Note

Organ and Tissue Distribution of Phencyclidine in the Rat[†]Megumi FUKUSHI*¹, Yoko ISHII*¹, Yuji NAKAHARA*² and Akira TANAKA*¹*¹ Showa Pharmaceutical University

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The excretion, distribution and metabolism of ³H-phencyclidine (PCP) were studied in male rats following oral administration. The radioactivity was mainly excreted in urine and to a lesser extent in feces. Most of the urinary radioactivity was excreted in the form of glucuronide conjugates of hydroxylated PCP metabolites and urinary excretion of unchanged PCP was minimal. Distribution study showed no marked affinity for any particular tissue, although the levels in liver and prostate were a little high within 24 h after dosing. PCP was detected in brain by GC-MS.

Key Words : ³H-phencyclidine, rats, oral dosing, distribution, metabolites

1. Introduction

Phencyclidine, 1-(1-phenylcyclohexyl) piperidine (PCP) is called Angel Dust or Crystal in the United States and a very popular drug of abuse, as well as being a legitimate veterinary tranquilizer. In Japan PCP has been classified as a narcotic since 1990.

PCP was first developed for possible use as an intravenous anesthetic drug in the late 1950's. However, clinical use of PCP was afterwards banned because of its unacceptable side effects such as confusion, excitation, paranoia, aggression, hallucinations, and violent, homicidal, and suicide behavior of its users,

although PCP is still a useful chemical for pharmacological studies in brain research fields^{1), 2)}.

No detailed papers have been reported on the organ and tissue distribution of PCP in animals and humans³⁾⁻¹³⁾, except for the special organs such as brain, adipose tissue, testis and liver as well as blood. This paper deals with organ and tissue distribution of PCP in rats using tracer technique in addition to the identification of PCP in brain by GC-MS SIM method. Extrapolation of the animal data of PCP to humans would be useful for assessment of human safety from the view point of forensic medicine.

2. Experimental

2.1 Standard reference compounds

PCP standard sample, mp 42.0 °C (Fig.1) and its HCl salt, mp 241 - 242.0 °C were synthesized according to the modified method of Maddox et

[†] ラットにおけるフェンシクリジンの臓器分布。

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Fig. 1 Structure of PCP.

al.¹⁴⁾, and ^3H -PCP [piperidyl-3, 4- ^3H (N)] was purchased from New England Nuclear (NEN, USA). The radiochemical purity of ^3H -PCP showed above 99% by thin-layer chromatography on Silica Gel using the following solvent systems; methanol: ammonium hydroxide (99:1) and 2-butanol: acetic acid: water (25:4:10), respectively.

^3H -PCP (1 535.5 GBq/mmol) was diluted with non-radioactive PCP to the appropriate specific activity (0.445 MBq/mg as HCl salt) for the experiment to be undertaken. The syntheses of PCP and its metabolites, 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (PCHP, mp 117 - 118.0 °C), 4-phenyl-4-piperidinocyclohexanol (PPC, mp 178 - 179.0 °C), 1-phenyl-1-(4-hydroxypiperidino)-4-cyclohexanol (PCP-diol, mp 193.0 °C) and 1-phenylcyclohexylamine (PCA, HCl salt, mp 232.0 °C) were carried out by the method of literatures^{5), 10), 11), 14), 15)}.

The chemical structures of synthetic compounds were confirmed by their spectral data such as IR and NMR, and elementary analysis^{16), 17)}.

2·2 Reagents and analytical instruments

Beta-glucuronidase (from *Helix pomatia*, glucuronidase activity 10 000 units per ml at pH 5.0, sulfatase activity <1 000 units) and sulfatase (from *Helix pomatia*, sulfatase 9.9 units) were obtained from Sigma Chemical Co. (St. Louis, Mo. USA). Bond-Elut Certify cartridge was purchased from Varian sample preparation

products (Harbor City, Calif., USA). GC (Hewlett Packard 5890 Series II)-MS (MSD 5971) SIM (Selected Ion Monitoring) method was used for identification of PCP (m/z : 186, 200, 243) in rat brain using a 1:1 mixture of PCP and PCP-D₅ as an internal standard. Capillary column (0.25 mm I.D. × 15 m) packed with 100% dimethyl polysiloxane was used as a column for gas chromatography. Other chemicals were of reagent grade quality.

2·3 Animal experiments and radioassay

2·3·1 Tracer experiment

Male Wistar rats (400 ± 20 g, body weight) and (200 ± 20 g, body weight) were used in the excretion and distribution studies, respectively, after fasting overnight before experiments. Excretion of PCP was studied with three rats. Rats were given a single dose of ^3H -PCP·HCl as 0.2% aqueous solution at a dose of 1 mg/body. After dosing each rat was kept in metabolic cages for the collection of daily urine and feces for 7 days. The feces were extracted with excess methanol. An aliquot of urine and fecal extract solution was mixed with a suitable volume of scintillation cocktail, Atomlight (Packard), for radioactivity counting.

The animals in individual cages were divided into the group of three rats each. The animals for distribution studies were sacrificed at 4, 8, 12 and 24 h after the first dose of ^3H -PCP·HCl (0.445 MBq/mg). Blood samples were taken by heart puncture from anesthetized rats and each organ and tissue was removed, weighed, dissolved in 1 ml of Soluene 350 (Packard). The colored Soluene solution was decolorized with some drops of 30% hydrogen peroxide solution before the addition of scintillator, Aquasol (Packard). The intestine was digested in 0.5 mol/L NaOH solution under gentle reflux for

1 h. An aliquot of the alkaline solution was mixed with Aquasol for measurement of radioactivity in the intestine. A model LS 6000 LL scintillation spectrometer (Beckman) was used for determination of the radioactivity.

The pooled ^3H -urine was condensed and diluted with an equal amount of water. The diluted urine was extracted with butyl chloride of the same volume to determine the ratio of the conjugated and free forms of PCP metabolites. An aliquot of the above water-soluble fraction (non-butyl chloride extractable fraction) was mixed with 1 ml acetate buffer (pH 5.4) and incubated with 1 ml β -glucuronidase and 3 mg sulfatase for 24 h at 37°C , respectively. After reaction an aliquot of the incubation mixture was dissolved in 0.5 ml ethanol and subjected to the Bond-Elut cartridge (2.8 ml capacity) to isolate hydroxylated PCP metabolites. Analytical conditions for the identification of PCP and its metabolites were as the same as those in the previous paper¹⁷⁾.

2.3.2 Non-radioactive experiment

Five rats weighing about 200 g were used for identification of metabolites in brain. Each rat was orally given PCP-HCl as 0.5% aqueous solution at a dose of 5 mg/body. The animals

were sacrificed 24 h after the first dosage. Brain samples were combined and digested with 50 ml of 0.5 mol/L HCl under reflux. The digested brain sample was filtered and the filtrate was lyophilized. The lyophilizate was subjected to analysis of PCP metabolites. In this experiment a control rat was also used for comparison of metabolites in the brain.

3. Results and Discussion

After oral dosing of ^3H -PCP·HCl to rats PCP-derived radioactivity was excreted mainly in urine (ca. 60%) in which conjugates and free forms were detected in the ratio of 86% and 14%, respectively, and, to a lesser extent, in feces (ca. 10%) as shown in Fig. 2. Fecal and urinary excretion reached a plateau on Day 2 and 3, respectively. Residual radioactivity was excreted slowly for long time after dosing. This fact is accord with the result by Misra et al.¹³⁾, although the dose and given route of PCP were different from those of us. Table 1 shows the organ and tissue distribution, and concentration in rats. The concentration ratio of each organ or tissue to that of blood is shown in Table 2.

These data show that brain, target organ of

Fig. 2 Cumulative excretion of radioactivity after a single oral administration at a dose of ^3H -PCP·HCl 1 mg/rat. Vertical bars show S. D. of 3 rats.

Table 1 Organ distribution (%) of radioactivity and concentration (%/g) after a single oral administration of ^3H -PCP·HCl at a dose of 1 mg/body

Time(h)	4	8	12	24
Organs				
Brain	0.12±0.07 (0.077)	0.13±0.04 (0.071)	0.17±0.03 (0.097)	0.22±0.09 (0.13)
Thymus	0.04±0.02 (0.083)	0.02±0.01 (0.048)	0.04±0.02 (0.083)	0.03±0.01 (0.07)
Lung	0.21±0.14 (0.11)	0.11±0.09 (0.068)	0.02±0.44 (0.28)	0.11±0.02 (0.10)
Heart	0.01±0.00 (0.013)	0.08±0.03 (0.097)	0.09±0.04 (0.12)	0.11±0.01 (0.14)
Stomach	0.26±0.14 (0.20)	0.11±0.02 (0.081)	0.32±0.30 (0.22)	0.16±0.06 (0.13)
Liver	2.07±1.37 (0.30)	4.55±4.27 (0.48)	2.34±3.20 (0.30)	2.92±1.47 (0.38)
Spleen	0.09±0.05 (0.12)	0.12±0.05 (0.18)	0.07±0.04 (0.10)	0.05±0.02 (0.098)
Kidney	0.50±0.20 (0.32)	0.32±0.04 (0.19)	0.32±0.06 (0.20)	0.28±0.05 (0.19)
Intestine	9.49±3.84 (0.73)	9.82±2.20 (0.70)	10.96±5.15 (0.86)	4.20±1.10 (0.38)
Testis	0.22±0.08 (0.12)	0.16±0.12 (0.067)	0.28±0.04 (0.13)	0.31±0.15 (0.13)
Prostate	0.03* (0.24)	2.84±4.88 (0.23)	0.02±0.01 (0.20)	0.02±0.01 (0.18)
Muscle	7.43* (0.10)	11.0* (0.14)	8.12* (0.012)	6.19* (0.0075)
Adipose	1.22±0.64 (0.045)	0.20±0.36 (0.0077)	0.34±0.16 (0.012)	0.28±0.12 (0.0099)
Blood	1.09±0.26 (0.075)	0.92±0.32 (0.061)	1.20±0.30 (0.079)	2.33±0.71 (0.15)

The values are mean data ± S.D. for three rats and numbers in the parentheses indicate concentrations. a) Intestine contains its contents. b) Assumes 40% of body weight to be muscle. c) Assumes 14% of body weight to be muscle. d) Assumes 7.69% of body weight to be blood. *The mean of two rats.

PCP, did not deposit so high label compared with other organs and tissues. It can be said in general that level in liver was highest and that in prostate was the second highest during the first 24 h. Low level radioactivity in adipose tissue may reflect that more hydrophilic metabolites of PCP and their conjugates were not deposited in the tissue, since PCP itself is very lipophilic attribute which shows high affinity for fatty tissues. In fact the above result

is supported by our experimental data that only a small amount (0.3%) of unchanged PCP was detected in the pooled urine. Main urinary metabolites of PCP were conjugated products of hydroxylated PCP derivatives, because PCP metabolites, PCHP, PPC and PCP-diol were detected after enzymatic or acid hydrolysis of conjugated fractions. Most conjugates were found to be glucuronides as a result of enzyme hydrolysis. PCA was not detected in urine

under the present condition.

Detailed studies on urinary metabolites of PCP as well as separation of isomeric metabolites, *cis*- and *trans*-PPC were previously reported by the author's group¹⁶⁾⁻¹⁸⁾.

Heart and brain were lower levels than blood

Table 2 Concentration ratio of organs to that of blood after oral dosing of ³H-PCP·HCl into rats

Time (h)	4	8	12	24
Organs				
Brain	1.0	0.8	1.2	0.9
Thymus	1.1	0.8	1.0	0.5
Lung	1.5	1.1	3.5	0.9
Heart	0.2	1.6	1.5	0.9
Stomach	2.7	1.3	2.8	0.9
Liver	4.0	7.9	3.8	2.5
Spleen	1.6	3.0	1.3	0.7
Kidney	4.3	3.1	2.5	1.3
Intestine	9.7	11.5	10.9	2.5
Testis	1.6	1.1	1.6	0.9
Prostate	3.2	3.8	2.5	1.2
Adipose	0.6	0.1	0.2	0.1
Muscle	1.3	2.3	1.2	0.5

Values are calculated according to the following equation:

Concentration ratio = organ level (%/g) / blood level (%/g).

at 4 h after dose. PCP alone was identified in the brain by GC-MS under our experimental conditions. The gas chromatogram/SIM of PCP is shown in Fig. 3. The peak had a retention time identical with that of the authentic sample of PCP under the known analytical conditions¹⁶⁾.

Microautoradiographic studies using labeled PCP with high specific activity would be useful to know exactly the site such as hippocampus in which the PCP receptor is located. This time other metabolites were not detected probably because of their low level in organs and tissues.

At present neurochemical effects of PCP are not well defined, although it is supposed that the serotonergic, opioidergic and GABAergic neuronal systems may regulate the dopaminergic system in the development of PCP actions²⁾. PCP is a dangerous drug from the point of forensic science, but very interesting tool from the view point of brain research.

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