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# THE UPTAKE OF PENTAZOCINE INTO BRAIN

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Summary

A simple procedure for the extraction from tissues and determination by gas chromatography of pentazocine was developed. The mean recovery from brain was 96%.

The time- and dose-dependent uptake of pentazocine into the brain was investigated and correlated with the plasma levels of the drug. The results showed a rapid entry of pentazocine into the brain; peak concentrations in plasma and brain were obtained at 2 min and 10 min, respectively, after the i.p. administration. After the injection of a single dose, the ratio, concentration of pentazocine in plasma/concentration of pentazocine in brain was constant from 10-90 min. This ratio remained unchanged over the range of 25-100 mg/kg of administered drug.

After the observation that phenazocine (1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-phenethyl-2,6-methano-3-benzazocin-8-ol), a benzomorphan (BM) derivative was a strong analgesic (1), a number of other compounds with similar chemical structures were prepared (2,3) and subjected to pharmacological and clinical evaluation (4,5,6,7). Pentazocine (1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(3-methyl-2-butenyl)-2,6-methano-3-benzazocin-8-ol), a clinically used BM derivative has been reported to be a potent analgesic in man, with low incidence of adverse effects (8) and minimum addiction liability (4,5). The distribution of cyclazocine (1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-cyclopropylmethyl-2,6-methano-3-benzazocin-8-ol), another BM derivative and pentazocine has been studied in dogs and cats using the tritiated drugs (9,10). In these experiments radioactivity in the brain was higher than in blood or plasma suggesting an easy penetration of the drugs through the blood-brain barrier.

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However, no identification of the radioactivity was made.

The present investigation was undertaken to study the entry of pentazocine into the brain of rats and correlate its concentration in this organ with that in plasma. Existing methods of determination of pentazocine (11,12) do not include estimation of the drug from tissues. Therefore, a procedure was developed for the extraction of pentazocine from brain and its quantitation by gas chromatography.

## Experimental Procedure

Pentazocine and cyclazocine were supplied as bases. Stock solutions were prepared in 0.9% NaCl by adjusting the pH to 4 with HCl. Cyclazocine was used as the internal standard in the gas chromatographic procedure. The chemicals used were of analytical reagent grade.

Male Sprague-Dowley rats weighing 300 g were used. In the first set of experiments seven groups of four rats each were injected i.p. (1 ml) with 50 mg/kg pentazocine in 0.9% NaCl. The rats in the individual groups were decapitated at 2, 5, 10, 30, 60, 90 and 150 min after administration of the drug. Ten min prior to decapitation, heparin, 0.15 ml of 1 mg/ml solution in 0.9% NaCl was administered, i.p. to all animals. The rats sacrificed at 2 and 5 min received heparin 8 and 5 min prior to the administration of the drug, whereas those decapitated at 10 min received pentazocine and heparin simultaneously by separate injections. The blood was collected into centrifuge tubes; the brain (excluding the cerebellum) was immediately removed, quickly rinsed in cold 0.9% NaCl, blotted dry and stored at -20°C. The separated plasma was kept at -20°C until analysis.

In the second set of experiments, four groups of four rats were injected i.p. (1 ml) with 25, 50, 75 and 100 mg/kg pentazocine respectively. These animals were decapitated at 60 min after the administration of the drug. The further treatment of the animals as well as the sampling of plasma and brain were as described before.

Pentazocine in plasma was analyzed according to the gas chromatographic procedure developed for the determination of BM derivatives in human plasma (13)

The brain was homogenized with 0.1 N HCl (10 ml/g tissue) in a Potter-Elvehjem all-glass homogenizer. The homogenate was centrifuged at 9000 x g for 10 min and the supernatant was collected. The sediment was suspended in 5 ml of 0.1 N HCl, centrifuged and the supernatant was added to that previously collected. Cyclazocine, used as internal standard, was added (0.2 ml of 100 μg/ml) to 12 ml of the acidic extract. After adjustment of the pH to 8 with 0.5 ml of 1 N NaOH, the drug was extracted with 20 ml benzene for 10 min on a horizontal shaker at medium speed. The samples then were centrifuged at 1900 x g for 20 min. Fifteen ml of the organic layer was transferred to conical tubes and evaporated to dryness on a water bath at 65°C under a stream of nitrogen. Just prior to analysis the residue was dissolved in 50 μl acetone and 1-2 μl injected into the gas chromatograph.

The amount of pentazocine in a sample was determined by measuring the ratio, peak height of the drug/peak height of internal standard, and relating this ratio to previously constructed standard curves obtained by using rat plasma and rat brain, respectively. These standard curves were linear over a wide range (25 to 500 ng). A dual column gas chromatograph (Hewlett-Packard, Model 402) equipped with flame ionization detectors was used. Glass columns 6 feet long, 1/4 inch O.D. were packed with 3% OV-1 gas chrom Q (100/120 mesh). The columns were conditioned for 48 hours at 260°C. Operating conditions were: column temperature 210°C, injection port 250°C and flame detector 260°C. Nitrogen flow was 35 ml/min; hydrogen and air was adjusted to give optimum detector response.

## Results

Typical chromatograms of pentazocine extracted from plasma and brain after the i.p. administration to rats are shown in Fig. 1. The retention time for the internal standard (cyclazocine) was 5.0 min and for pentazocine 5.7 min.

No interference by the biological material was encountered.

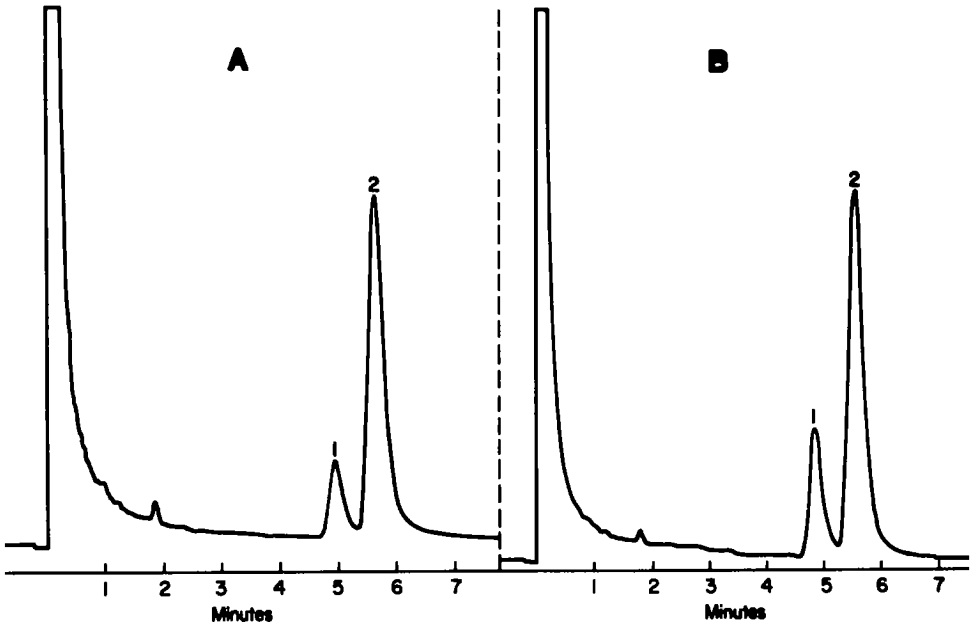


FIG. 1

Gas chromatographic separation of A: 60 ng of cyclazocine and 240 ng of pentazocine from rat plasma and B: 100 ng of cyclazocine and 300 ng pentazocine from rat brain homogenate, after the i.p. administration of pentazocine.

Table 1 shows the recovery of pentazocine from plasma and brain of rats. In these experiments known amounts of pentazocine (2 to 16 μg/ml plasma or 4.98 to 38.46 μg/g brain tissue) were added to plasma or brain homogenate of untreated rats and the drug extracted as described. The mean recovery of pentazocine from plasma and brain was 100 and 96%, respectively.

TABLE I

Recovery of pentazocine from rat plasma and rat brain homogenate. The results represent the mean ± S.D. of 4 rats. The figures in parenthesis express % recovery of the drug.

| Amount of drug added | | Amount recovered | |
|---|---|---|---|
| to plasma (μg/ml) | to brain homogenate (μg/g) | from plasma | from brain homogenate |
| 2 | 4.98 | 1.96 ± 0.12 (98) | 4.68 ± 0.42 (94) |
| 4 | 9.90 | 4.12 ± 0.31 (103) | 9.75 ± 1.01 (98) |
| 8 | 19.61 | 7.96 ± 0.26 (99) | 18.14 ± 1.96 (92) |
| 16 | 38.46 | 16.36 ± 0.41 (102) | 38.20 ± 2.21 (99) |
| | | Mean: 100% | 96% |

The concentration of pentazocine in plasma and brain of rats after an i.p. dose of 50 mg/kg is shown in Fig. 2. The plasma concentration dropped rapidly from 10.18 μg/ml at 2 min to 2.92 μg/ml at 10 min after the administration. During this time the concentration of the drug in the brain rose from 3.50 μg/g to 11.92 μg/g. At all times the concentration of the drug in brain was higher than in plasma. The mean ratio, concentration in plasma/concentration in brain (P/B) of 0.24 reached at 10 min, was maintained until 90 min. The ratio rose to 0.48 at 150 min after the administration of pentazocine.

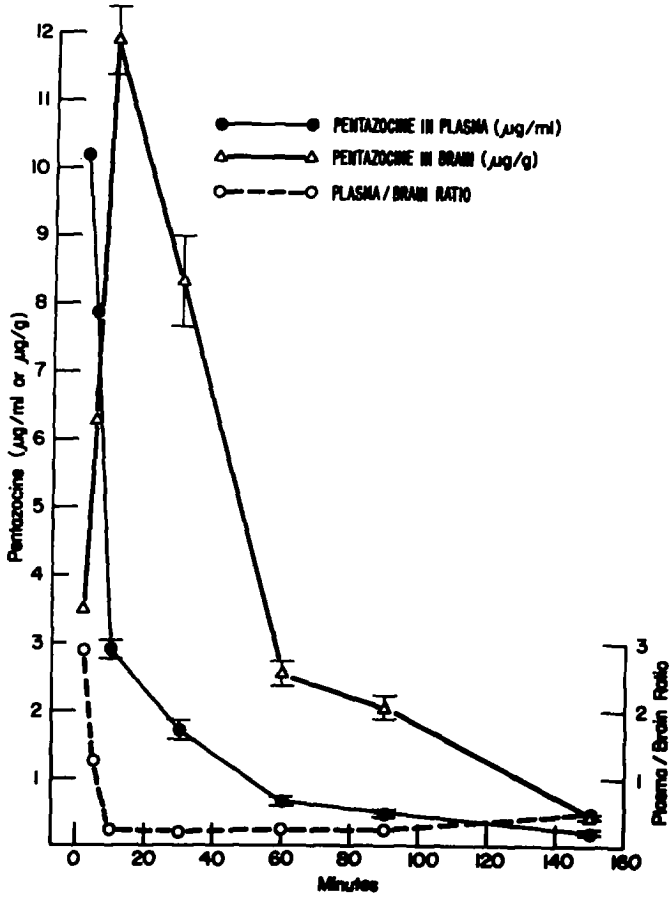


FIG. 2

Concentration of pentazocine in plasma (●—●) and brain (Δ—Δ) of rats at various times after the i.p. administration of 50 mg/kg of the drug. Each point is the mean ± S.D. of 4 rats. The S.D. at 2 and 5 min has been omitted for clarity. The broken line (O---O) shows the ratio, concentration of drug in plasma/concentration of drug in brain.

Fig. 3 shows the concentration of pentazocine in plasma and brain of rats at 60 min after the i.p. administration of 25, 50, 75 and 100 mg/kg. With increase of dose, the drug concentration in plasma and brain rose proportion-

ately resulting in a constant mean P/B ratio of 0.28.

In control experiments, rats received 0.15 ml of 0.9% NaCl, instead of heparin, and subsequently the concentration of pentazocine in brain was determined. The results agreed well with those obtained after the administration of the corresponding doses of pentazocine to heparinized rats.

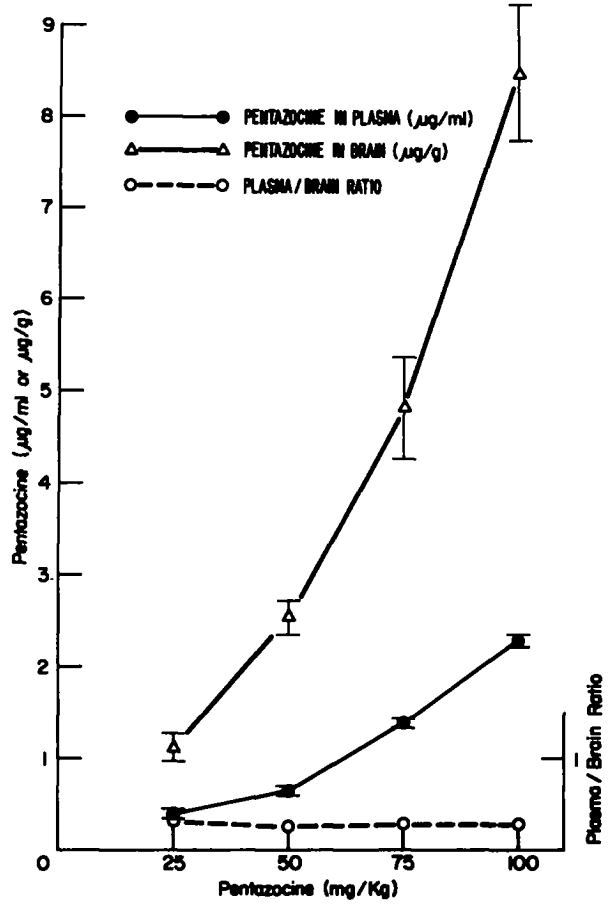


FIG. 3

Concentration of pentazocine in plasma (●—●) and brain (△—△) of rats at 60 min after the i.p. administration of increasing doses of the drug. Each point is the mean ± S.D. of 4 rats. The broken line (O---O) shows the ratio, concentration of drug in plasma/concentration of drug in brain.

## Discussion

The previously reported tissue levels of pentazocine were obtained by the use of the radioactively labeled drug (10, 14). The simple procedure for the determination of pentazocine in brain described in this communication allows the quantitation of the drug by gas chromatography. Good chromatographic separation of the internal standard and of pentazocine was achieved, and no interfering peaks were obtained from the biological material (Fig. 1). The high recovery of the drug from brain homogenates (Table 1) suggest the applicability of the procedure to other tissues as well. In combination with the described gas chromatographic determination of other benzomorphine derivatives (13) the procedure presented here could be used for the analysis of these compounds in tissues.

The analysis of plasma and brain after the addition of known amounts of pentazocine, as well as after the i.p. administration of the drug to rats, yielded identical chromatograms both showing single well resolved peaks with a retention time of 5.7 minutes. No interference by peaks having similar retention times was encountered. Pentazocine is mainly metabolized by oxidation of the terminal methyl groups of the dimethylallyl side-chain to yield two isomeric alcohols. The relative retention times of these metabolites as compared to pentazocine (1.00) were determined to be 2.06 and 2.30 respectively (14).

In agreement with the rapid onset of action observed clinically (11) the concentration of pentazocine in brain reached a high level quickly: peak levels were obtained 10 min after the i.p. administration of the drug (Fig. 2). Pentazocine also leaves the brain rapidly: at 60 min after the administration, the level in brain represented less than 25% of the peak concentration. After 60 min the decline was considerably slower. The peak of plasma levels obtained at 2 min after administration rapidly declined at 60 min to 10% of its original value. Of particular interest was the finding

that during these rapid and large changes in concentration of the drug, the P/B ratio remained essentially constant from 10 min to 90 min after the administration. The constancy of the blood/tissue ratio was even more accentuated in the experiments when increasing doses of pentazocine were injected i.p.: over the range of 25-100 mg/kg, the P/B ratio remained unchanged. Therefore, plasma levels are apparently indicative of the concentration of pentazocine in the brain.

The results presented in this communication show that pentazocine with a molecular weight of 285 obviously penetrates biological membranes quite easily and the blood/brain barrier seems to have little restricting effect on the entry of this compound into the brain. Pentazocine as a basic amine is likely to concentrate in tissues, and its high partition coefficient between organic and aqueous phase (10) emphasizes its lipophilic nature. However, the present study demonstrates a pronounced and rapid exchange of the drug between compartments separated by biological membranes and justifies further work to elucidate the mechanism of this transport. Such work is in progress.

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