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Synthesis and evaluation of ^{11}C-PK 11195 for *in vivo* study of peripheral-type benzodiazepine receptors using position emission tomography

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The biodistribution of ^{3}H-PK 11195, an antagonist of the peripheral-type benzodiazepine receptors, was studied in mice. High accumulations of radioactivity in the heart, lung, spleen, kidney and adrenal were observed after intravenous injection of tracer amounts of ^{3}H-PK 11195 into the mice. The radioactivity in the heart, lung, spleen, kidney and adrenal was significantly decreased by the coadministration of carrier PK 11195, which indicated that PK 11195 specifically binds to the receptors. No radioactive metabolites were observed in the heart, lung and brain 20 min after intravenous administration of ^{3}H-PK 11195. The accumulation of ^{3}H-PK 11195 in the lung was not affected by pretreatment with either α-methyl benzylamine or imipramine, suggesting that ^{3}H-PK 11195 specifically binds to the receptors. The ratios of radioactivity of the kidney, adrenal and spleen to blood increased as a function of time, whereas that of the lung and heart rapidly reached to a steady state. ^{11}C-PK 11195 was synthesized by the N-methylation of desmethyl precursor yielding more than 100 mCi with high specific activity (more than 1.4 Ci/μmol). The labeling and purification procedure was completed within 23 min after the end of bombardment (EOB). The ^{11}C-PK 11195 solution for injection seems to have a high potential for the *in vivo* study of the peripheral-type benzodiazepine receptors in the living human by means of positron emission tomography (PET).

Key words: PK 11195, Peripheral-type benzodiazepine receptor, Positron emission tomography, *In Vivo* binding, Carbon-11

INTRODUCTION

In vivo studies of the central-type benzodiazepine receptors in the living human brain using positron emission tomography (PET) have recently been reported.[1–5] Benzodiazepine receptors are classified as central-type or peripheral-type on the basis of on their relative affinities for clonazepam and Ro 5-4864[7-chloro-1,3-dihydro-1-methyl-5-(p-chlorophenyl)-2H-1, 4-benzodiazepin-2-one].[6] The results of a thermodynamic analysis indicated that PK 11195 [1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide] might be an antagonist and Ro 5-4864 an agonist at the peripheral-type benzodiazepine receptors.[7] ^{3}H-Ro 5-4864 and ^{3}H-PK 11195 have been used to identify, characterize and localize the peripheral-type benzodiazepine receptors in the peripheral organs as well as in the central nervous system.[7–12] In the brain, high densities of

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peripheral-type benzodiazepine receptors have been observed in the choroid plexus, ependyma and olfactory bulb.[11,13-15] Recently, high accumulations of ^{3}H-PK 11195 in the transplanted glioma in the rat were reported.[16] In the periphery, high concentrations of the peripheral-type benzodiazepine receptors have been reported in several organs including the kidney, nasal epithelium, lung, heart and endocrine organs such as the adrenal, testis and pituitary gland.[17-20] Although the precise physiological function of the peripheral-type benzodiazepine receptors is still unclear, several laboratories have reported interaction between these receptors and calcium channels in the heart[21-23] or anion transport system in the kidney.[24] And changes in the density (Bmax) of the peripheral-type benzodiazepine receptors in the kidney following pretreatment with diuretic furosemide have recently been reported.[24]

In a previous paper, we reported that ^{3}H-PK 11195 had a high potential as a radiotracer for the *in vivo* study of the peripheral-type benzodiazepine receptors.[25] In the present paper, we studied in more detail the biodistribution and *in vivo* stability of ^{3}H-PK 11195 in mice. In order to determine whether a high accumulation of ^{3}H-PK 11195 in the lung was due to the specific binding with receptors or to linked to the active transport system for basic amines, the effects of basic amines on the lung uptake of ^{3}H-PK 11195 were also examined. Finally, a high specific activity ^{11}C-PK 11195 solution for injection was prepared for the *in vivo* study of the peripheral-type benzodiazepine receptors in man with PET.

MATERIALS AND METHODS

1. Materials

^{3}H-PK 11195 (87 and 90 Ci/mmol) was obtained from New England Nuclear, Boston, MA, USA. PK 11195 and desmethyl PK 11195 were donated by Dr. G. Le Fur (Pharmuka Laboratories, Gennevillers, France). Paraquat (Sigma, St Louis, MO, USA), 6-hydroxydopamine hydrobromide (Sigma) and furosemide (WACO Pure Chemical Industries Ltd.,) were used. Other chemicals were purchased commercially.

2. Distribution of radioactivity in mice after intravenous administration of ^{3}H-PK 11195

In this study, male ddy mice (30–35 g) were used. Two-tenths mL of ^{3}H-PK 11195 solution (1 μCi) was intravenousely injected into the mice, which were then killed by decapitation at 1, 5, 10 and 20 min after injection of the tracer. The blood, heart, lung, liver, spleen, kidney, adrenal and brain were removed and weighed, each sample being incinerated with a sample oxidizer (Aloka, ACS-113) and the percentage of injected dose per gram tissue (% dose/g) in each sample was determined with a liquid scintillation counter (Aloka, LSC-1000).

In the carrier-added experiment, two-tenths mL of ^{3}H-PK 11195 (1 μCi, 5 mg/kg) was intravenously injected into the mice, and the radioactivity in the tissues was determined as described above. The solution for injection of ^{3}H-PK 11195 (5 mg/kg) was a 10% emulsion with Nikkol HCO 40 (emulsifier, Nikko Chemicals, Tokyo).

3. In vivo competitive inhibition of specific binding by carrier PK 11195

Two-tenths mL of ^{3}H-PK 11195 solution (0.1, 10, 30, 100, 300 and 1000 μg/kg, ca. 1 μCi) was intravenously injected into the male ddy mice (30–35 g), which were then killed by decapitation at 5 min after injection. The radioactivity in the blood, heart, lung and brain was determined as described above. Various doses of ^{3}H-PK 11195 solution were prepared by diluting 10% emulsion with Nikkol.

4. In vivo stability of ^{3}H-PK 11195 in the mice

About 12 μCi of ^{3}H-PK 11195 was intravenously injected into the male ddy mouse. Twenty min after injection of the tracer, the mouse was killed by decapitation, and the heart, lung and brain were quickly removed. Each organ was homogenized with 1 mL of saline, and 100 μL of the homogenate was sampled as a standard for the determination of extraction efficiency. One mg of carrier PK 11195 and 400 μL of ethyl acetate were added to the 400 μL of the homogenate, then radioactive materials were extracted. Extraction efficiencies in the heart, lung and brain determined by comparison with the standard were more than 95, 95 and 90% respectively. Organic extractable materials were analyzed by thin-layer chromatography (TLC, silicagel; chloroform: methanol= 95: 5, Rf value=0.48, chloroform: diethyl ether: hexane=1: 1: 1, Rf value=0.16).

5. Effects of basic amines on the lung uptake of ^{3}H-PK 11195

α-Metyl benzylamine (1 and 10 mg/kg) and imipramine (25 mg/kg) were intraperitoneally injected into the male ddy mice. Two-tenths mL of ^{3}H-PK 11195 (1 μCi) was intravenously injected into the mice 10 min after pretreatment with these amines. The radioactivity in the blood, heart, lung and brain 5 min after injection of the tracer was determined as described above. α-Metyl benzylamine hydrochloride and imipramine hydrochloride solutions for injection were diluted with distilled water in a volume of 0.1 mL/10 g body weight.

6. Effect of various drugs on the biodistribution of ^{3}H-PK 11195 in the mice.

Two-tenths mL of ^{3}H-PK 11195 solution (1 μCi) was

intravenously injected into the male C3H mice 2 days after intraperitoneal administration of paraquat (30 mg/kg), which were than killed by decapitation 5 min after injection of the tracer. The blood, heart, lung, adrenal and brain were removed and the radioactivity in each sample was determined as described above.

Two-tenths mL ^{3}H-PK 11195 (1 μCi) was intravenously injected into the male C3H mice 1 week after the second injection of 6-hydroxydopamine (50 mg/kg/day$\times$2, i.v.) or vehicle. The radioactivity in the blood, heart, lung, kidney, adrenal and brain 5 min after injection of the tracer was determined as described above.

Two-tenths mL of ^{3}H-PK 11195 (1 μCi) was intravenously injected into the male ddy mice 6 hr after the last injection of furosemide (50 mg/kg/day$\times$5, i.p.) or vehicle (5% Tween-80 suspension in saline). The radioactivity in the blood, heart, lung, liver, spleen, kidney, adrenal and brain 5 min after injection of the tracer was determined as described above.

7. Preparation of ^{11}C PK 11195 solution for injection

^{11}C-Methyl iodide was prepared as described previously.[26] ^{11}C-PK 11195 was synthesized by the alkylation of the desmethyl precursor with ^{11}C-methyl iodide in dimethylformamide solution (DMF: DMSO: NaH=350: 150: 15, 500 μL) at room temperature for 1 min, and purified by preparative high performance liquid chromatography (HPLC) (Megapack SIL C18-column, 7.2$\times$250 mm, JASCO, Tokyo, Japan) eluting with a solvent (acetonitrile: 0.01 M ammonium acetate: phosphoric acid=70.9: 29.1: 0.05) at a flow rate of 6 mL/min. The radioactive peak corresponding to PK 11195 was corrected in a sterile flask containing 150 μL of Tween-80, evaporated to dryness in a rotary evaporator and dissolved in 10 mL of saline with 80 μL of ethyl alcohol filtered through a 0.22 μm Milex filter. Radiochemical purity and specific activity of the ^{11}C-PK 11195 solution were determined by analytical HPLC (Finepack SIL, C18-column, acetonitrile: 0.01M ammonium acetate: phosphoric acid=195: 65: 0.125) at a flow rate of 3 mL/min.

RESULTS

As shown in Table 1, high accumulations of radioactivity in the heart, lung, spleen, kidney and adrenal after intravenous administration of ^{3}H-PK 11195 were observed. A moderate accumulation of radioactivity in the brain after injection of the tracer was also observed. The time courses of radioactivity in the heart, lung and brain rapidly decreased, whereas increases in radioactivity in the spleen, kidney and adrenal were observed. The half-lives of radioactivity in the heart and lung were almost the same as that of radioactivity in the blood, which indicated that the tracer kinetics rapidly reached a steady state after intravenous administration.

In the carrier-added experiment, the radioactivity in the heart, lung, spleen, kidney and adrenal were much less than in the carrier-free state, as shown in Table 2. The biodistributions of radioactivity in the blood, heart, lung and brain 5 min after intravenous administration of various doses of ^{3}H-PK 11195 are summarized in Fig. 1. The radioactivity in the heart and lung was significantly decreased in a dose dependent manner, whereas the radioactivity in the blood and brain was unchanged by the coadministration of carrier PK 11195.

Thin-layer chromatograms of radioactive materials in the heart, lung and brain 20 min after intravenous administration of ^{3}H-PK 11195 indicated that almost all of the radioactivity in the heart, lung and brain was due to unmetabolized PK 11195 (Fig. 2).

The effects of α-methyl benzylamine (1 and 10 mg/kg) and imipramine were examined in order to determine whether the high accumulation of ^{3}H-PK 11195 in the lung was due to the specific binding with the receptors or linked to the amine uptake system.

Table 1 Distribution of radioactivity in mice after intravenous administration of ^{3}H-PK 11195.

(% dose/g)

	Control			
	1 min	5 min	10 min	20 min
Blood	5.60±0.22	2.02±0.18	1.43±0.25	0.939±0.08
Heart	28.67±3.16	20.00±2.23	14.11±0.87	10.52±0.87
Lung	72.25±13.6	32.00±7.39	22.21±3.08	13.63±2.69
Liver	3.32±0.31	6.03±1.03	7.36±0.52	7.81±1.14
Spleen	5.23±1.61	10.79±2.11	10.77±0.42	10.91±1.50
Kidney	14.02±3.82	19.52±1.11	19.88±0.57	19.72±1.93
Adrenal	40.50±15.6	61.08±15.9	82.84±14.5	100.2±23.6
Brain	4.14±0.34	2.33±0.16	1.45±0.10	1.09±0.18

Three mice in each group; average±1 SD

Table 2 Distribution of radioactivity in mice after intravenous administration of carrier-added ^{3}H-PK 11195 (% dose/g)

	Carrier-added (5 mg/kg)			
	1 min	5 min	10 min	20 min
Blood	2.74±0.21	1.60±0.07	1.09±0.02	0.709±0.10
Heart	8.30±0.52	2.80±0.27	1.65±0.15	0.996±0.03
Lung	3.91±0.46	2.71±0.26	1.90±0.20	1.33±0.17
Liver	5.91±1.91	12.77±0.51	11.25±1.22	7.03±0.39
Spleen	2.23±0.30	1.89±0.16	1.33±0.14	0.991±0.05
Kidney	10.10±0.61	4.70±0.11	3.97±0.16	3.25±0.28
Adrenal	52.88±4.96	18.35±3.48	18.24±5.34	16.97±6.91
Brain	5.01±0.22	2.16±0.19	0.963±0.13	0.398±0.03

Three mice in each group; average±1 SD

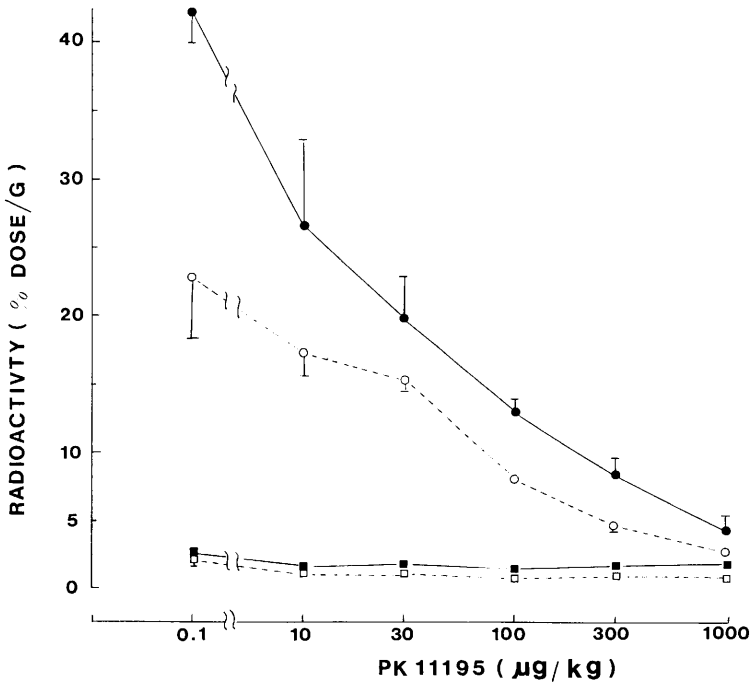

Fig. 1 *In vivo* competitive inhibition of specific binding by carrier PK 11195. The radioactivity in the heart (○···○), lung (●—●), brain (■—■) and blood (□···□) was expressed as percentage pet gram organs (% dose/g) as described in Materials and Methods. Values are presented as the average±1 SD for three mice of each point.

As shown in Table 3, the uptake of ^{3}H-PK 11195 in the lung as well as the other organs 5 min after injection of the tracer was not significantly changed by pretreatment with these basic amines. Further, we studied the effects of paraquat, which was used as an animal model of the lung damage,[27,28] neurotoxin 6-hydroxydopamine and diuretics furosemide on the biodistribution of ^{3}H-PK 11195 in the mice after injection of the tracer. No significant changes in the biodistribution of ^{3}H-PK 11195 were found following pretreatment with these drugs, as shown in Table 4.

^{11}C-PK 11195 was synthesized by the alkylation of N-desmethyl PK 11195 with ^{11}C-methyl iodide (Fig. 3) (29), and purified by radio-UV HPLC (Fig. 4) yielding more than 100 mCi of ^{11}C-PK 11195 solution. The labeling and purification procedure was completed within 23 min after the end of bombardment (EOB). The radiochromatogram of the ^{11}C-PK 11195 solution is shown in Fig. 5. Radiochemical purity was more than 99.9%, and specific activity of the ^{11}C-PK 11195 solution was determined to be more than 1.4 Ci/μmol by radio-HPLC.

DISCUSSION

The *in vivo* mapping of various receptors in the brain and heart in the intact human with PET has recently

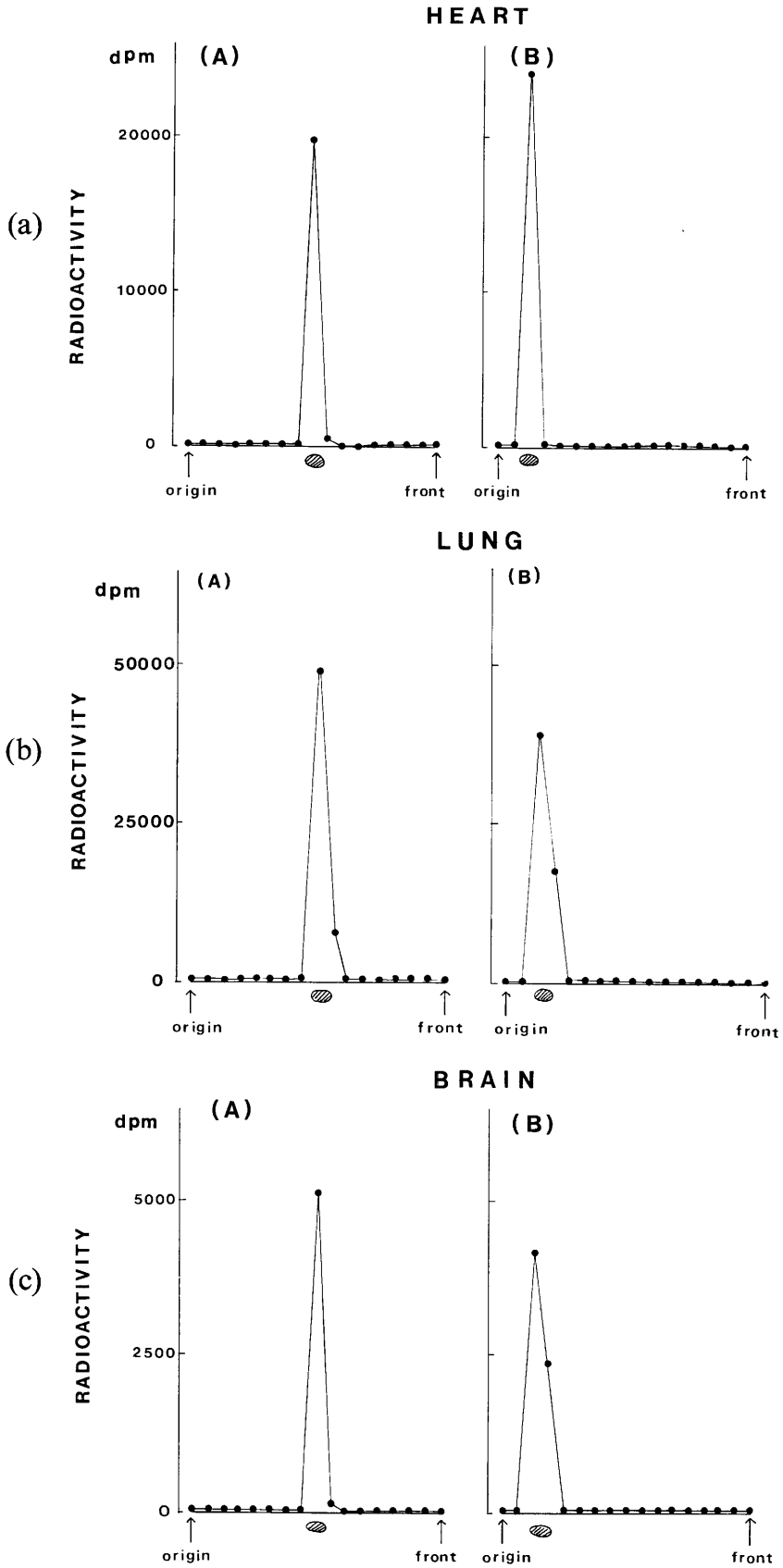

Fig. 2 Thin-layer chromatograms of radioactive materials in the heart, lung and brain removed 20 min after intravenous administration of ^{3}H-PK 11195 (12 μCi) into the mouse. ▨: Cold PK 11195 detected by u.v. lump. Solvent system; (A), chloroform: methanol=95: 5, Rf value=0.48 (B), chloroform: diethyl ether: hexane=1: 1: 1, Rf value=0.16

Table 3 Effects of basic amines on the distribution of radioactivity in mice at 5 min after intravenous administration of ^{3}H-PK 11195

(% dose/g)

	Blood	Heart	Lung	Brain
Control	1.35±0.21	16.58±1.92	34.47±8.39	1.91±0.33
α-Methyl Benzylamine (1 mg/kg)	1.66±0.45	17.55±2.28	37.52±2.23	2.02±0.34
α-Methyl Benzylamine (10 mg/kg)	1.67±0.03	15.59±1.39	29.46±5.89	1.86±0.17
Imipramine (25 mg/kg)	1.44±0.14	16.52±2.95	33.41±10.86	1.81±0.27

Three mice in each group; average±1 SD
α-Methyl benzalamine (1 and 10 mg/kg) and imipramine (25 mg/kg) were intraperitoneally injected into the mice 10 min before i.v. injection of the tracer.

Table 4 Effects of various drugs on the distribution of radioactivity in mice at 5 min after intravenous administration of ^{3}H-PK 11195

(% Dose/g)

	Control	Paraquat
Blood	2.95±0.23	3.03±0.61
Heart	24.1±2.68	26.0±4.04
Lung	43.5±7.10	51.0±10.2
Adrenal	107±38.5	90.7±12.3
Brain	3.07±0.09	3.85±0.52

Three mice in each group; average±1 SD

(% Dose/g)

	Control	6-OHDA
Blood	1.51±0.15	2.22±0.26
Heart	19.6±2.65	19.3±1.17
Lung	40.8±8.40	64.7±11.3
Kidney	16.8±3.63	13.0±2.44
Adrenal	63.5±12.7	64.7±11.3
Brain	2.67±0.29	2.88±0.05

Three mice in each group; average± 1 SD

(% Dose/g)

	Control	Furosemide
Blood	1.49±0.23	1.73±0.10
Heart	15.1±1.38	18.7±2.28
Lung	24.2±8.16	31.6±1.98
Liver	5.50±1.06	5.93±0.89
Spleen	8.73±2.00	8.47±1.38
Kidney	18.1±3.87	20.3±2.92
Adrenal	87.6±4.01	99.9±23.8
Brain	1.77±0.23	2.25±0.16

Three mice in each group; average±1 SD

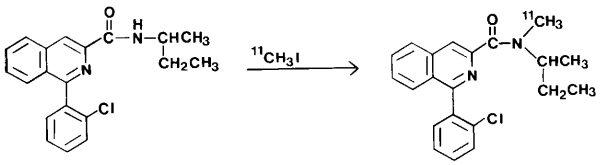

Fig. 3 Synthesis of ^{11}C-PK 11195

been performed. For this purpose, the selection of a suitable radioligand is very important in order to obtain high permeability to the cell membrane, high affinity with the receptor and high stability *in vivo*. ^{3}H-PK 11195 was found to be satisfied in the above critical conditions as follows: 1) High uptake of radioactivity in each organ was observed 1 min after intravenous administration of ^{3}H-PK 11195. 2) The radioactivity in the heart, lung, spleen, kidney and adrenal was found to be specifically bound with receptors in the carrier-added experiments. 3) TLC analysis showed ^{3}H-PK 11195 to be quite stable in the heart, lung and brain for at least 20 min after injection of the tracer.

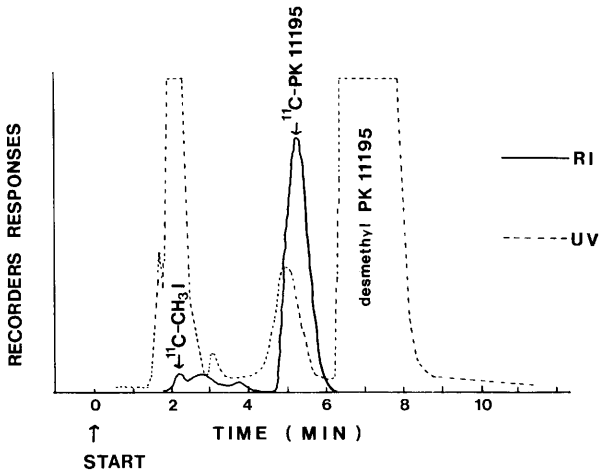

Fig. 4 Purification of ^{11}C-PK 11195 by HPLC

Very high accumulations of radioactivity in the heart, lung, spleen, kidney and adrenal following intravenous administration of ^{3}H-PK 11195 were observed. More than 80% of the total radioactivity in the lung and heart was found to be due to the

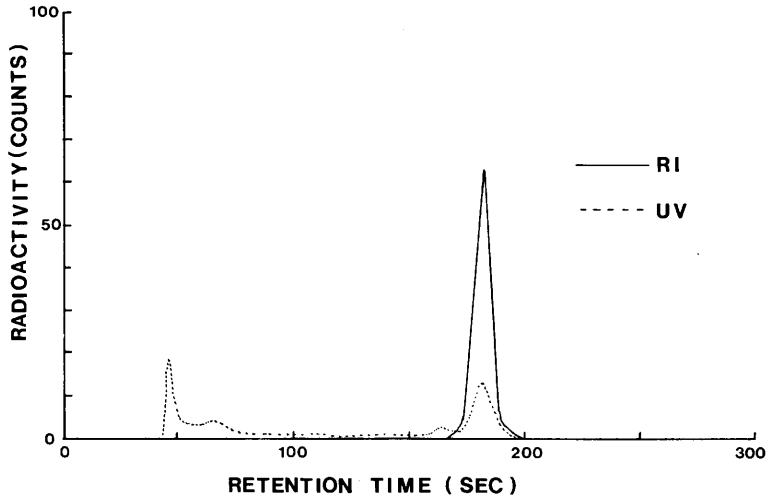

Fig. 5 HPLC of ^{11}C-PK 11195 solution for injection: conditions Finepack SIL (JASCO); solvent system, CH_3CN: 0.01 M CH_3COONH_4: H_3PO_4=195: 65: 0.125
Retention time of ^{11}C-PK 11195 (3 min), Flow rate 3 ml/min

specific binding with receptors. The time courses of radioactivity in the heart and lung after injection of the tracer were parallel to that in the blood, which suggested that the *in vivo* kinetics of ^{3}H-PK 11195 would reach the steady state soon after intravenous administration. These *in vivo* characteristics of ^{3}H-PK 11195 simplify the estimation of the binding potential (BP=Bmax/Kd) of the receptors. Since a considerable amount of peripheral-type benzodiazepine receptors exists in the platelets (30), a rapid estimating method for the determination of the free ligand concentration in the blood is required for the quantitative analysis of receptors.

From the previous data *in vitro*,[17] high densities of the peripheral-type benzodiazepine receptors have been observed in the heart, lung, kidney, adrenal and other organs, while the physiological functions of this receptor were not clearly understood. In order to examine the relationship between the peripheral-type benzodiazepine receptors and the catecholaminergic neurons, we studied the effect of neurotoxin 6-hydroxydopamine on the biodistribution of ^{3}H-PK 11195 in the mice. The present results indicated that the peripheral-type benzodiazepine receptors might not be regulated by the catecholaminergic neurons. Since it has been shown that the peripheral-type benzodiazepine receptors could be coupled to the calcium channel in the heart,[21,22] the *in vivo* study of the peripheral-type benzodiazepine receptors with ^{11}C-PK 11195 and PET would be of great value in the investigation of the living human heart.[31,32] In fact, Charbonneau et al. had already started a clinical study with ^{11}C-PK 11195 and PET.[33]

It has been recognized that the lung plays an important role in the regulation of a lot of circulating substances, including endogenous and exogenous

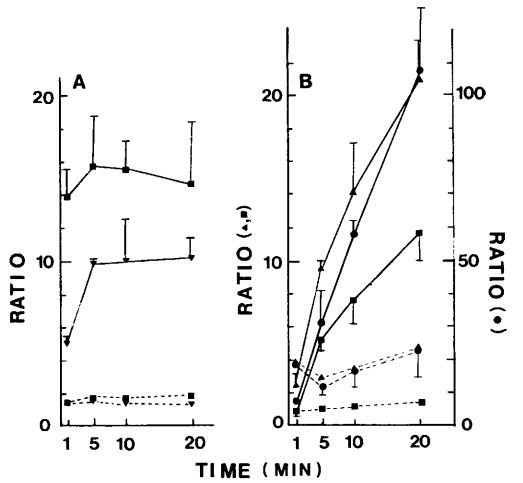

Fig. 6 Time courses of ratio of radioactivity in each organs to that in blood. (A) (▼—▼) and (■—■) are expressed as ratios of radioactivity in the heart and lung to the radioactivity in the blood in the carrier-free state. (▼---▼) and (■---■) are ratios in the carrier-added state. Three mice in each group; average±1 SD
(B) (■—■), (▲—▲) and (●—●) are ratios of radioactivity in the spleen, kidney and adrenal to the radioactivity in the blood in the carrier-free state. (■---■), (▲---▲) and (●---●) are ratios in the carrier-added state. Three mice in each group; average±1 SD

amines, and high accumulations of the basic amines in the lung have been widely reported.[34-38] We examined the effects of basic amines (α-methyl benzylamine and imipramine) on the lung uptake of ^{3}H-PK 11195 in order to determine whether the high accumulation of ^{3}H-PK 11195 in the lung was due to the specific binding with receptors or to the uptake system of basic amines. These results indicate that

the uptake of ^{3}H-PK 11195 in the lung might be due to the specific binding with receptors. It is also of interest to investigate the lung functions as receptor levels under various diseases with ^{11}C-PK 11195 and PET. However, the lung uptake of ^{3}H-PK 11195 in the paraquat pretreated mice, which was used as animal model of lung damage,[27,28] was unchanged when compared with that in the control mice. Further studies on the functional role of peripheral-type benzodiazepine receptors in the lung are necessary.

The distributions of radioactivity in the spleen, kidney and adrenal were increased over a period of 20 min after injection of the tracer. The time courses of the ratio of radioactivity in each organ to that in blood are shown in Fig. 6. The ratios in these organs were significantly decreased by treatment with carrier PK 11195. Since these ratios in the spleen, kidney and adrenal were increased over a period of 20 min after injection of the tracer, this radioligand would be suitable for the imaging of the spleen, kidney and adrenal. One possible reason for the difference between the binding kinetics of this radioligand *in vivo* in these three organs and other organs such as heart might be due to the different subclass of peripheral-type benzodiazepine receptors. It has recently been reported that the peripheral-type benzodiazepine receptors in the kidney was regulated by an anion transport system.[24] In the present study, the biodistribution of ^{3}H-PK 11195 in the kidney as well as other organs was not significantly changed by pretreatment with diuretic furosemide. Studies to further determine the relationship between the biodistribution of ^{3}H-PK 11195 in the kidney and anion transport system are necessary.

The distribution of radioactivity in the mouse brain 5 min after injection of ^{3}H-PK 11195 was not decreased by treatment with various doses of PK 11195. However, the radioactivity in the brain 10 and 20 min after injection of the tracer was slightly reduced by carrier PK 11195. Autoradiographic distribution of ^{3}H-PK 11195 5 min after intravenous injection was found in the ventricular structures, such as the choroid plexus and ependyma (data not shown). However, it was recently reported that marked species differences exist in the distribution and density of the peripheral-type benzodiazepine receptors in the brain.[39,40] It is necessary to remember this in connection with the *in vivo* study of the peripheral-type benzodiazepine receptors in the living human brain.

Furthermore, the ^{11}C-PK 11195 solution for injection was able to be obtained in high yield with high specific activity and high radiochemical purity.

In conclusion, ^{11}C-PK 11195 would be a suitable radioligand for the *in vivo* study of the peripheral-type benzodiazepine receptors in the living human with PET.

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