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Note

Gas–liquid chromatographic determination of dextromethorphan in serum and brain

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Dextromethorphan is widely accepted as a safe and effective antitussive agent, for its activity seems generally to be the same as that of codeine both in terms of potency and in mechanism of action. Unlike codeine and related drugs, dextromethorphan and its active metabolite dextrorphan are in a sense unique in that, although chemically related to opioids, they seem to be non-addictive[1–8]. Nevertheless, some information from toxicologists and neuropsychiatrists regarding rapid deterioration owing to habituation to dextromethorphan[9] has stimulated experimental research showing that this agent can prevent morphine abstinence and induce addiction in dog[10], and in the mouse and rat[11].

On the other hand the W.H.O. Expert Committee on Drug Dependence, examining the available reports, considered that the frequency of abuse was so low that it does not constitute a serious social problem. However, the Committee did recommend strict monitoring of dextromethorphan consumption to detect any sign of addiction[12]. Subsequently, a W.H.O. Scientific Group on Opiate Analgesics and Antitussive Agents remarked that, in high doses, dextromethorphan can cause mental changes in some people, although the drug seems to be "little or not addicting" (ref. 13).

Thus we have attempted to achieve a reliable procedure for the qualitative and quantitative evaluation of dextromethorphan and dextrorphan in serum and brain in order to assess whether the addiction induced in animals might be related to the persistence of these agents, or to some conformation changes in the opiate receptors independently of stereospecific binding affinities[14].

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EXPERIMENTAL

Reagents

Dextromethorphan and dextrorphan were obtained from Roche (Basel, Switzerland), imipramine from Geigy (Basel, Switzerland) and chloroform and diethyl ether from Merck (Darmstadt, G.F.R.).

Apparatus

A Carlo Erba Fractovap GV gas chromatograph equipped with a dual flame ionization detector was used. The glass columns (4 mm I.D.) were packed with 3% OV-17 on Gas-Chrom Q (100–200 mesh) (Carlo Erba, Milan, Italy). Operating conditions: Injection temperature, 280°; column temperature, 250°; detector temperature, 270°; nitrogen flow-rate, 40 ml/min; hydrogen flow-rate, 35 ml/min; air flow-rate, 400 ml/min.

Extraction procedure (Fig. 1)

Rat serum. 20 μl of methanol containing 1000 μg/ml of imipramine (internal standard), 0.2 ml of 1 *N* NaOH and 8 ml of chloroform were added to 1 ml of serum in a glass centrifuge tube, where they were mixed for 30 sec on a rotatory mixer and then centrifuged for 10 min at 2000 *g*. The aqueous phase was discarded, and the chloroform layer was transferred to a clean tube and evaporated to dryness under a stream of nitrogen in a water bath at 50°. After evaporation, the residue was redissolved in 50 μl of methanol and 1 μl was injected into the gas chromatograph.

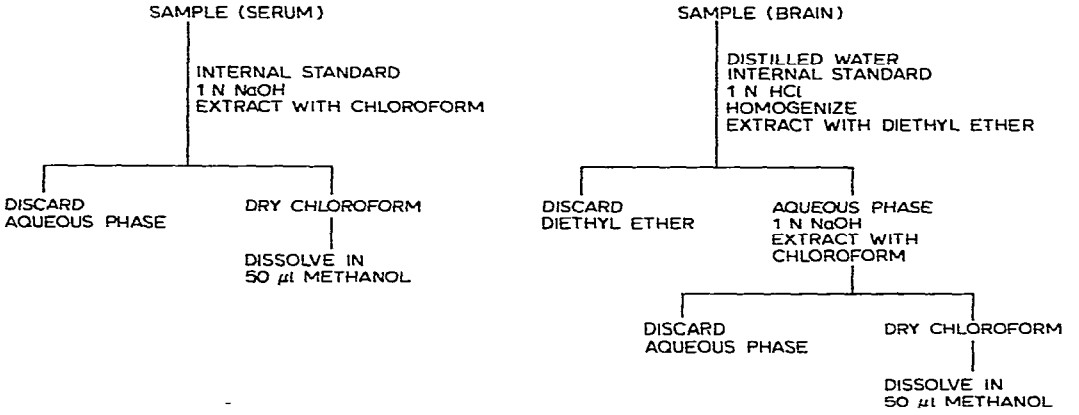

Fig. 1. Outline of the extraction procedure.

Rat brain. 300 mg wet weight of rat brain were homogenized in a glass potter in the presence of 8 volumes of distilled water, 20 μl of methanol containing 1000 μg/ml of imipramine (internal standard) and 0.2 ml of 1 *N* HCl. The homogenate was transferred to a glass centrifuge tube, and 10 volumes of diethyl ether were added (with respect to the initial fresh tissue). The reagents were mixed for 30 sec on a rotatory mixer and then centrifuged for 20 min at 2000 *g*. The organic phase was discarded and the aqueous phase was transferred to another centrifuge tube, alcalinized with 0.2 ml of 1 *N* NaOH and extracted with 6 ml of chloroform by mixing for 30 sec on a rotatory

mixer and centrifuged for 10 min at 2000 *g*. The aqueous phase was discarded, and the chloroform layer was transferred to a clean tube and finally evaporated to dryness as for the serum extract. After evaporation, the residue was treated in the same way as the serum extract and 1 μl was injected into the gas chromatograph.

Calculations

Known amounts of dextromethorphan (0.5–30 μg) and 20 μg of imipramine (internal standard) were added to serum or rat brain and extracted as previously described. The extracts were chromatographed and calibrations curves were constructed by measuring the peak-area ratio of dextromethorphan to the internal standard. These standard curves (Fig. 2) were used to calculate the unknown concentrations of samples.

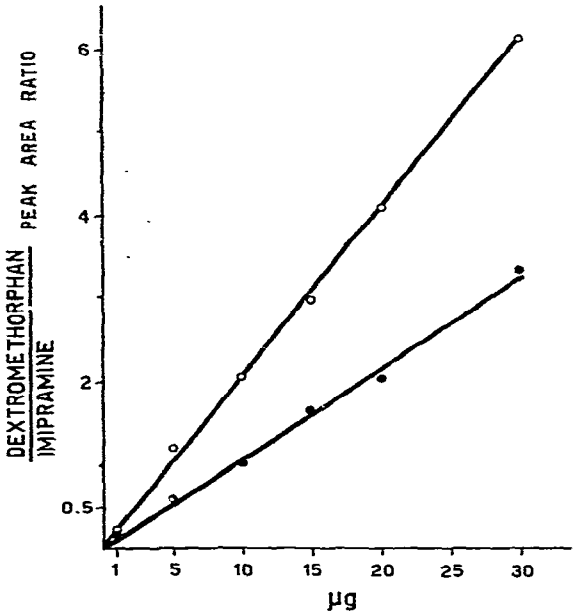

Fig. 2. Standard calibration curves for concentrations of dextromethorphan extracted from serum (○—○) and from brain (●—●).

RESULTS AND DISCUSSION

Under the gas chromatographic (GC) conditions described above, dextromethorphan and imipramine (internal standard) appeared within 6 min in the order shown in Fig. 3. Dextromethorphan exhibited a retention time of *ca.* 4 min, and the internal standard of *ca.* 5 min. The peak area ratio for various concentrations of drug and internal standard were plotted against drug concentration in μg per ml of serum or μg per 300 mg of rat brain and gave good linearity up to 30 μg of drug added. The back-extraction of brain samples, by acidification in diethyl ether followed by alkalinization and re-extraction in chloroform, removed interfering peaks and thus allowed a better measurement of the peak.

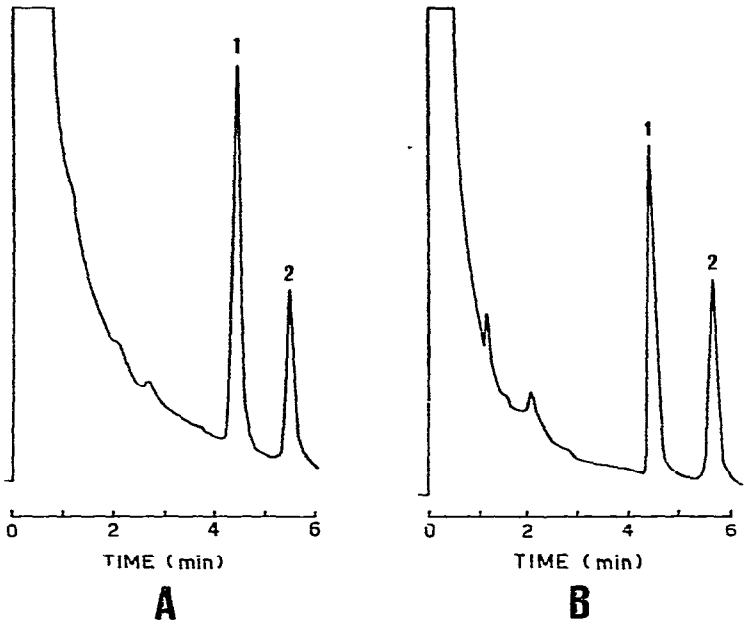

Fig. 3. Gas chromatograms of dextromethorphan (1) and imipramine (2) (internal standard) obtained from serum (A) and brain extracts (B).

The recovery of dextromethorphan from samples and the reproducibility of the method are illustrated in Table I. The reproducibility of the method was tested by analyzing five times a mixture of the two drugs extracted from specimens. The results demonstrate that there is a good recovery and an excellent reproducibility. It was demonstrated that the extracts (stored at room temperature) were stable for almost a week.

TABLE I

RECOVERY OF DEXTROMETHORPHAN FROM RAT SERUM AND BRAIN, AND THE REPRODUCIBILITY OF THE METHOD FROM FIVE ANALYSES

	Serum		*Brain*	
	10 μg/ml	*20 μg/ml*	*5 μg/300 mg*	*10 μg/300 mg*
Recovery (%) (mean ± S.D.)	85 ± 5.5	97 ± 4.7	87 ± 4.7	93 ± 3.7
Reproducibility	10 ± 0.16	20 ± 1.0	5 ± 0.1	10 ± 1.0

By use of the same procedure, we achieved good results for the determination of the metabolite dextrorphan through a further step in the extraction: the alkaline aqueous phase, after extraction of dextromethorphan, was acidified, then made alkaline (pH 8.5) with solid sodium bicarbonate and finally extracted with ethyl acetate. Dextrorphan exhibited a retention time of *ca.* 6 min under the above GC conditions. Therefore the peak of the internal standard lies between those of dextromethorphan and its metabolite.

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