BRAIN LEVELS OF CHLORDIAZEPOXIDE IN RATS

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Summary: The time- and dose-dependent uptake of chlordiazepoxide by the brain of rats was investigated and correlated with the blood levels of the drug. Peak concentration in the brain was reached in 10 min after i.p. injection of 25 *mg/kg*, and in 5 min with a dose of 50 *mg/kg* of the drug. Retention of chlordiazepoxide by the brain showed different characteristics depending on the size of the dose used.

Key words: chlordiazepoxide rat brain

INTRODUCTION

Benzodiazepine derivatives used as minor tranquillisers selectively act on the limbic system (hippocampus, amygdala, hypothalamas) which is concerned with control of emotion (4). Individual members of this group of drugs differ from one another in potency and duration of action (1).

Although benzodiazepine derivatives are used commonly, few quantitative data regarding their distribution are available. Autoradiographic (9), colorimetric (2) and spectrofluorometric (3,7) methods have been utilised to determine chlordiazepoxide and diazepam in tissues, organs and body fluids. Radioimmunoassay and gas-liquid chromatography have been used to determine diazepam in blood (6). It was observed that diazepam accumulates rapidly in the brain, kidney, liver and myocardium. The penetration of chlordiazepoxide in the brain was slower than that of diazepam. However, the distribution of both the drugs in the brain was similar (9).

The present investigation was undertaken to study the entry of chlordiazepoxide into the brain of rats and to correlate the concentration in this organ with that in blood.

MATERIALS AND METHODS

Chlordiazepoxide hydrochloride (LIBRIUM, Roche) was obtained as a dry substance and solutions made in the diluting fluid supplied. All other chemicals used were of analytical reagent grade.

Male Long-Evans rats weighing 200 to 250 g were used. Twelve groups of 10 rats each were selected. Two rats from each group were used as controls. Six groups of 8 rats were injected with 25 *mg/kg*, and the other six groups with 50 *mg/kg* chlordiazepoxide intraperitoneally (i.p.). The control rats (corresponding to each group) were injected with an equivalent volume of normal saline i.p. The rats in the individual groups were decapitated at 5, 10, 30, 60, 90 and 120 min after administration of the drug or normal saline. Ten min prior to decapitation, 0.1 *ml* of heparin (5,000 units/*ml*) was administered i.p. to all the animals. The rats sacrificed at 5 min received heparin 5 min before the administration of chlordiazepoxide and the rats decapitated at 10 min received heparin and the drug simultaneously by separate injections. The blood was collected into heparinised tubes; the brain removed immediately (within 1 min), quickly rinsed in cold normal saline, blotted dry and stored at -20°C until analysis. The blood was analysed soon after collection. The brain was homogenised (1 g/10 *ml*) in 0.9% NaCl in a glass homogeniser before analysis.

Determination of chlordiazepoxide from blood and brain was based on the method described by Natelson (5). The drug was hydrolysed to form 2-amino-5-chloro-benzophenone which is an aniline derivative. This was diazotised and coupled with N-(1-naphthyl) ethylene diamine to produce a pink colour which was read in a spectrophotometer (Beckman, Model B) at 550 *mμ*. End products obtained by identical processing of blood and brain homogenates from the control animals were used as blank. The actual concentration of chlordiazepoxide was estimated by consulting a previously constructed standard curve prepared by using different concentration of the pure material.

A series of experiments were carried out to determine the recovery of chlordiazepoxide from blood and homogenised brain. In these experiments known amounts of chlordiazepoxide were added to the blood and brain homogenates obtained from untreated rats which were then analysed for recovery of chlordiazepoxide.

RESULTS

Table I shows the recovery of chlordiazepoxide from brain and blood of rats. The mean recovery from blood and brain was 100 and 84% respectively.

The mean concentration of chlordiazepoxide in blood and brain of rats after i.p. injection of 25 *mg/kg* is shown in Fig. 1. Peak concentration in blood (13.2 *μg/ml*) was reached at 5 min, but peak brain level (21.8 *μg/ml*) reached at 10 min. The decline of chlordizepoxide level in both blood and brain was sharp at 30 min (61.9 and 61.7% respectively of the peak concentration in the blood and brain). From this time, the blood level of the drug was constant upto 120 min when a concentration of 56.1% of the peak level was observed. The brain level of chlordiaze-

poxide, however, exhibited a further fall at 60 min (45.8% of the peak concentration). After thi time, the concentration of the drug in the brain was constant upto 120 min when a concentratio of 43.29% of the peak level was detected. The mean ratio, concentration in blood/concentratio in brain (B/B) of 0.88 reached at 5 min dropped to 0.58 and 0.60 at 10 and 30 min respectivel but reverted to 0.78 at 60 min. The B/B ratio was 0.74 and 0.76 at 90 and 120 min respectivel

TABLE I: Recovery of chlordiazepoxide from rat blood and rat brain homogenates. The results represent mean ± SD of 6 rats. Figures in parenthesis express % recovery of the drug.

Amount of chlordiazepoxide added to blood or brain homogenate μg/ml or μg/g	*Amount recovered*	
	Blood	*Brain homogenate*
10	10.36 ± 0.31 (103)	8.60 ± 0.59 (86)
25	24.63 ± 0.62 (98)	20.46 ± 1.27 (82)
100	not done	84.03 + 3.82 (84)
Mean	100%	84%

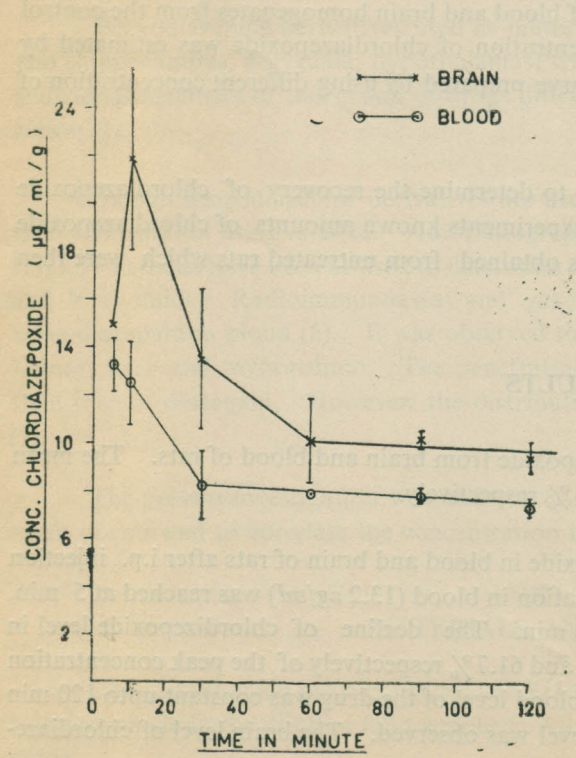

Fig. 1: Concentration of chlordiazepoxide in blo and brain of rats at various times after i injeciton of 25 *mg/kg* of the drug. Ea point is the mean ± SD of 8 rats. T SD at 5 min for brain and at 60 min f blood has been omitted for clarity.

With i.p. injection of 50 *mg/kg* chlordiazepoxide, the peak concentration in blood (18.4 *μg/ml*) and brain (26.3 *μg/g*) were reached at 5 min which was maintained with slight variations upto 60 min (Fig. 2). After this time, there was a decline in the level of the drug both in blood and brain. At 90 min, the concentrations in the blood and brain were 10.6 *μg/ml* and 19.5 *μg/g* (57.4 and 74.0% respectively of the peak concentrations), and at 120 min, these were 8.2 *μg/ml* and 11.2 *μg/g* (corresponding to 44.5 and 42.5 % of the peak concentrations respectively). The B/B ratio of 0.70 reached at 5 min was maintained upto 60 min (0.71, 0.72 and 0.68 respectively at 10, 30 and 60 min) with slight alterations, dropped to 0.54 at 90 min, but reverted to 0.74 at 120 min.

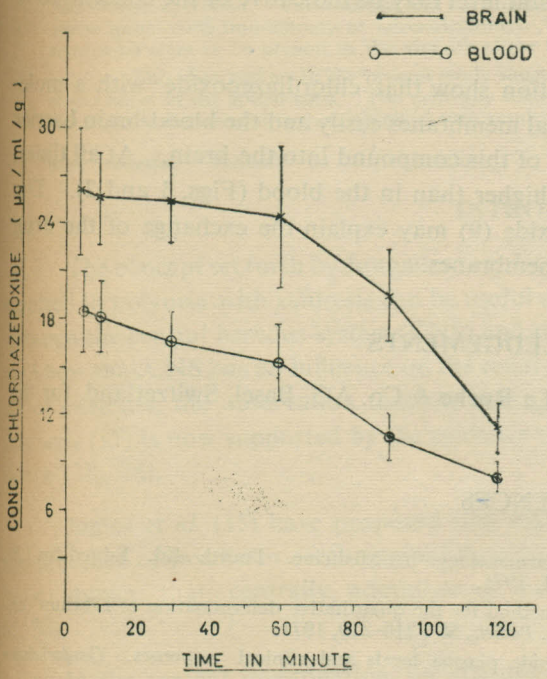

Fig. 2: Concentration of chlordiazepoxide in blood and brain of rats at various times after i.p. injection of 50 *mg/kg* of the drug. Each point is the mean ±SD of 8 rats.

In separate experiments rats received 0.1 *ml* of 0.9% NaCl instead of heparin, and subsequently, the concentration of chlordiazepoxide in brain was determined. The results agreed well with those obtained after the administration of the corresponding doses of chlordiazepoxide to heparinised rats.

DISCUSSION

The recovery of chlordiazepoxide from blood agrees with the results of Frings and Cohen (2), and confirms the validity of the procedure used.

In autoradiographic studies (9), selective retention of chlordiazepoxide in the white matter of the brain of mice was observed from 30 min onwards after intravenous injection. After 45 min, and later, detailed differences in the brain pattern of chlordiazepoxide were found. In the present study, the initial changes (from 5 to 60 min) in the brain concentration of chlordiazepoxide with i.p. injection of 25 *mg/kg* (Fig. 1) seem to be due to the normal delay in the onset of selective retention of the drug by the white matter of the brain. The alterations in the brain content observed after 60 min of administration of 50 *mg/kg* (Fig. 2) were probably due to rearrangement of brain pattern of the drug. These results suggest that size of the dose of chlordiazepoxide has a role to play on the onset and maintenance of its retention by the brain . The attainment of similar B/B ratio (0.76 and 0.74) at 120 min after different doses (25 and 50 *mg/kg*) of chlordiazepoxide suggest that around this time the blood level may be indicative of the concentration of the drug in the brain.

The results presented in this communication show that chlordiazepoxide with a molecular weight of 299.75 (8) penetrates the biological membranes easily and the blood/brain barrier seems to have little restricting effect on the entry of this compound into the brain. At all times, the concentration of the drug in the brain was higher than in the blood (Figs. 1 and 2). The basic and lipophilic properties of chlordiazepoxide (9) may explain the exchange of the drug between compartments separated by biological membranes.

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