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Note

High-performance liquid chromatographic determination of imipramine and its metabolites in rat brain

SOTARO SUGITA, AKIMARO KOBAYASHI, SHIGERU SUZUKI, TOSHIHIRO YOSHIDA and KINYA NAKAZAWA*

Department of Neuropsychiatry, Aichi Medical College, Yazako, Nagakute-cho, Aichi-gun, Aichi 480-11 (Japan)

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It has recently been reported from several laboratories [1–6] that the pharmacokinetics of antidepressant drugs and their metabolites in the brain are observed to be markedly different from those in blood. This suggests that pharmacokinetic studies on antidepressant drugs with the active metabolites in the brain might be essential for elucidation of their actions on the central nervous system. The present studies were carried out to establish a laboratory method capable of assaying simultaneously imipramine, desipramine and their 2-hydroxy compounds in the brain by high-performance liquid chromatography (HPLC). Although several laboratories [7–15] have already presented HPLC methods for quantitative determination of imipramine and its metabolites in blood, these methods appeared in our hands to be unsuitable for brain samples, because a major portion of imipramine and its derivatives was readily coprecipitated with macromolecules following the addition of acid. Attempts to prevent such coprecipitation by addition of an excess of an organic solvent, such as methanol, to the homogenization medium caused a marked increase of unidentified interfering peaks in the chromatogram. The present chromatographic quantitative analyses of the drug and metabolites in brain were reproducible with good recoveries. The pharmacokinetic differences between the serum and brain in rats receiving imipramine are briefly described.

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EXPERIMENTAL

HPLC conditions

The equipment used for HPLC was a Toyo Soda (Tokyo, Japan) Model CCPD solvent-delivery system with a Rheodyne injector, Model CP-8000 computing integrator and Yanagimoto (Kyoto, Japan) Model M-315 spectrometer fixed at 254 nm. A reversed-phase column, Cosmosil $5C_{18}$ column (Nakarai, Kyoto, Japan; 25 cm × 4.6 mm I.D.; particle size 5 μm) was employed at 40°C. The operating conditions were follows: mobile phase, 1% triethylamine (pH 3.0, adjusted with phosphoric acid)–methanol–tetrahydrofuran (88:45:17) at a flow-rate of 1 ml/min; injection volume, 100 μl; detector sensitivity, 0.005 a.u.f.s.

Sample preparations

Male Wistar rats weighing ca. 300 g were used. Food and water were provided ad libitum. They were intraperitoneally injected with imipramine (20 mg/kg), and then decapitated at arbitrary times. The trunk blood was collected from the severed cervical vessels, and the serum was conventionally prepared by centrifugation and kept frozen at −20°C until the following procedures were carried out. To the serum (1 ml) were added 800 ng of nortriptyline as an internal standard, followed by 4 ml of methanol and 5 ml of 2.5% perchloric acid. This mixture was vigorously shaken. After centrifugation at 11 000 *g* for 15 min, the resulting supernatant was added to 1 ml of 4 *M* potassium hydroxide and centrifuged again. The resultant supernatant (9 ml) was shaken with 10 ml of diethyl ether–ethyl acetate (85:15) for 15 min. An aliquot (8 ml) of the organic phase was evaporated under a stream of nitrogen gas. The residue was dissolved in 200 μl of 1% triethylamine (pH 2.0, adjusted with phosphoric acid)–methanol (9:1), and an aliquot (100 μl) was injected into the HPLC system.

The whole brain (ca. 2 g) was rapidly removed, weighed and homogenized in 10 volumes of 2.5% perchloric acid and 4 volumes of methanol with nortriptyline (800 ng/g of tissue) in a Polytron homogenizer. After the homogenate was centrifuged at 11 000 *g* for 15 min, the supernatant was added to 4 ml of 4 *M* potassium hydroxide and centrifuged again. The resultant supernatant was shaken with 20 ml of diethyl ether–ethyl acetate (3:1) for 15 min. An aliquot (8 ml) of the organic phase was evaporated and the residue was dissolved in 200 μl of the same solution as described above for serum samples, followed by injection into the HPLC system.

Materials

Imipramine, 2-hydroxyimipramine and 2-hydroxydesipramine were donated by J.R. Geigy (Basle, Switzerland). Desipramine and nortriptyline were given by Dainihon Pharmaceutical (Osaka, Japan). Tetrahydrofuran, methanol and triethylamine, which were prepared for HPLC, were obtained from Nakarai. The conventional distilled water was further purified by a Milli-Q water purification system for HPLC.

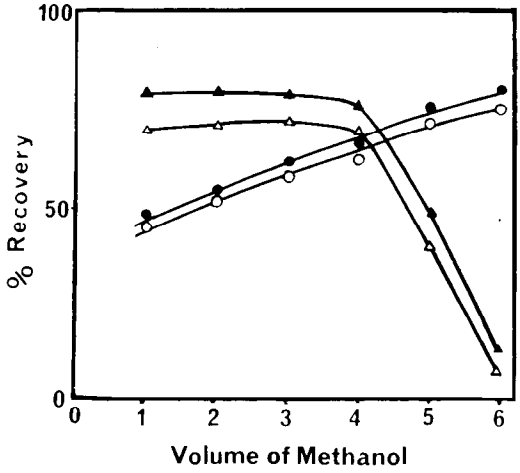

Fig. 1. Effect of methanol on the recoveries of imipramine (●), desipramine (○), 2-hydroxyimipramine (▲) and 2-hydroxydesipramine (△). In their presence (each 50 μg/g of tissue) brain tissue was homogenized with various volumes of methanol and 10 volumes of 2.5% perchloric acid. As described in Experimental, the homogenate was centrifuged and the supernatant was added to potassium hydroxide, followed by centrifugation. An aliquot of the resulting supernatant was injected into the HPLC system, and the recovery of each compound was calculated.

RESULTS AND DISCUSSION

As rat brain was homogenized with a few volumes of 6% perchloric acid in the presence of imipramine and its metabolites, almost 100% of the compounds was coprecipitated with the macromolecular fraction. When 10 volumes of 2.5% perchloric acid were used as the homogenization medium, the recoveries of imipramine and desipramine into the supernatant fraction following the removal of the macromolecular fraction were less than 40%, whereas the recoveries of the 2-hydroxy compounds were 70–80%. Furthermore, methanol was added to the medium with 10 volumes of the acid at the lower concentration. The recoveries of imipramine and desipramine depended on the volume of methanol in the medium, as shown in Fig. 1, but more than 5 volumes of methanol caused marked loss of the hydroxy compounds with the appearance of many unidentified peaks in the chromatogram. Such loss was observed to be caused by coprecipitation not with the macromolecular fraction, but with the precipitant produced by the addition of potassium hydroxide. Therefore, for brain samples, 4 volumes of methanol were used with 10 volumes of 2.5% perchloric acid as the homogenization medium. In addition, the proportion of ethyl acetate in extraction medium and pH of the buffer in which the residue was dissolved after evaporation of the organic phase were both crucial to obtain good recoveries for quantitative analyses. Consequently, the total recoveries for imipramine and desipramine were 45–50% in the present determinations, whereas the recoveries for the hydroxy compounds were 50–55%.

Calibration curves were produced as follows. Working solutions were prepared by homogenizing rat brain (2 g) in the medium containing 20–1600 ng of each

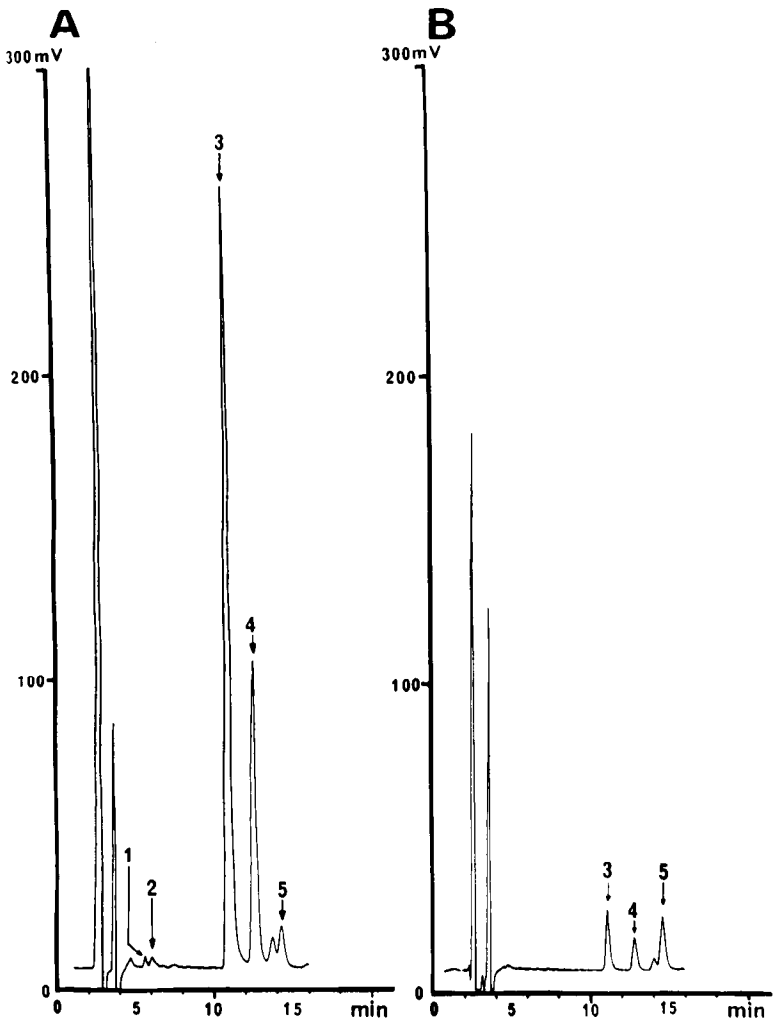

Fig. 2. Chromatograms of the brain (A) and serum (B) from a rat receiving a single intraperitoneal injection of imipramine. Detailed experimental conditions are described in the text. The arrows at 1, 2, 3, 4 and 5 indicate the peaks of 2-hydroxyimipramine, 2-hydroxydesipramine, imipramine, desipramine and nortriptyline, respectively. The retention time were 5.2, 6.2, 11.2, 12.3 and 14.2 min, respectively.

imipramine derivative together with 1600 ng of nortriptyline. These solutions were processed by the HPLC method described in Experimental. The ratios of the peak area of each compound to the peak area of nortriptyline in the working solution were plotted versus the concentrations of imipramine and the metabolites. The curves exhibited linear relationships over the concentration range (10–800 ng/g of tissue), with each detection limit being ca. 10 ng/g of tissue. The reproducibilities of the calibration curves were determined by performing replicate analyses on each homogenate from eight rat brains. At four different concentrations (50, 100, 200 and 500 ng/g of tissue) of imipramine derivatives, the

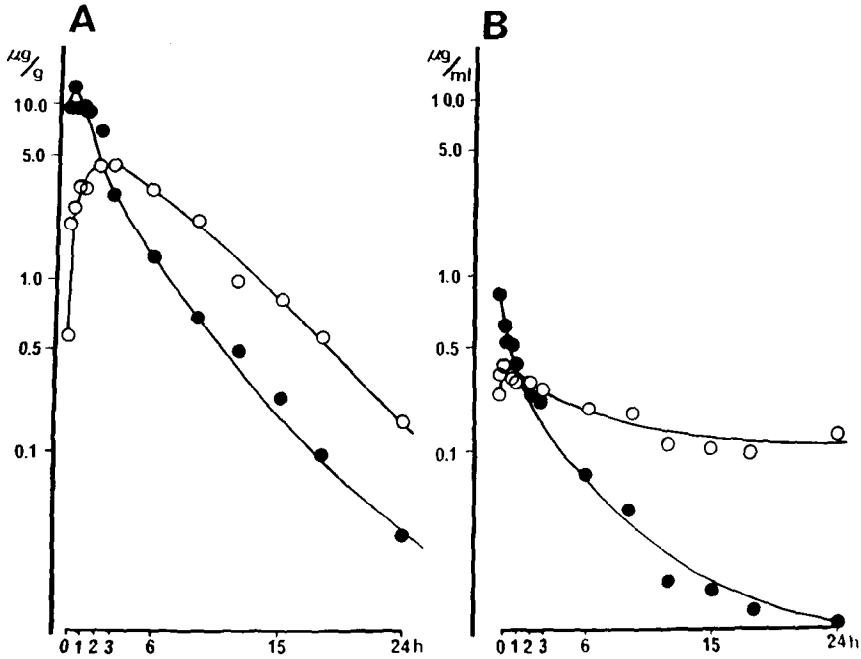

Fig. 3. The concentrations of imipramine (●) and desipramine (○) in brain (A) and serum (B) after a single intraperitoneal injection of imipramine. Detailed experimental conditions are described in the text. Each data point is the mean of five animals, and the standard deviations are less than 10%.

coefficients of variation (C.V.) were between 1.9 and 9.5% for imipramine and desipramine, and between 6.0 and 9.7% for the hydroxy compounds. For quantitative determinations with better reproducibility, the use of a separate internal standard for the tertiary amines and the hydroxy metabolites might be needed.

Rats were intraperitoneally injected with 20 mg/kg imipramine and then sacrificed 1 h after the injection. As described in Experimental, the serum and brain were removed, and HPLC samples were prepared with nortriptyline. In the chromatograms of the brain and serum samples the peaks derived from each compound were distinguishable as sharp and symmetrical peaks. The results showed that the concentrations of imipramine and desipramine were much higher in brain than in serum (Fig. 2); the ratio of the brain level (ng/g tissue) of the drug to the serum level (ng/ml) was ca. 20. On the other hand, the concentrations of the 2-hydroxy compounds were less than 10 ng/g of tissue in the brain, and not detectable in the serum. However, when rats were given 100 mg/kg imipramine, the level of 2-hydroxyimipramine in the brain and serum was 182 ng/g of tissue and 20 ng/ml, whereas that of 2-hydroxydesipramine was 29 ng/g of tissue and 31 ng/ml, respectively. Such limited hydroxylation of imipramine and desipramine is consistent with studies of the enzymic conversion of imipramine by rat microsomal fraction [16,17].

After rats received a single intraperitoneal injection of the drug (20 mg/kg) and were sacrificed at various time intervals, imipramine as well as desipramine

in the brains and sera was quantitatively determined (Fig. 3). The time required to achieve the maximal concentrations (C_{max}) of imipramine and desipramine in brain was 15 min and 2 h, respectively, whereas the time for C_{max} of the latter compound in serum was 30 min. As mentioned above, the levels of imipramine and its metabolite in brain were observed to be much higher than those in serum. The semilogarithmic plots of time curves for imipramine in both tissues, as well as for desipramine in serum, showed some deviations from linearity, indicating that the pharmacokinetics followed the equations for a multi-compartment system. The plot for the metabolite in brain, however, appeared to show linearity after the maximal level was attained.

Using the second decay (β) phase in Fig. 3, the elimination half-lives of both compounds were calculated. The half-lives for imipramine and desipramine were 3.6 and 10.1 h in serum, and 3.5 and 4.3 h in brain, respectively. These observations on imipramine are in good agreement with the findings reported by other laboratories [1,2], although our data on desipramine are incompatible with theirs, which demonstrate that the high level of desipramine in brain does not appear to be reduced for more than 20 h, following its maximal level. This discrepancy might be due to the experimental conditions, since DeVane et al. [2] used pregnant rats and Barkai et al. [1] do not seem to have examined the recoveries of imipramine and desipramine from brain homogenate. However, the observations described by Hrdina and Dubas [3], where rats were administered desipramine itself, appear to be in good agreement with ours.

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