The Physiological Disposition of Chlorpromazine in the Rat and Dog (34784)

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Previous studies of the physiological disposition of chlorpromazine have reported concentrations in tissues and plasma of laboratory animals after high doses (1–4), but not after doses comparable with those used in behavioral studies in animals (5, 6) and clinically in man (7). This laboratory has developed a new method which is sensitive enough to measure low concentrations (8). The present paper compares concentrations in plasma of rats during the first 24 hr after intravenous and intraperitoneal injections. Concentrations in liver, brain, and muscle are compared with the plasma levels during the same period after intraperitoneal injection in rats. In dogs, chlorpromazine levels are followed for 7 hr after the intravenous administration of three different doses, all in the range commonly used in pharmacologic studies.

Methods and Materials. Male Sprague-Dawley rats (150–200 g) were injected intravenously (tail vein) or intraperitoneally with chlorpromazine hydrochloride (10 mg/kg in 0.85% NaCl). At suitable intervals, the rats were anesthetized lightly with ether, and blood (up to 7 ml) was drawn by cardiac puncture and placed in tubes containing 0.1 ml of 5% sodium citrate solution. Tissues were removed immediately, frozen on Dry Ice, and stored frozen. In preparation for assays, the frozen tissues were thawed and homogenized with 9 vol of 0.05 *N* HCl in a Sorvall Omni-mixer.

Male beagle dogs (11–18 kg, 4–5 years old) were given chlorpromazine hydrochloride (1, 3, and 10 mg/kg in 0.85% NaCl) intravenously (saphenous vein). Blood was collected from the jugular vein through an indwelling polyethylene catheter into vacuum tubes containing potassium oxalate.

Nonradioactive chlorpromazine was assayed by gas chromatography (8). For tissue distribution studies, ^{35}S-chlorpromazine was used (Nuclear-Chicago). When these studies were begun, the specific activity of the chlorpromazine was 12.7 mCi/mmole but it had decreased to 1.5 mCi/mmole (approx 3 half-lives) by the end of the studies. Total radioactivity in plasma and homogenates of tissues from animals injected with ^{35}S-chlorpromazine was determined by direct sampling of 0.1–0.5 ml of the solution. In studies that required the specific assay of chlorpromazine, the following solvent extraction procedure was used. A sample (1–5 ml) of plasma or tissue homogenate was added to 1 ml of 5% NaOH and 5–10 ml of *n*-heptane containing 1.5% isoamyl alcohol in a 20-ml screw-capped polycarbonate centrifuge tube and the aqueous volume was adjusted to 6 ml. The tube was shaken for 30 min and then centrifuged. An aliquot of the heptane layer was transferred to a second polycarbonate tube, shaken with an equal volume of 0.1 *M* acetate buffer (pH 4.7) for 15 min, and the mixture was then centrifuged. The heptane layer was assayed for radioactivity by liquid scintillation spectrometry.

The radioactivity in the heptane layer was shown to exist only in chlorpromazine by the technique of comparative distribution ratios (9). Thus, the radioactivity in the heptane extract, after washing with buffer solution, (pH 4.7) distributed between *n*-heptane containing 1.5% isoamyl alcohol and buffer solutions with partition coefficients similar to

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those for the distribution of authentic chlorpromazine (Table I). Recovery, by the above extraction procedure, of chlorpromazine added to plasma or tissue homogenates was 72 ± 2%.

Radioactivity measurements were expressed as chlorpromazine hydrochloride by comparison with the radioactivity of chlorpromazine hydrochloride solutions of known strength and specific activity. Quenching corrections were made by adding known quantities of ^{35}S-chlorpromazine to the counting vials and redetermining the radioactivity. The liquid scintillation fluid consisted of toluene containing 2,5-bis[5′-*tert*-butylbenzoxazoly (2′)]-theophene (BBOT), 0.4%; naphthalene, 0.8%; and methylcellosolve, 40%.

The gas-chromatographic method accurately analyzed chlorpromazine added to plasma, with a recovery of 100% and a standard deviation of 6%. Recovery of radioactive chlorpromazine added to plasma and extracted into *n*-heptane containing 1.5% isoamyl alcohol, omitting the buffer wash, was the same, also with a standard deviation of 6%. Washing the extracts with a buffer solution at pH 4.7 removed 28% of the chlorpromazine. This last procedure was introduced to increase specificity, since certain metabolites of chlorpromazine are heptane-extractable (8). In previous studies, a buffer wash of pH 5.6 was used (2); this resulted in higher recovery, but it was recently shown that this procedure only partially removed demonomethylchlorpromazine from the heptane layer (8).

TABLE I. Distribution of Apparent Chlorpromazine Extracted from Plasma, and Authentic Chlorpromazine, Between *n*-Heptane Containing 1.5% Isoamyl Alcohol and Various Solutions of Controlled pH Values.

The extract was prepared as described under Methods; aliquots were shaken with equal volumes of the aqueous solutions, and the concentration of the radioactivity extracted into the aqueous phase was expressed as a percentage of the radioactivity originally present in the aliquot of the extract. The standard (authentic chlorpromazine) was prepared by dissolving chlorpromazine hydrochloride in water, totally extracting the material into *n*-heptane containing 1.5% isoamyl alcohol, and treating aliquots of the extract as described for the test material.

Solution	pH	Percentage extracted: Authentic chlorpromazine	Percentage extracted: Test material
0.01 *N* HCl	2.2	100	100
0.1 *M* Phthalate buffer	3.5	95	96
0.1 *M* Acetate buffer	4.0	63	65
	4.8	19	20
	5.6	0	0

Because of high concentrations and large samples of plasma obtained in dogs, multiple assays (2–4) of chlorpromazine were usually made with samples from this species. The range on such multiple assays was larger than that on multiple assays of standard solutions of chlorpromazine added to plasma. For each point on the graph of plasma chlorpromazine levels in dogs (Fig. 4), the multiple assays on each plasma sample were averaged, and the mean of the averages obtained with three dogs was determined.

Results. Plasma chlorpromazine levels after iv and ip doses. In rats, plasma chlorpromazine concentrations were comparable after intravenous and intraperitoneal injections (Fig. 1). The similarity in the CPZ plasma

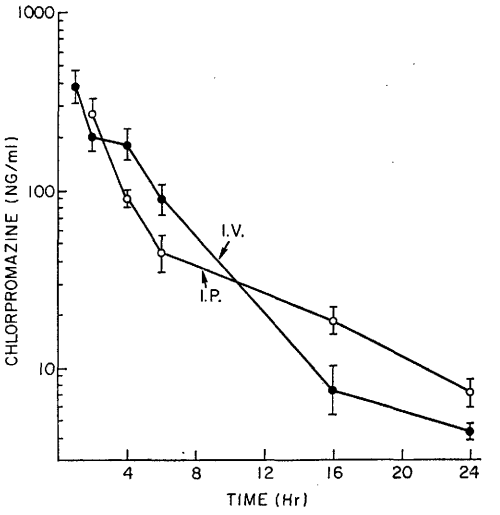

FIG. 1. Concentrations of chlorpromazine in the plasma of rats given 10 mg/kg, iv (●); and ip (○); each point is the mean from 10 rats; limits are ± SE.

levels indicated that the intraperitoneal dose was absorbed into the general circulation rapidly.

Chlorpromazine in tissues. Chlorpromazine concentrations after intraperitoneal doses in rats declined steadily in liver, brain, muscle, and plasma (Fig. 2). Concentrations in liver and brain greatly exceeded plasma levels, with liver/plasma and brain/plasma ratios greater than 14 during the period 2 to 6 hr after administration. In contrast, muscle/plasma ratios never exceeded 10 during this time period.

Chlorpromazine metabolites in tissues. In rats injected with ^{35}S-chlorpromazine, concentrations of total radioactivity in plasma, muscle, brain and liver declined as shown in Fig. 3. At all times, liver concentrations of radioactivity were much greater than the concentrations of radioactivity in brain, muscle, or plasma. During the first 6 hr, concentrations of radioactivity were also much greater in brain and muscle than in plasma. At 16 and 24 hr, however, brain and plasma levels of

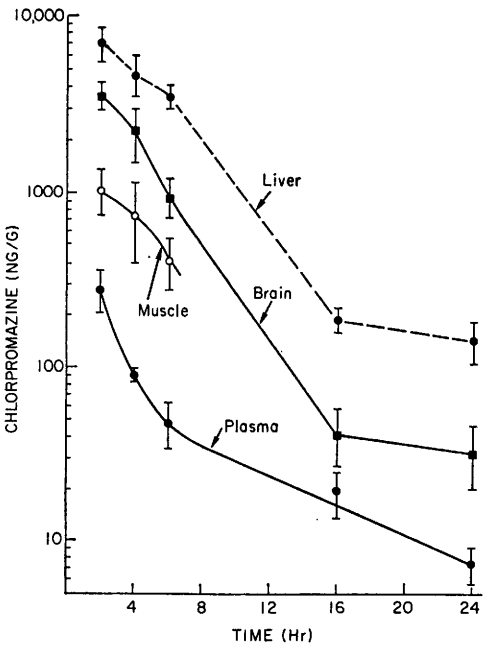

FIG. 2. Concentrations of chlorpromazine in plasma (●); brain (■); liver (●); and muscle (O) of rats given 10 mg/kg, ip. Values are means of 10 rats at each point ± SE.

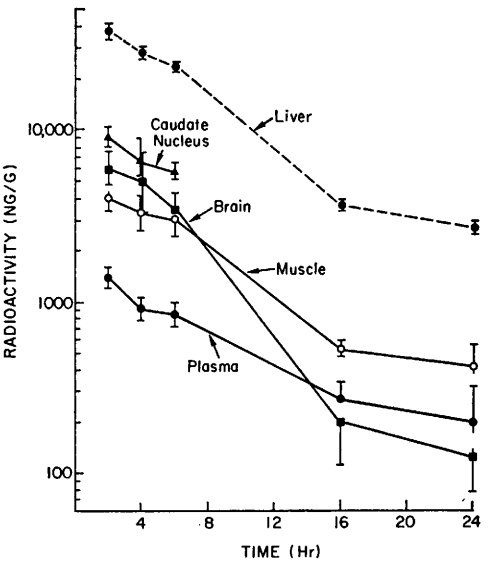

FIG. 3. Concentrations of total radioactive material (calculated as chlorpromazine hydrochloride) in plasma (●); brain (■); liver (●); muscle (O), and caudate nucleus (▲) of rats given 10 mg/kg, ip. Values are means of 10 rats at each point, ± SE.

radioactivity did not differ significantly. This reflects a more rapid decline of radioactivity in brain than in plasma, liver, and muscle. This presumably resulted from the formation of chlorpromazine metabolites, which failed to cross the blood–brain barrier and accumulated in plasma, liver, and muscle.

Radioactivity in the caudate nucleus was measured separately from the whole brain at 2, 4, and 6 hr because of the possibility of preferential uptake of chlorpromazine or its metabolites into basal ganglia; this hypothesis was proposed on the assumption that chlorpromazine or its metabolites might have a high affinity for sites of high dopamine content. Concentrations slightly higher than those for whole brain were recorded, but the significance of this, if any, is unknown.

Chlorpromazine in dog plasma after intravenous doses. The mean concentration of chlorpromazine in dog plasma appeared to decline in two phases, the first lasting about 1 hr and the second continuing through the remaining 6 hr of the experiments (Fig. 4). It can be presumed by analogy with thiobarbiturates (10) that the first phase mainly indi-

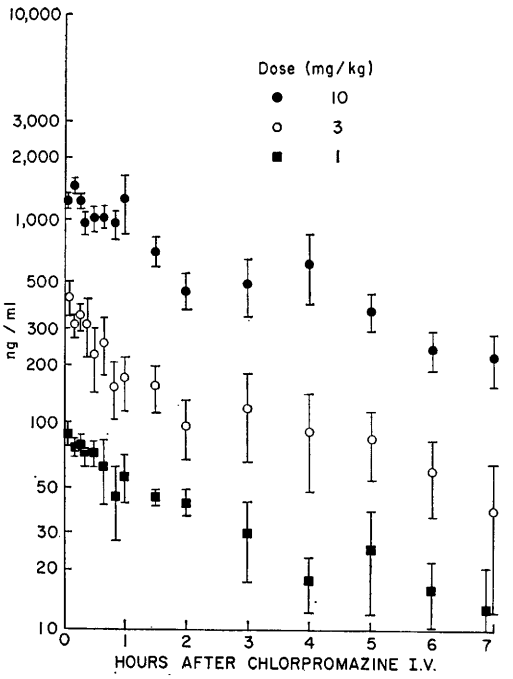

FIG. 4. Plasma chlorpromazine levels in dogs; concentrations of chlorpromazine in plasma of dogs given various doses iv: Each point is the mean ± SE, of three dogs. For each point in each dog, the mean of 1–4 assays was used. Each dose was given to the same 3 dogs with at least 3 weeks between successive doses. The order of administration of the 3 doses was randomized.

cated gradual achievement of equilibrium of distribution between tissues and plasma, and that the second phase mainly indicated metabolism of the drug. Although the magnitudes of concentrations in plasma were approximately proportional to the dose, there was a tendency towards a slightly higher proportion of the dose persisting in plasma at higher doses. This is shown by the values for the apparent volumes of distribution (V) of chlorpromazine in dogs, calculated at the three dose levels from the formula:

$$V = \frac{\text{Dose (mg/kg)}}{\text{extrapolated conc of drug in plasma at the time of dosage (mg/liter)}}.$$

The values obtained were 10.5 after 10 mg/kg, 12.0 after 3 mg/kg, and 15.6 after 1 mg/kg.

Discussion. These studies should be considered in the interpretation of chlorpromazine concentrations in human plasma for at least three reasons. Firstly, very low concentrations have been measured in man after oral doses, in comparison with concentrations after intramuscular doses (11). This discrepancy could reflect a loss of drug by metabolism in the portal circulation before reaching the general circulation, but this seems unlikely in view of the similar concentrations measured in rat plasma after intravenous and intraperitoneal doses. Secondly, the data suggest that chlorpromazine and its metabolites distribute largely by reversible processes between plasma and tissues. This observation in rats together with the near proportionality of doses and concentrations in plasma over a 10-fold range of doses, justifies the use of plasma concentrations in man as indicators of tissue concentrations. Thirdly, after a single dose, chlorpromazine and its metabolites were apparently cleared rapidly from rat vascular tissues and plasma.

Summary. Chlorpromazine (10 mg/kg) was rapidly absorbed after intraperitoneal adminstration in rats. Concentrations in liver and brain greatly exceeded plasma levels. Chlorpromazine and its metabolites rapidly disappeared from plasma and vascular tissues. In dogs, chlorpromazine levels in plasma were approximately proportional to the dose during the first 7 hr after the intravenous administration of three different doses (1, 3 and 10 mg/kg), all in the range commonly used in pharmacological studies.

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Received Feb. 2, 1970. P.S.E.B.M., 1970, Vol. 134.