Measurement of desipramine in brain tissue by a radioisotope derivative technique

The content of imipramine-like drugs in tissues has usually been measured by the spectrophotofluorometric method of Dingell, Sulser & Gillette (1964) which is, however not sensitive enough to detect low amounts of these compounds. A radioisotope derivative technique of Hammer & Brodie (1967) has been widely used to determine the concentration of secondary amino tricyclic antidepressants in biological fluids, particularly in plasma (Hammer & Sjöqvist, 1967; Hammer, Mårtens & Sjöqvist, 1969; Borgå, Azarnoff & others, 1969; Jori, Bernardi & others, 1971). While this method is highly sensitive for plasma (with a lower limit of 5 ng ml^{-1}), we have found that it could not be applied to brain tissue because the complex macromolecular compounds present in brain homogenate interfered with the reaction. For experimental studies on drug kinetics (e.g. tissue–plasma ratios) a single method applicable to both plasma and brain tissue would be desirable. We now report on an adaptation of the radioisotope derivative technique of Hammer & Brodie (1967) to allow the measurements of low quantities of desipramine in the brain tissue.

The method of Hammer & Brodie (1967) for determination of desipramine in plasma is based on extraction of the drug followed by acetylation with tritium-labelled acetic anhydride. The labelled acetyl-desipramine derivative is then extracted into heptane and assayed by scintillation counter; the concentration of desipramine is determined from standards containing known amount of the drug added to plasma.

The present procedure to determine desipramine in brain tissue involved an initial basic extraction of the drug with heptane, followed by an acid back-extraction with heptane and by a final basic extraction with hexane. The acid brain homogenate (200 mg in 2 ml of 0·1 N HCl), with known amounts of drug (100–400 ng) added, was made basic (pH 13) by 0·2 ml of 5 N NaOH and gently shaken with 3 ml of n-heptane for 15 min. After centrifugation (500 *g* for 5 min) the tubes were placed on a dry ice-acetone mixture, thus breaking any emulsion. The contents of tubes were allowed to thaw and then recentrifuged. The organic phase (2·5 ml) was transferred to a 12 ml glass tube (previously silanized with 1% aqueous solution chlorotrimethylsilane) containing 3 ml of 0·5 M HCl (acid back-extraction). After reshaking (for 15 min) and centrifugation, the heptane was discarded, the aqueous phase was made alkaline with 0·5 ml of 5N NaOH and the drug extracted with 3 ml of n-hexane (final basic extraction). Adsorption of desipramine onto the surface of the pipettes was prevented by wetting them with isoamylol.

The reaction with the tritiated acetic anhydride was then carried out and the radioactivity measured as described by Hammer & Brodie (1967).

For simultaneous estimation of desipramine concentrations in the brain and plasma, Wistar rats were injected with a 10 mg kg^{-1} dose of the drug and killed 15 or 60 min later. The brains were rapidly removed, homogenized and treated as described above. The drug in plasma was extracted and measured as described by Hammer & Brodie (1967). Known amounts of desipramine were added to the rat brain homogenates, rat plasma or water and carried through the entire procedure to serve as internal standards. Samples of either rat brain homogenate, rat plasma or water without the drug were carried through the entire procedure to serve as corresponding blanks.

Desipramine hydrochloride was kindly donated by Ciba-Geigy Ltd., Canada. [^{3}H]Acetic anhydride (Amersham, Searle) had a specific activity of 100 mCi $mmol^{-1}$. The scintillation mixture consisted of 7% (v/v) butyl-PBD (Amersham/Searle) in toluene. The purity of the labelled[^{3}H]acetic anhydride at the time of shipping was more than 98% and the rate of decomposition was given by the manufacturer as being 0·3% per month. The radioactivity of the labelled desipramine derivative was

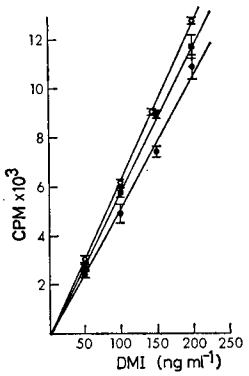

FIG. 1. Plots of radioactivity recovered from samples of water (■), plasma (○) or brain homogenate (●) against the concentration of desipramine added to these samples. For details see the text.

measured in a Beckman 150 Liquid Scintillation counter. The counting efficiency ranged from 37 to 40%.

Data presented in Fig. 1 show that the radioactivity recovered was proportional to the amount of drug added to the brain homogenate, plasma or water in the concentration range of 50–200 ng ml^{-1}. The recovery of drug from the rat plasma was 79 ± 1% (mean ± s.e.m. of 31 estimations) and the lower level of sensitivity about 5 ng ml^{-1}. This compares favourably with both the range of absolute recovery (72–91%) and the sensitivity of the method of Hammer & Brodie (1967) for human plasma. The mean recovery of desipramine from brain homogenate (n = 6) and water (n = 4) was 65 ± 2 and 74 ± 1·2%, respectively. Tissue blanks gave radioactivity levels equivalent to those expected from about 15 ng of drug. The lower limit of sensitivity for rat brain tissue was thus estimated to be about 30 ng. Since the concentration of desipramine in the rat brain 1 h after a 10 mg kg^{-1} dose was over 4 $\mu g\ g^{-1}$, it should be possible to measure the concentration of the drug in discrete brain areas weighing less than 50 mg by using this technique. In the spectrophotofluorometric method (Dingell & others, 1964) as used to measure the brain concentration of both desipramine and imipramine (Jori & Bernardi, 1968; Jori, Bernardi & others, 1970), adequate recovery has been reported after addition of 5–900 μg of the appropriate compound to tissue homogenate.

Concentrations of desipramine in rat plasma and brain tissue simultaneously measured 15 and 60 min after intraperitoneal administration of 10 mg kg^{-1} dose of the drug were: at 15 min plasma 259 ± 33 (n = 6), brain 1510 ± 170 (n = 5) brain: plasma ratio 5·8; at 60 min plasma 161 ± 13 (n = 8), brain 4060 ± 140 (n = 6) brain : plasma ratio 25·2. The brain concentrations of drug 60 min after injection (4·06 ± 0·14 $\mu g\ g^{-1}$) determined by using the radioisotope derivative technique are concordant with that (4·24 $\mu g\ g^{-1}$) measured spectrophotofluorometrically by Jori & others (1971) 1 h after the administration of 8 mg kg^{-1} of desipramine. The brain tissue to plasma ratio of drug increased from 5·8 at 15 min to 25·2 at 60 min after administration of a single dose which indicates that this tricyclic antidepressant rapidly leaves the blood and is accumulated in the brain.

It is hoped that the adaptation of the radioisotope derivative technique of Hammer & Brodie (1967) for estimation of desipramine in brain tissue will be of help in studies concerning the brain distribution, brain to plasma ratios and pharmacokinetics of secondary amino tricyclic antidepressant compounds.

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Department of Pharmacology, University of Ottawa, Ottawa, Ontario, Canada

E. RIVA*
P. D. HRDINA**

Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy

P. L. MORSELLI

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* Visiting research assistant from the Istituto di Ricerche Farmacologiche "Mario Negri" Milan, Italy.
** To whom reprint requests should be addressed.

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Agonist and antagonist potencies of isomeric 2,3-dimethyl-3-aryl-piperidines

Iorio & Casy (1975) recently reported on the antinociceptive effects of diastereoisomers of 2,3-dimethyl-3-aryl-piperidines and on their antagonist properties in rats and monkeys. These compounds have now been assayed on the guinea-pig isolated ileum by the method previously described (Kosterlitz & Watt, 1968). The results (Table 1) indicate general agreement with the observations obtained *in vivo*. The *N*-phenethyl isomers have relatively weak agonist activity, the α-isomer being more potent than the β-isomer. The *N*-allyl isomers are devoid of agonist activity. The antagonist potencies of all 4 compounds are low, the β-isomer of the allyl analogues being more active than the α-isomer while the reverse relationship holds for the phenethyl analogues. These observations agree with the data obtained on morphine-dependent monkeys.

Table 1. *Assessment of 2,3-dimethyl-3-*m*-hydroxyphenyl-1-R_1-piperidines*

R_1	Isomer	ID50 (nM)	Ke (nM)	Relative agonist potencies (morphine or normorphine = 1) Ileum	Nilsen	Relative antagonist potencies (naloxone = 1) Ileum	Dependent monkey
Phenethyl	α	291 ± 51	99·7 ± 10·3	0·21 ± 0·01	0·17	0·012 ± 0·001	Mild-intermediate withdrawal
Phenethyl	β	2138 ± 354	389 ± 57	0·03 ± 0·005	0·07	0·003 ± 0·001	Very mild withdrawal
Allyl	α	infinite	141 ± 16	0	0	0·009 ± 0·001	<0·05
Allyl	β	infinite	56·7 ± 7·9	0	0	0·023 ± 0·003	0·05–0·1

The values are the means ±s.e. of 4 observations (5 with α-allyl). α is cis and β trans in respect of Me_3/Ph_3. The results on the morphine-dependent monkey (Dr. E. L. Harris & Dr. M. Aceto, Virginia Medical College) and those of the Nilsen antinociceptive tests in mice have been supplied by Dr. M. A. Iorio and Dr. E. L. May.