific localization to the receptor-rich fractions such as microsomes.[6] We demonstrated a specific accumulation of [^{11}C]NMSP in the striatum, reversibility of [^{11}C]NMSP binding in the striatum and frontparietal cortex and the specific localization to N+CM+MIC fractions. These data fulfilled the three criterias, indicating that [^{11}C]NMSP is a suitable radiopharmaceutical for the *in vivo* dopamine$_2$ and serotonin$_2$ receptors binding experiments.

N-dealkylation, a common oxidative biotransformation, occurred after [^{11}C]NMSP injection and final product could be detected as [^{11}C]CO_2 in expired gas. But amount of expired [^{11}C]CO_2 was little and [^{11}C]CO_2 is expected to be rapidly excreted from the lung. Therefore the metabolism of [^{11}C]NMSP is not considered to influence too much the evaluation of dopamine$_2$ receptors.

Striatum-to-cerebellum ratio was a function of time after injection and administrated dose of [^{11}C]NMSP. We might be able to evaluate dopamine$_2$ receptor in the striatum with this ratio because the ratio remained constant in low doses at fixed time.

Saturation kinetics of [^{11}C]NMSP could allow the determination of Bmax and Kd. Therefore B, concentration of [^{11}C]NMSP specifically bound to neuroleptic receptors at fixed time, is defined by this equation.

$$B=Bmax/(1+Kd/D)$$

In this equation, Bmax=the number of binding sites, Kd=the binding affinity, and D=administrated dose of [^{11}C]NMSP. This equation demonstrated that Bmax and Kd were not separably ascertainable in a single PET study and that the amount of specific binding in each experiment is influenced by both Bmax and Kd. B is not always elevated when Bmax is increased. If not only Bmax but also Kd were elevated in some subjects(e.g. tardive dyskinesia), the binding of [^{11}C]NMSP to the specific receptors (B) might be lowered in some cases, compared with control.

[^{11}C]NMSP may be considered to be rapidly distributed to three kinetically changing pools: 1) a pool for dopamine$_2$ or serotonin$_2$ receptors, 2) nonspecific binding pool, and 3) a pool for unbound (free) [^{11}C]NMSP. It is the most important to distinguish the specific binding pool from the other pools. In our experiments, the saturable component was not observed in the cerebellum and a large dose of [^{11}C]NMSP diminished the regional difference of distribution in the rat brain except for white matter. Moreover time-activity curve of cerebellum in control PET study was very analogous to that of striatum in the dog brain pretreated with spiperone. These data indicated that cerebellum could be regarded as a nonspecific binding pool and unbound pool.

Amount of [^{11}C]NMSP binding displaceable by spiperone was about 50% of the concentration obtained by subtracting the radioactivity of striatum in the pretreatment study from that of striatum in control study. The data obtained by pretreatment study was quite different from that of displacement in both rats and a dog. It might be suggested that displacement experiment were not a suitable method for quantitative measurement of receptor binding sites.

A pharmacological treatment required to demonstrate specific binding of radioligand to brain neurotransmitter receptors *in vivo* may cause unfavourable side effects, for example, hypotension, tachycardia and shock. In order to avoid these effects and maintain a high concentration of spiperone for a long time we administrated a large amount of spiperone to a dog continuously during 15 min instead of rapid injection.[23)]

It is necessary that the data obtained by PET study are analyzed using a mathematical kinetic model for the purpose of characterizing quantitatively, locally and *in vivo*, neuroleptic binding sites in the brain.

Acknowledgments

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Table 1. Regional distribution of [^{11}C]NMSP in the rat brain. The loading dose is less than 10 nmol/kg of body weight.

	10 min n=3	30 min n=3	45 min n=5	60 min n=3
striatum	0.98±0.14	1.4 ±0.1	1.6 ±0.4	1.9 ±0.2
frontparietal cortex	0.78±0.11	0.86±0.03	0.64±0.06	0.68±0.12
posterior cortex	0.60±0.15	0.59±0.01	0.42±0.11	0.44±0.06
cerebellum	0.29±0.06	0.22±0.04	0.19±0.06	0.19±0.02
hippocampus	0.38±0.09	0.32±0.01	0.29±0.09	0.29±0.09
meddula oblongata	0.46±0.10	0.41±0.04	0.33±0.12	0.29±0.01
hypothalamus,midbrain	0.43±0.06	0.46±0.05	0.35±0.04	0.36±0.05
forebrain	0.48±0.04	0.67±0.02	0.52±0.03	0.59±0.08
blood	0.15±0.02	0.16±0.02	0.16±0.02	0.16±0.01
striatum/cerebellum	3.4 ±0.2	6.3 ±0.5	8.4 ±2.4	10.5 ±0.7

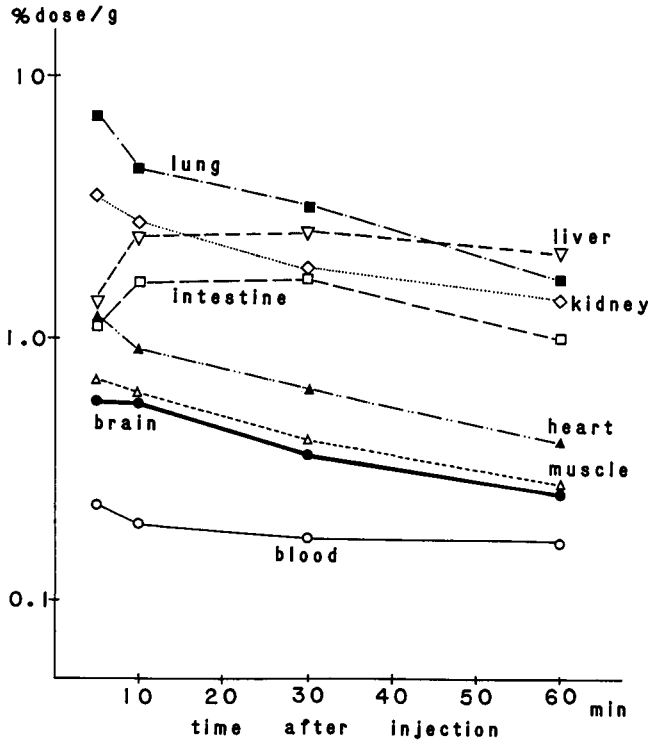

Fig. 1. Organ distribution of [^{11}C]NMSP in rats. Each point represents the mean of 3 rats.

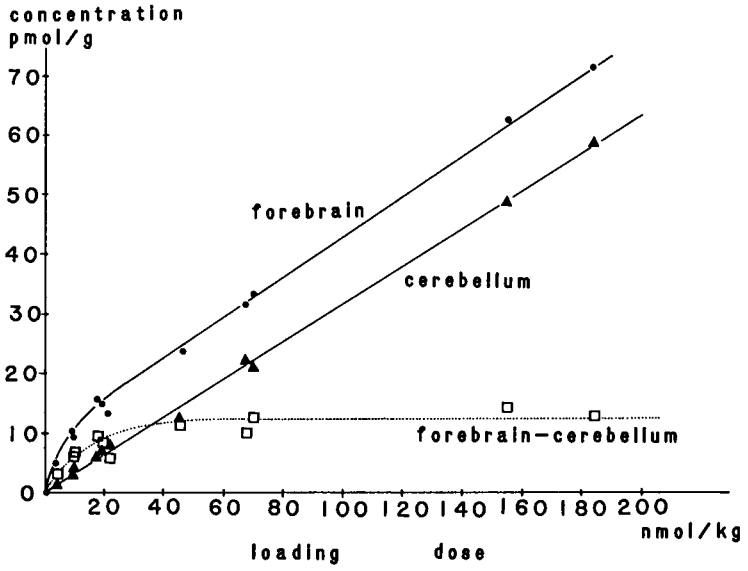

Fig. 2. Saturation kinetics of [^{11}C]NMSP in the forebrain(●——●) and cerebellum (▲——▲) at 45 min after the injection. The concentration of [^{11}C]NMSP in the brain was calculated with %dose/g in each region and the loading dose.

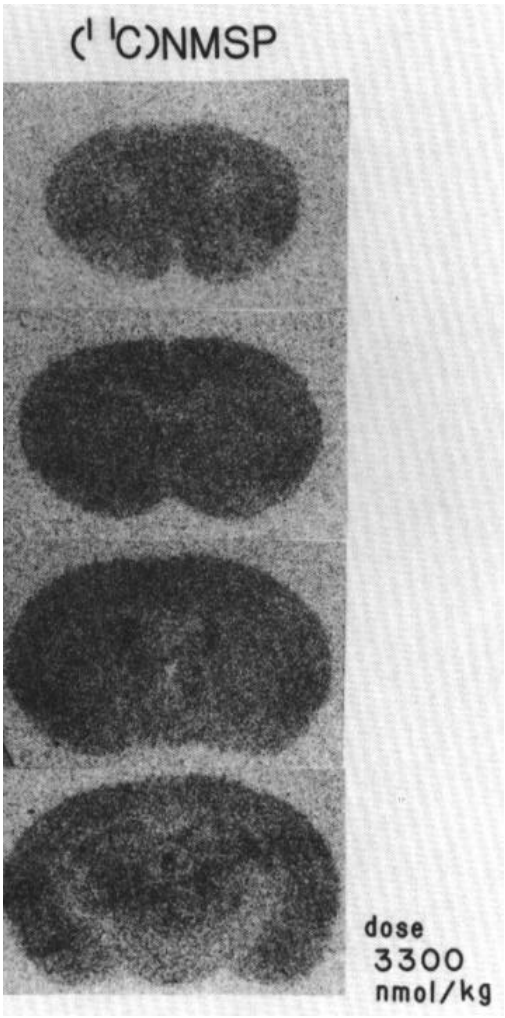

Fig. 3. The ARG of [^{11}C]NMSP. The ARG images are expected to show the nonspecific binding pool of NMSP.

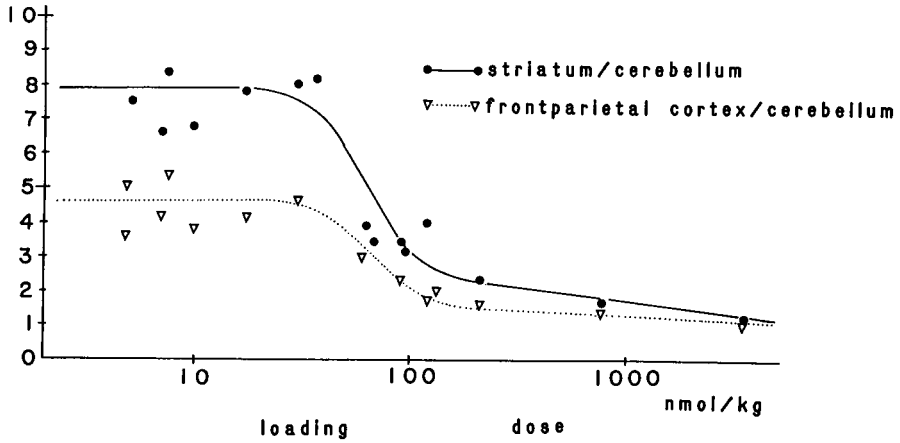

Fig. 4. Striatum-to-cerebellum(●——●) and frontparietal-to-cerebellum(▽---------▽) ratios in rats 45 min after the injection of [^{11}C]NMSP as a function of administrated dose of NMSP.

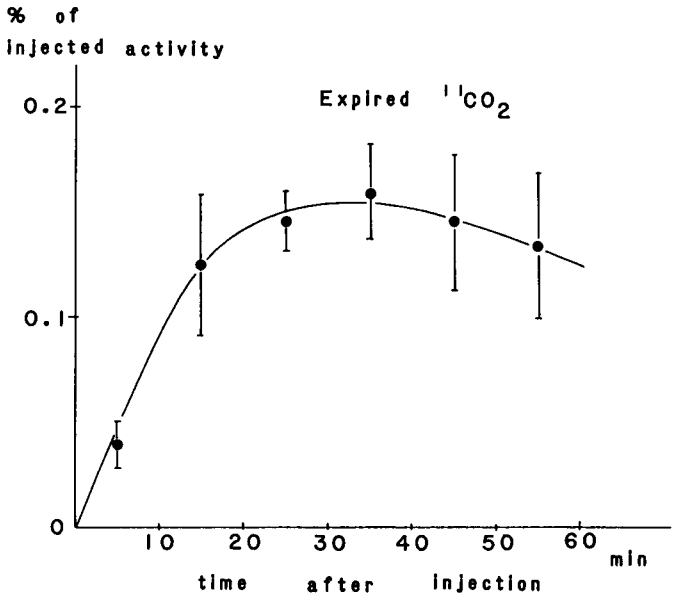

Fig. 5. Detection of expired [^{11}C]CO_2 in rats.
Each point repreasents the mean of 5 determinations.

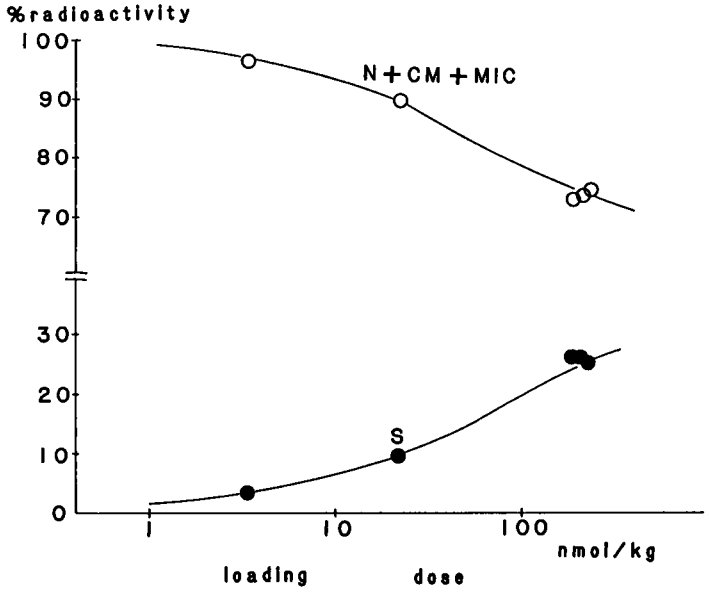

Fig. 6. The effect of administrated dose on the
subcellular distribution of [^{11}C]NMSP in rats.

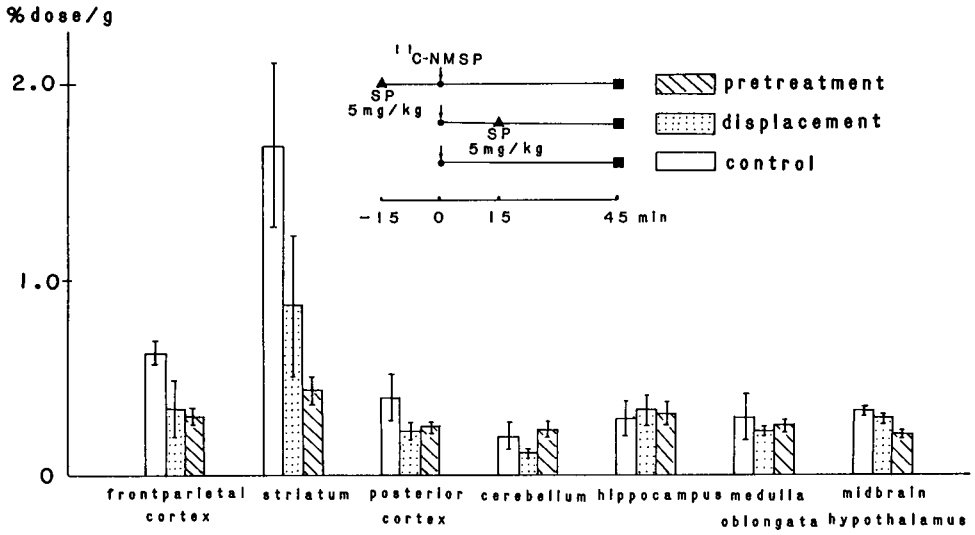

Fig. 7. The effects of pretreatment and displacement on the regional distribution of [^{11}C]NMSP in the rat brain. Spiperone(5 mg/kg) was pretreated 15 min before [^{11}C]NMSP administration. Regional distribution was examined at 45 min after the injection. In displacement study, spiperone (5 mg/kg) was administrated 15 min after [^{11}C]NMSP injection.

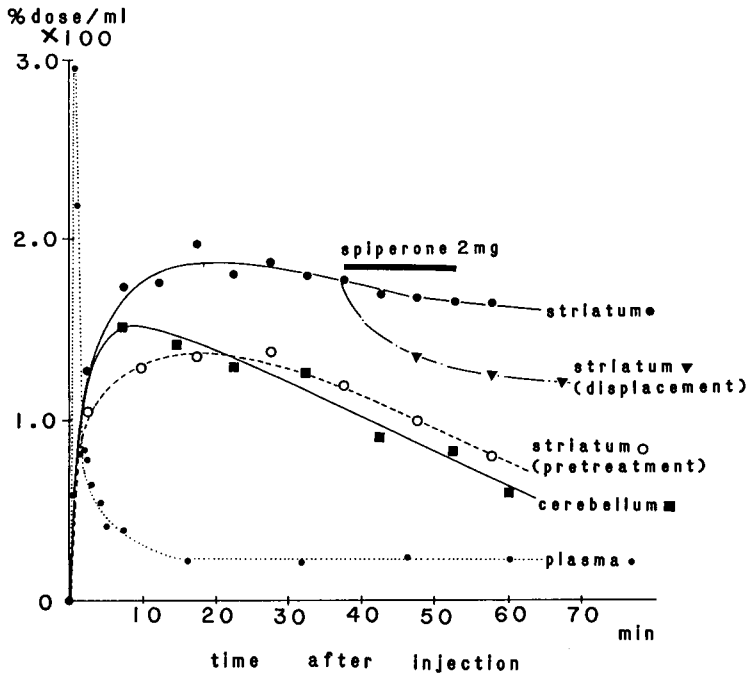

Fig. 8. The PET study of [^{11}C]NMSP.