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The pharmacokinetics of phentermine and chlorphentermine in chronically treated rats

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Young rats were treated with [^{3}H]labelled phentermine or chlorphentermine for varying periods (1 day to 8 weeks). The plasma tissue and concentrations of the drugs were determined. The distribution of phentermine reflected a partition, the tissue : blood ratios remaining constant for the entire period. In contrast, chlorphentermine was increasingly accumulated the longer the treatment lasted, as indicated by rising tissue : blood ratios. Chlorphentermine proved to be tightly bound to tissue components. The highest tissue : blood ratio (160 after 8 weeks) was found in lungs and the highest increase in the accumulation rate (10 fold in 8 weeks) was for the adrenals. These results, together with biochemical and ultra-structural findings, suggest that the highly amphiphilic chlorphentermine induces an impairment of phospholipid metabolism resembling lipidosis.

Chronic treatment with anorexigenic drugs may induce pulmonary hypertension in patients (Gurtner, Gertsch & others, 1968; Gurtner, 1969; Lang, Haupt & others, 1969; Osterman & Tegner, 1969; Schwingshackl, Amor & Dienstl, 1969; Gahl, 1970; Harmjanz, Gahl & others, 1968; Thorspecken, Hassenstein & others, 1970). Lüllmann, Parwaresch & others, 1972 found that chlorphentermine after chronic administration increased the pulmonary blood pressure of rats whereas phentermine did not. Microscopical examination of lungs of animals treated with chlorphentermine, revealed numerous large cells of "foamy" appearance freely located in the alveoli (Franken, Lüllman & Siegfriedt, 1970) but few were present after phentermine. Therefore we decided to compare the pharmacokinetic data of the two drugs, their only chemical difference being a *p*-chlorine substitution on the benzene ring.

Randomly tritiated phentermine and chlorphentermine were administered to rats for periods of 1 day to 8 weeks, and the concentrations of the drugs in blood and several tissues were determined 24 h after the last injection. In additional experiments, the blood level and the urinary and biliary excretion of radioactivity were measured in anaesthetized rats after a single injection of drug. The results demonstrate that the difference of the pharmacokinetics between phentermine and chlorphentermine becomes more pronounced with increasing time of treatment.

METHODS

To determine the time course of the plasma level and the excretion *via* bile and urine, male Sprague-Dawley rats (mean weight 350 g) were anaesthetized with pentobarbitone 50 mg kg^{-1}. A carotid artery and the bile duct were exposed, and small polyethylene tubes inserted. Urine was obtained by bladder puncture. The drugs were injected intra-arterially in pharmacologically inactive doses, the radioactivity given amounted to 100 μCi kg^{-1}.

For the chronic treatment with [^{3}H]phentermine or [^{3}H]chlorphentermine, male Sprague-Dawley rats (100 g) received daily doses of 10^{-4} mol kg^{-1} intraperitoneally for periods ranging from 1 day to 8 weeks. At the end of the appropriate experimental periods the rats were anaesthetized with pentobarbitone, and killed by cutting the carotid arteries, the blood was collected and 100 mg samples of the different tissues were taken.

The tissue samples were dried for 12 h at 50° and incinerated using a "Sample Tritium Oxidizer" (Packard, Model 300). Plasma and blood samples (0·1 ml) were applied to filter paper, dried and also incinerated. Bile, urine and blood samples were solubilized in "Soluene TM 100" (Packard). The tritium was counted in a "Tricarb-Liquid-Scintillation-Spectrophotometer" (Packard, Model 3380) using the following scintillation fluid: 0·4% PPO, 0·01% POPOP, 20% ethanol in toluene.

RESULTS

Single injection of phentermine or chlorphentermine

The blood and urine drug levels were followed for 5 h after injection. The concentration of radioactivity in the blood declined rapidly during the first 30 min (the distribution phase). The subsequent steady state blood levels were similar for the two drugs.

The total excretion of radioactivity with bile and urine is shown in Fig. 1. Within 5 h, about 8% of the applied doses of either drug were eliminated via the liver, whereas

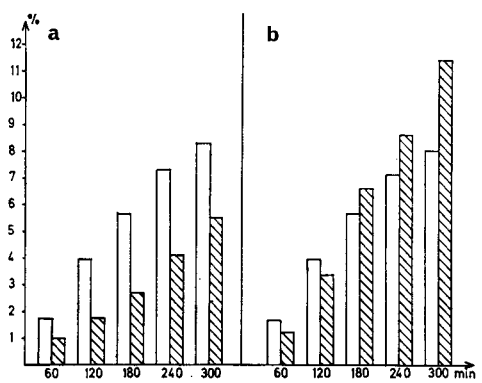

FIG. 1. Excretion of radioactivity with bile (open columns) and urine (hatched columns) after injection of tritiated chlorphentermine (a) and phentermine (b). Ordinate: in percentage of the doses applied; Abscissa: time after injection in minutes.

the urinary excretion of phentermine-equivalents was twice that of chlorphentermine-equivalents, i.e. 11% and 5% of the doses given. According to radiochromatographic analyses, the greater part of radioactivity excreted in the bile consisted of polar metabolites of phentermine and chlorphentermine. In urine, unchanged compounds and polar metabolites were present. Upon treatment by acid hydrolysis, some of the polar metabolites could be converted into substances possessing R_F values identical with those of phentermine and chlorphentermine.

The tissue content of phentermine and chlorphentermine was determined 1 and 24 h after a single injection. The results expressed as tissue : blood ratios (T/M ratio), are presented in Fig. 2A. The lungs displayed the highest T/M ratios of all tissues investi-

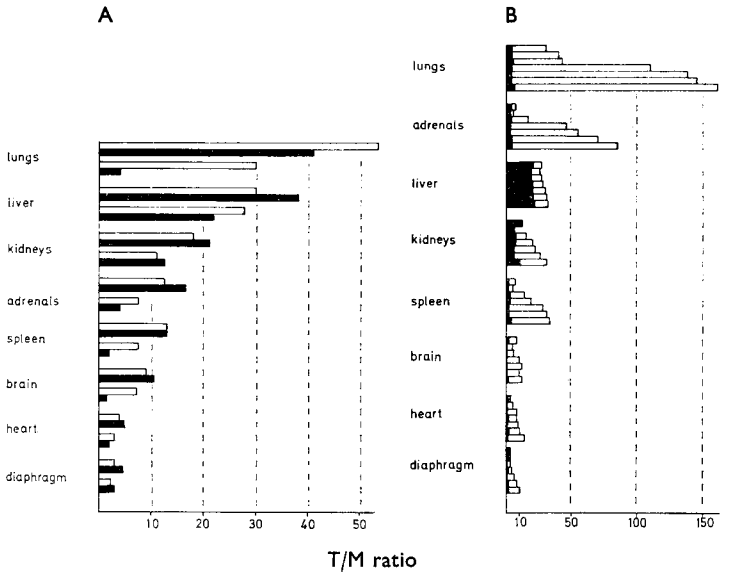

FIG. 2A. Tissue: blood (T/M) ratios at 1 and 24 h (upper pair and lower pair of columns respectively) after injection of chlorphentermine (open columns) and phentermine (solid columns).

B. Tissue: blood (T/M) ratios after chronic treatment by phentermine (solid columns) and chlorphentermine (open columns), determined 24 h after the last application. Duration of treatment 1 and 3 days, 1, 2, 3, 4 and 8 weeks in descending order of columns for each tissue.

gated: for chlorphentermine the ratio amounted to about 50 and for phentermine to about 40. High T/M ratios were also found in the excretion organs liver and kidneys. With the exception of the excretion organs, the T/M ratios of chlorphentermine decreased less than those of phentermine within 24 h, indicating that chlorphentermine is more tightly bound than phentermine.

Tissue concentration of phentermine and chlorphentermine after chronic treatment

The compounds were determined 24 h after the last injection of 10^{-4} mol kg^{-1}. The periods of treatment lasted for 1 or 3 days, 1, 2, 3, 4 or 8 weeks. During treatment the blood levels of both drugs increased only moderately (2 and 5 times for phentermine and chlorphentermine, respectively). The tissue concentrations expressed as T/M ratios are depicted in Fig. 2B for the different periods of treatment. A striking difference between phentermine and chlorphentermine is readily seen: The T/M ratios of phentermine remained constant over the entire period of treatment while the T/M ratios of chlorphentermine significantly increased with time in all tissues investigated except liver. The highest T/M ratios were found in lungs (170) and adrenals (80). To demonstrate more clearly the increasing binding capacity of some tissues, induced by the chronic treatment, the relative T/M ratios expressed as multiples of the T/M ratios after one day of application are depicted in Fig. 3. Except for the liver, the T/M ratios began to rise after 3–7 days of treatment, and approached values of 3 to 5 times the initial values; a value of almost 10 fold was reached in the adrenals. This suggests the occurrence of new binding sites caused by the treatment.

A separate set of experiments was performed to investigate the tightness of binding and exchangeability of the compounds present in the tissues. Rats, chronically treated

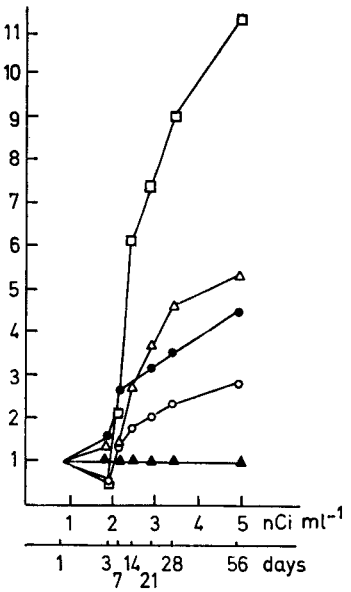

FIG. 3. Increase of tissue : blood (T/M) ratios in different tissues with time of treatment by chlorphentermine. Ordinate: multiples of the T/M ratio found 24 h after *one* injection; Abscissa: duration of treatment in days and blood level of chlorphentermine in nCi ml^{-1}, respectively. □ Adrenals. △ Lungs. ○ Kidney. ▲ Liver. ● Heart.

with unlabelled phentermine or chlorphentermine for different periods of time, received one single injection of a tritiated drug. The distribution of the labelled compound in tissues of pretreated animals, was compared with the distribution of the drug in animals treated exclusively with [^{3}H]phentermine or [^{3}H]chlorphentermine. For phentermine, the distribution pattern at 1 and 24 h after the injection was unchanged after pretreatment periods of up to 8 weeks, indicating a lack of tight binding and suggesting that partition was according to the solubility. In contrast, the distribution pattern of chlorphentermine changed markedly with time of pretreatment, demonstrating a low exchangeability of the compound present in some tissues, particularly in the lungs and adrenals.

DISCUSSION

During the first hours after injection, phentermine and chlorphentermine displayed comparable pharmacokinetics, only the renal excretion of the chlorinated compounds was lower. After a single injection, phentermine was taken up by different tissues to a moderate degree. After chronic treatment, the tissue blood ratios (T/M ratio) remained constant while the phentermine blood level rose slowly. On the first day of treatment, the tissue accumulation of chlorphentermine was greater than that of phentermine, particularly in the lungs. During chronic treatment, however, the accumulation of chlorphentermine became more and more pronounced, indicated by the rising T/M ratios for adrenals, lungs, heart and kidneys (Figs 2 and 3). In contrast to phentermine, which demonstrated pharmacokinetic properties that indicated simple partition, chlorphentermine displayed unusual behaviour: it became tightly bound in some tissues and induced new binding sites.

This marked difference between the two closely related drugs can only be caused by the *p*-chlorine substitution, which increases the amphiphilic nature of chlorphentermine and its affinity to certain tissue components.

Ultrastructural observations might contribute to a possible explanation of the effects of chlorphentermine: The huge, free intra-alveolar "foam cells" (Franken & others, 1970), and nearly all sessile cell types of pulmonary tissue, have been shown to contain great numbers of lamellar inclusion bodies, which probably consist mainly of phospholipids (Lüllmann-Rauch, Reil & others, 1972). Similarly, in adrenal cortex and medulla, but not in liver tissue, numerous lamellar inclusions have been found. These alterations have been interpreted as a manifestation of a drug-induced accumulation of phospholipids, which might serve as binding sites for the amphiphilic chlorphentermine.

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