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Regional Distribution and Elimination Kinetics of Imipramine in Rat Brain after a Single Intraperitoneal Administration

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The elimination of imipramine (IMP) from the brain regions was investigated in rats after a single 5 mg/kg or 20 mg/kg intraperitoneal administration. At both 5 mg/kg and 20 mg/kg IMP injection, the brain regions could be divided into two classes in terms of IMP elimination. IMP levels in plasma and class A brain regions (cerebellum, mid-brain and medulla, striatum, posterior cortex and frontal cortex) declined according to a monoexponential profile with the same elimination rate constant and the time courses only shifted in parallel depending on the dose. On the other hand, the disappearance of IMP in class B brain regions (hypothalamus, thalamus, hippocampus and nucleus accumbens) followed a biexponential profile. The initial phase was similar to that in the class A brain region at each dose. However, the later phase of the curves was identical regardless of the dose. It is suggested that the class B brain region may possess the high-affinity and low-capacity binding site for IMP.

Keywords——imipramine; rate brain region; elimination; distribution; pharmacokinetics; intraperitoneal dose

Introduction

The tricyclic antidepressant, imipramine (IMP), is commonly used for the treatment of depression. Studies on tissue distribution and pharmacokinetics of IMP[1-7] seem to be important for understanding the mechanism of action. Also, the brain is a target organ of IMP, so the elimination pattern is pharmacodynamically important. However, there is little information available on elimination of IMP from the brain regions. On the other hand, many workers have demonstrated the presence of high-affinity binding sites for IMP in rat brain by *in vitro* experiments.[8-14] However, the role of the IMP binding sites in the pharmacological action is not known yet. In the course of studies on the disappearance and binding of psychotropic drugs from various brain regions in rats, we found[15] that the brain regions can be divided into two classes regarding IMP elimination.

In this study, we investigated the variation of the regional distribution and elimination kinetics of IMP in rat brain after a single intraperitoneal (i.p.) administration at two different doses (5 mg/kg and 20 mg/kg). Further, we discuss whether or not, the IMP level in each region is affected by blood remaining there.

Materials and Methods

IMP hydrochloride was obtained from Ciba-Geigy (Japan) Limited (Takarazuka, Japan). All other chemicals were analytical grade products available commercially.

Male Wistar rats, weighing 200—230 g were dosed i.p. with IMP (5 or 20 mg/kg). At various intervals after the administration (5 mg/kg: 0.25, 0.5, 1, 2, 4, 8 and 12 h. 20 mg/kg: 1, 2, 4, 8, 12, 16, 20 and 24 h), the animals were sacrificed. Blood samples were collected in heparinized tubes and the plasma was stored at −30 °C. The brain was quickly removed and dissected into 9 regions according to the technique of Glowinski and Iversen.[16] These regions were cerebellum, mid-brain and medulla, hypothalamus, thalamus, striatum, hippocampus, posterior cortex, nucleus accumbens and frontal cortex. After being weighed, the brain regions were also stored at −30 °C until analysis. The analytical method for IMP was described in our previous paper.[15]

The blood volume in the brain regions was determined by the use of ^{14}C-inulin. Male Wistar rats, weighing 200—230 g were dosed intravenously with ^{14}C-inulin-carboxyl (New England Nuclear, 10.4 μCi/rat). At 15 min after the injection, the animals were sacrificed. Blood samples were collected, and the brain was quickly removed and dissected into 9 regions. Each brain region was homogenized in saline solution. The brain homogenates and blood were placed in scintillation vials and dissolved in 1 ml of Soluene 350 (Packard). After the materials had been completely dissolved, 10 ml of scintillation fluid (Aquasol II®, New England Nuclear) was added to each vial. Radioactivities were measured by using a liquid scintillation counter (Aloka, LSC-651) and the amount of ^{14}C-inulin in the samples was calculated.

Results and Discussion

After the injection of a low dose (5 mg/kg) or high dose (20 mg/kg) of IMP into rats, the time courses of IMP concentration in plasma and in 9 brain regions (cerebellum, mid-brain and medulla, striatum, posterior cortex, frontal cortex, hypothalamus, thalamus, hippocampus and nucleus accumbens) were examined. The results for plasma, the first 5 brain regions (termed class A) and the last 4 brain regions (termed class B) are shown in Figs. 1, 2 and 3 at the low dose, and Figs. 4, 5 and 6 at the high dose, respectively. Also, it is necessary to investigate the influence of IMP in localized blood on the level in each brain region. The blood volume in brain regions as calculated from the inulin space, the IMP levels in brain regions at 4 h after a single i.p. 5 mg/kg dose, the IMP levels originating from local blood in each brain region and the ratios of the IMP level of localized blood to the apparent IMP level in the brain region are shown in Table I. In all 9 brain regions, the IMP levels originating from localized blood in the brain regions were less than 0.4% of apparent IMP levels in the brain region, that is, the determination of IMP levels in brain regions would not be significantly influenced by the blood remaining in any brain region. Consequently, the determination of IMP levels in

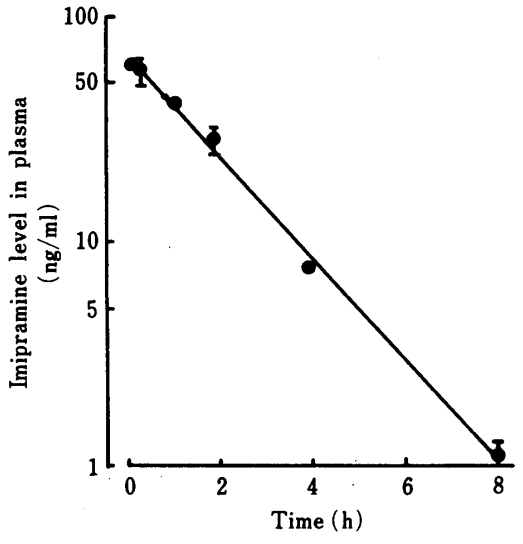

Fig. 1. Time Course of Imipramine Plasma Level (mean ± S.E.) after i.p. Administration of 5 mg/kg Imipramine

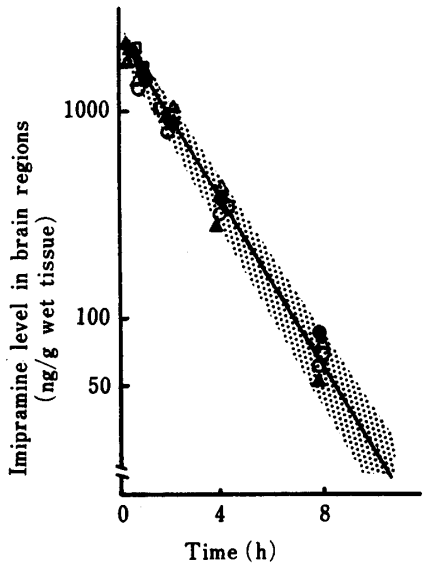

Fig. 2. Time Course of Imipramine Levels Area (mean ± S.E.) in Class A Brain Regions after i.p. Administration of 5 mg/kg Imipramine

○, cerebellum; ●, mid-brain and medulla; △, striatum; ▲, posterior cortex; □, frontal cortex.

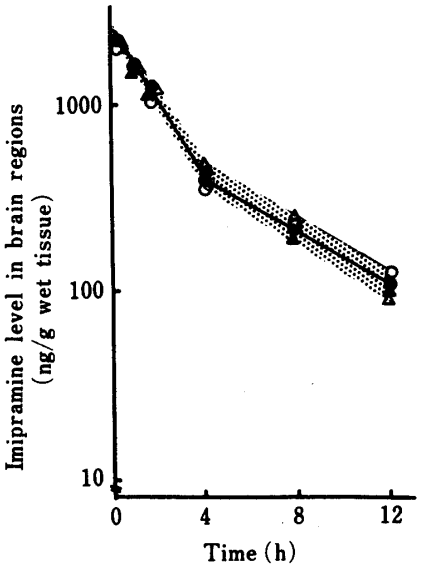

Fig. 3. Time Course of Imipramine Levels Area (mean ± S.E.) in Class B Brain Regions after i.p. Administration of 5 mg/kg Imipramine

○, hypothalamus; ●, thalamus; △, hippocampus; ▲, nucleus accumbens.

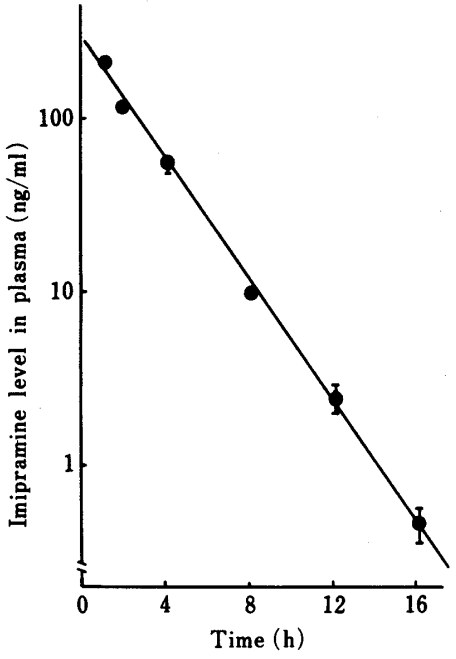

Fig. 4. Time Course of Imipramine Plasma Level (mean ± S.E.) after i.p. Administration of 20 mg/kg Imipramine

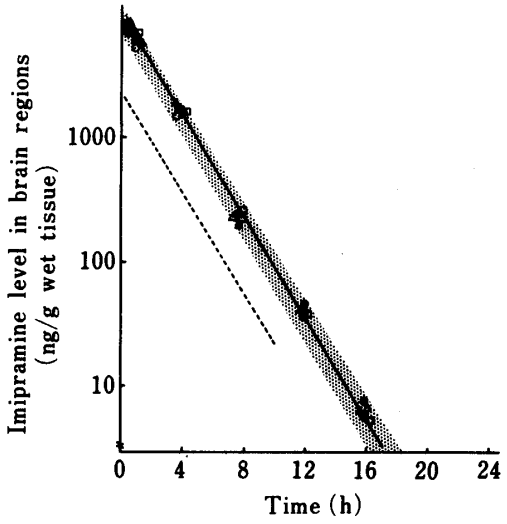

Fig. 5. Time Course of Imipramine Levels Area (mean ± S.E.) in Class A Brain Regions after i.p. Administration of 20 mg/kg Imipramine

○, cerebellum; ●, mid-brain and medulla; △, striatum; ▲, posterior cortex; □, frontal cortex. The dotted line represents the result shown in Fig. 2.

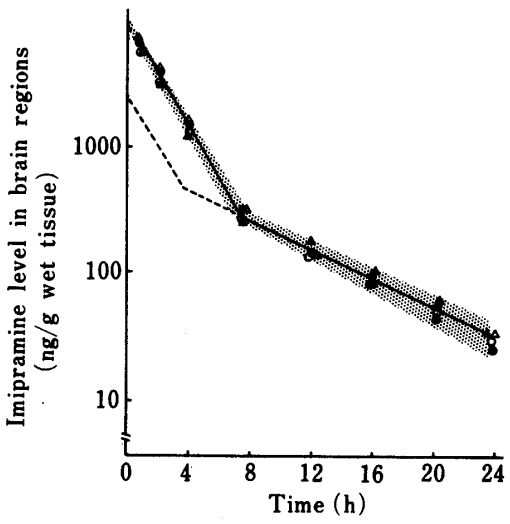

Fig. 6. Time Course of Imipramine Levels Area (mean ± S.E.) in Class B Brain Regions after i.p. Administration of 20 mg/kg Imipramine

○, hypothalamus; ●, thalamus; △, hippocampus; ▲, nucleus accumbens. The dotted line represents the result shown in Fig. 3.

brain regions was done without dehematization of the tissue. Each value represents the mean of 5 animals in all the experiments.

As shown in Figs. 1—6, the brain regions can be divided into two classes regarding IMP disposal *in vivo* at both the low and high doses. Under these experimental conditions, IMP plasma levels fell rapidly following a monoexponential profile with elimination rate constants

TABLE I. The Ratios of IMP Levels of Blood Origin to IMP Levels in the Brain Regions

Brain region	Blood volume in tissue (μl/g)	Drug conc. in tissue (ng/g)	Drug conc. of blood origin in tissue (ng/g)	Blood to tissue drug conc. (%)
Cerebellum	73.0	292.5	0.87	0.30
Mid-brain and medulla	62.2	349.2	0.74	0.21
Hipothalamus	99.4	354.2	1.18	0.33
Thalamus	59.7	385.8	0.71	0.18
Striatum	42.3	363.8	0.50	0.14
Hippocampus	55.9	461.3	0.67	0.15
Postreior cortex	72.5	244.8	0.86	0.35
Nucleus accumbens	109.0	468.2	1.30	0.28
Frontal cortex	65.4	339.8	0.78	0.23
Plasma	—	11.9 (ng/ml)	—	—

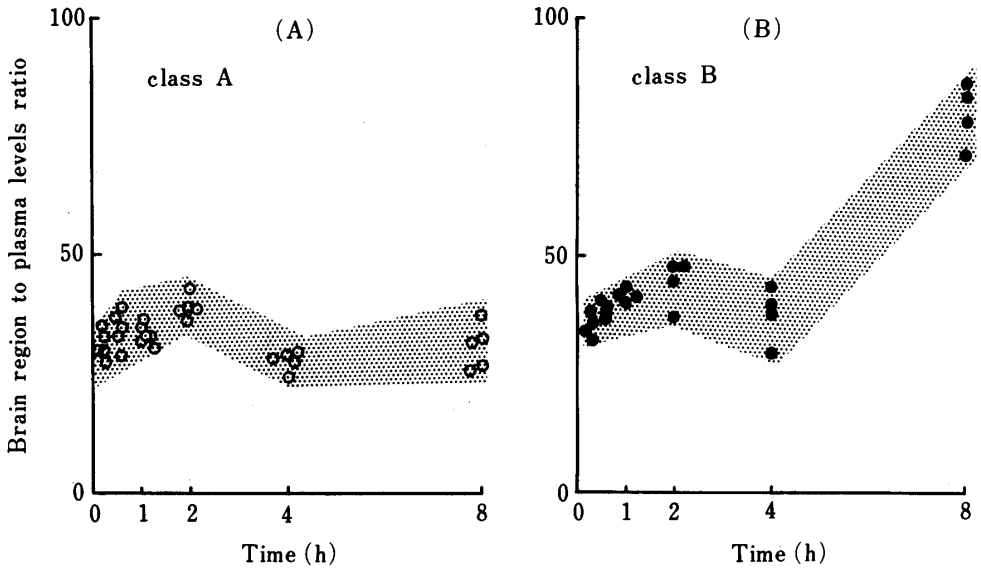

Fig. 7. Ratios of Imipramine Levels in Brain Regions ((A), Class A; (B), Class B) to Plasma Level after i.p. Administration of 5 mg/kg Imipramine

of 0.52 h^{-1} at the low dose (Fig. 1) and 0.48 h^{-1} at the high dose (Fig. 4). The elimination rate constant at the low dose was similar to that at the high dose.

Figure 7 shows the ratios of IMP levels in brain regions to that in plasma after a single 5 mg/kg i.p. dose. In the case of class A brain regions (Fig. 7(A)) the ratios were constant at about 30—40, indicating marked accumulation in the brain. On the other hand, while the ratios in class B brain regions (Fig. 7(B)) were constant at about 30—40, as in class A regions, in the initial period after the administration, the IMP levels in class B brain regions reached approximately 75 times the plasma level in the later period. The ratios at the high dose were the same as those at the low dose. The results suggest that the class B brain regions may possess a specific binding site for IMP.

IMP levels in class A brain regions showed monoexponential elimination similar to that of the plasma level, and the elimination rate constants were 0.46 and 0.47 h^{-1} at the low (Fig. 2) and high (Fig. 5) doses, respectively. The elimination rate constants were thus essentially identical at the low and high doses. In addition, at the 4-fold higher dose, IMP levels in the

plasma and the class A brain regions were only shifted in parallel to about 4-fold higher levels. The relationship between the plasma and class A brain regions levels was linear within these dose ranges.

On the other hand, the disappearance curve of IMP in class B brain regions followed a biexponential profile and could be resolved into two major components at both low (Fig. 3) and high (Fig. 6) doses. The initial phase (α-phase) of the curves in class B brain regions at each dose was similar to those in class A brain regions. The α-phase of the curve for the class B brain regions was also shifted in parallel depending on the dose. In contrast, in the later phase (β-phase), IMP levels were cleared more slowly from class B brain regions with elimination rate constants of 0.14 and 0.14 h^{-1} at the low and high doses, respectively. The elimination rate constant of β-phase was thus identical at the two doses. IMP levels in class B brain regions were maintained at around 100 ng/g wet tissue even at 12 h after administration at either dose. The initial concentration of the β-phase ($C_{0\beta}$) was 0.60 μg/g wet tissue for both doses. The only difference between the low and high doses in the β-phase of the curves in class B brain regions was in the starting points, which appeared at 3—4 h at the low dose and at 7—8 h at the high dose after the i.p. administration. The β-phase of the curve at low dose was essentially compatible with that at high dose. It is interesting that the IMP levels of the β-phase of the class B brain regions were unchanged regardless of the dose. Class B brain regions must be different from class A regions in regard to the binding affinity for IMP. That is to say, class B brain regions may possess the high-affinity and low-capacity binding site for IMP as judged from the behavior of the β-phase. The specific high-affinity sites for IMP shown by *in vitro* experiments[8-14] can be considered to be effective, but relatively little is known about binding in the brain regions. We have already shown that the class B brain regions possess the high-affinity and low-capacity binding site for IMP by *in vitro* binding experiments[17] (data not shown). The relationships between the behavior of IMP in the class B brain regions *in vivo* and the high-affinity and low-capacity binding site *in vitro* experiment are being investigated.

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