Biochemical Pharmacology, Vol. 44, No. 9, pp. 1894–1897, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00
© 1992. Pergamon Press Ltd

A gas chromatographic procedure for separation and quantitation of the enantiomers of the antidepressant tranylcypromine

(*Received* 2 *January* 1992; *accepted* 17 *July* 1992)

Abstract—A novel assay procedure has been developed that allows for the separation and quantification of the enantiomers of the monoamine oxidase inhibitor tranylcypromine (TCP) in brain and liver of rats. The analytical method involves extraction of the drug from rat tissue with an organic solvent. TCP is then derivatized with *S*-(−)-*N*-(trifluoroacetyl)-prolyl chloride to allow gas chromatographic analysis of the resulting diastereoisomers. Conditions for analysis by a gas chromatograph equipped with a nitrogen–phosphorus detector and a capillary column are described. The method has been applied to the separation and quantification of the enantiomers of TCP in samples of brain and liver of rats that had been injected with this drug alone and after pretreatment with iprindole, a drug known to block aromatic ring hydroxylation.

Tranylcypromine (TCP)*, a monoamine oxidase-inhibiting antidepressant, is a mixture of (−)- and (+)-*trans*-2-phenylcyclopropylamine. It is one of many psychotropic drugs administered as a racemic mixture. Theoretically, the use of such a mixture constitutes the administration of two separate drugs which may be absorbed and/or metabolized and/or excreted at different rates. Conventional analytical techniques often do not differentiate the enantiomers and it must not be assumed that the enantiomers will be present in equal amounts [1, 2]. Previous studies have shown that the enantiomers of TCP have markedly different pharmacological properties. (+)-TCP (1*S*, 2*R*) is a more effective monoamine oxidase inhibitor, while (−)-TCP (1*R*, 2*S*) is a better inhibitor of noradrenaline and dopamine uptake [3–8]. (+)-TCP has also been shown to increase levels of brain 5-hydroxytryptamine over those caused by (−)-TCP [9]. Despite these known differences, few studies have been conducted on the ratios of the two enantiomers in tissues and body fluids. This ratio may be of great importance when interpreting findings on the pharmacodynamics and the pharmacokinetics of TCP.

In the study reported here, a method has been developed which allows for the extraction, derivatization and separation of the enantiomers of TCP from brain and liver tissue. This method has been applied to such tissue from rats that had been injected with TCP, with or without pretreatment with iprindole, an antidepressant drug known to block aromatic ring hydroxylation. The results of this preliminary application are presented.

Materials and Methods

Drugs. (±)-Tranylcypromine·HCl was purchased from the Sigma Chemical Co. (St. Louis, MO) and the individual enantiomers were gifts from SmithKline & French (Mississauga, Canada). Iprindole·HCl was provided by Wyeth Research (U.K.) Ltd (Taplow, Maidenhead, U.K.).

Subjects. Male Sprague–Dawley rats were randomly allocated to drug or vehicle treatment groups. The rats were injected intraperitoneally with either saline vehicle or iprindole·HCl (11.2 mg/kg). After 1 hr the rats were injected intraperitoneally with either vehicle or 2.5 mg/kg TCP. The rats were killed by decapitation at predetermined time intervals. The brain and liver were removed, immediately put on dry ice, and stored at −80° until the time of analysis.

Derivatization. Tissue samples were allowed to partially thaw and then were weighed and homogenized in 5–10 vol. of cold 0.1 N perchloric acid containing EDTA (10 mg/100 mL) and ascorbic acid (0.88 mg/100 mL). Homogenates were centrifuged at 10,000 *g* for 15 min at 0–4°. Aliquots (2 mL) of each supernatant were transferred to clean test tubes and to each was added 200 ng of the internal standard, *p*-chlorophentermine. Potassium carbonate (25%) (400 μL) was added to all samples and the pH value was checked to ensure basicity. Ethyl acetate (4 mL) was added to each tube, and the tubes were shaken vigorously for 5 min and centrifuged at 1000 *g* for 5 min. The organic phase was carefully transferred to another tube and taken to dryness in a warm water bath under a gentle stream of nitrogen. The residue was dissolved in a mixture of toluene (100 μL) and the chiral derivatizing reagent *S*-(−)-*N*-(trifluoroacetyl)-prolyl chloride (2 μL). The samples were vortexed briefly and allowed to sit at room temperature for 15 min. Saturated sodium borate (1 mL) was added to each sample, and each was vortexed and centrifuged. The toluene layer was retained from each sample for injection of aliquots (1 μL) on the gas chromatograph. Brain and liver tissues from vehicle-treated rats were carried through each assay run to act as controls.

Gas chromatograph. A Hewlett–Packard (HP) model 5890A gas chromatograph equipped with a nitrogen-phosphorus detector and linked to an HP 3392A integrator was used. A fused silica capillary column (25 m × 0.32 mm i.d.) coated with a 0.52 μm film thickness of 5% phenyl methyl silicone (Hewlett Packard Co., Palo Alto, CA), was employed. The carrier gas was pure helium (Linde, Union Carbide) at a flow rate of 2 mL/min. The detector was purged with pure hydrogen (Linde, Union Carbide) at 3.5 mL/min mixed with dry air (Linde, Union Carbide) at 80 mL/min.

Injection port and detector temperatures were 250° and 325°, respectively. The oven temperature was programmed to increase from 105° (maintained for 0.5 min) at a rate of 10°/min to 270°.

Calibration. A standard curve ranging from 50 to 500 ng for each enantiomer was run in parallel with each assay. These were prepared by adding 200 ng of the internal standard, *p*-chlorophentermine, and various amounts of (±)-TCP to the appropriate volume of supernatant from brain or liver homogenate obtained from drug-naive rats. The ratio of the peak height of the individual derivatized enantiomers of TCP to that of the derivatized internal standard was calculated and plotted against the concentration of the individual enantiomers.

Statistics. Data were analyzed by analysis of variance followed by the Newman–Keuls test ($\alpha = 0.05$).

Results and Discussion

Reproducibility and recovery. Under the conditions described here, the enantiomers of TCP were readily

* Abbreviations: TCP, tranylcypromine; and IPR, iprindole.

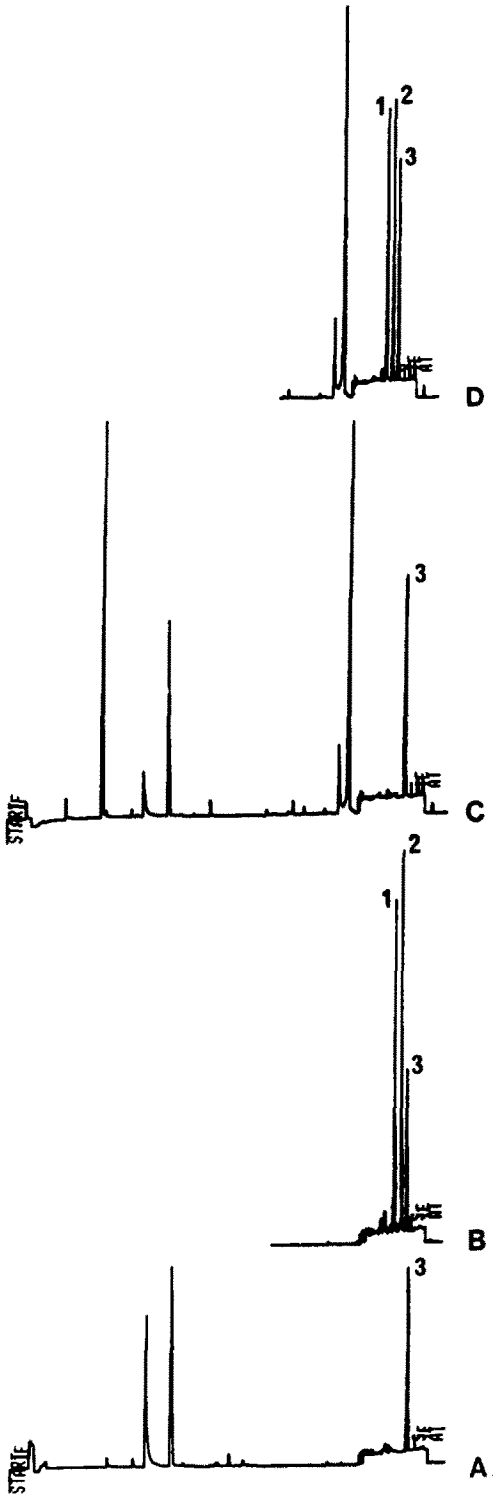

Fig. 1. Typical GC traces obtained in the separation of TCP enantiomers. Shown are derivatives of: (A) extract of liver tissue from a rat treated with vehicle; (B) extract of liver tissue from a rat treated with (±)-TCP; (C) extract of brain tissue from a rat treated with vehicle; and (D) extract of brain tissue from a rat treated with (±)-TCP. Peaks: derivatives of (−)-TCP (1); (+)-TCP (2); and added internal standard, *p*-chlorophentermine (3). Retention times for these peaks were 13.77 min (1), 14.00 min (2) and 14.19 min (3).

separated and quantified. Various temperature and time conditions were tested while developing the assay, and it was found that the derivatization was complete by 15 min at room temperature. Typical chromatograms of liver extracts and brain extracts from vehicle-treated and TCP-treated rats are shown in Fig. 1. The standard curve was linear over the range of 50–500 ng and a correlation coefficient > 0.99 was routinely obtained. Mean interassay coefficients of variation for 50- and 100-ng samples of the enantiomers were 3.5 and 4.8%, respectively, for (−)-TCP and 4.0 and 3.8%, respectively, for (+)-TCP. The practical limits of sensitivity (signal : noise ratio > 3) for (−)-TCP and (+)-TCP were 20 and 5 ng/g, respectively, in both brain and liver extracts; the difference in sensitivities was the result of a noisier baseline in the area of the retention time of the (−)-enantiomer. Mean percent recoveries of (−)-TCP and (+)-TCP from brain extracts were 82.7 and 75.2%, respectively.

Other procedures have been published for analysis of TCP [e.g. Refs. 6, 10–19], and in some cases [10, 14–16] the reported sensitivities were greater than for the present assay. However, these assays did not provide simultaneous analysis of both enantiomers and/or were not applied to brain or liver tissue.

Identification of the derivatives using combined gas chromatography–mass spectrometry (GC–MS). The structure of the derivative of TCP was confirmed by combined GC–MS with an HP 5840A GC inlet coupled to a mass spectrometer and an HP 7920 data system. The GC–MS system also consisted of an HP 2648A graphics terminal, HP 9876A printer, HP 7920 disc drive (software) and HP 21MX series E computer (hardware). Operating conditions were as follows: ion source temperature, 200°; interface temperature, 275°; column pressure, 34.5 kPa; accelerating voltage, 2200 eV; ionization voltage, 70 eV; scan speed, 100 amu/sec; and dwell time, 200 msec. The same column and oven conditions were used as for the GC analysis. The structures of all major ions in the spectrum of the derivative of TCP are identified in Fig. 2.

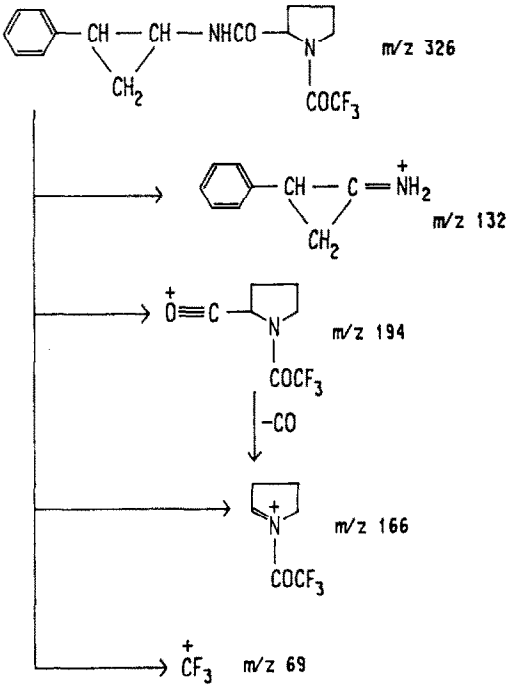

Fig. 2. Proposed electron impact mass spectrometric fragmentation pattern for the tranylcypromine derivative.

Table 1. Levels of (+)- and (−)-TCP in rat whole brain and liver after injection of (±)-TCP with and without pretreatment with iprindole

		(+)-TCP (ng/g)		(−)-TCP (ng/g)		Ratio (+)-TCP:(−)-TCP	
		SAL/TCP	IPR/TCP	SAL/TCP	IPR/TCP	SAL/TCP	IPR/TCP
Brain	1 hr	1071 ± 65	1712 ± 154*	1009 ± 150	1818 ± 205*	1.07	0.95*
	3 hr	312 ± 24	712 ± 129*	198 ± 17	530 ± 69*	1.60	1.32*
Liver	1 hr	1193 ± 111	2349 ± 441*	868 ± 84	1763 ± 327*	1.39	1.33
	3 hr	687 ± 87	1262 ± 145*	370 ± 70	806 ± 226*	1.83	1.57

Values for (+)- and (−)-TCP are expressed as means ± SEM (N = 5–10). Rats were injected with saline vehicle (SAL) or with iprindole (IPR)·HCl (11.2 mg/kg, i.p.) and 1 hr later with (±)-TCP (2.5 mg/kg). The rats were killed at either 1 or 3 hr following the (±)-TCP injection.

* $P < 0.05$, compared to values in rats pretreated with SAL/TCP.

Levels in rat tissues. The doses of TCP administered to rats reported in the literature vary widely (approximately 0.5 to 25 mg/kg). For the present investigation, a dose of TCP of 2.5 mg/kg was chosen since this is similar, on a mg/kg basis, to clinical doses which have been reported recently to be useful in treating refractory depressives [20]. The results obtained by measuring the concentrations of each enantiomer after the administration of racemic TCP (Table 1) suggest that there are differences in the pharmacokinetics of (+)-TCP and (−)-TCP. In the brain, the levels of (+)- and (−)-TCP were similar at 1 hr, but after 3 hr the change in the ratio indicated that the concentration of (+)-TCP was higher than that of (−)-TCP. A similar pattern of change was evident in rat liver. These preliminary results would appear to be due to the increased clearance of (−)-TCP over that of (+)-TCP, which would agree with the findings of Hampson *et al.* [7] who examined rat brain levels of each enantiomer after intraperitoneal administration of the individual enantiomers and found that the (−)-enantiomer is cleared faster than the (+)-enantiomer. Fuentes *et al.* [4] reported that rat brain levels of (−)-TCP are higher than those of (+)-TCP 15 min after separate administration of the enantiomers but that by 60 min concentrations of the enantiomers are similar. Reynolds *et al.* [6] in a study in which human subjects were administered (+)- and (−)-TCP on separate occasions found that plasma levels of (−)-TCP are higher than those of (+)-TCP after a single dose (3 hr) whereas the reverse effect is apparent after drug administration for 3 days. Mutschler *et al.* [17] reported that in humans (−)-TCP plasma levels are higher than those of (+)-TCP for up to 8 hr after drug administration, whether the enantiomers are given separately or the TCP is given as the racemate; interestingly, administration of racemic TCP resulted in higher plasma levels of both enantiomers when compared to levels observed after administering the individual enantiomers.

The levels of TCP both in the brain and the liver showed an increase after pretreatment with iprindole, a drug known to block aromatic ring hydroxylation. This elevation was observed at both 1 and 3 hr post-TCP injection. This is in agreement with a previous study conducted by Hampson *et al.* [7] in which an increase in whole brain levels of (±)-TCP after pretreatment with iprindole was reported, but it is our belief that this is the first time that the effects of iprindole on TCP enantiomers have been reported. Other studies have identified 4-hydroxy-TCP as a metabolite of TCP both in the brain and the heart [21, 22]. It is of interest that iprindole seems to have a similar effect on both enantiomers, as indicated by the similarity of the ratios of the enantiomers in both treatment groups. Although the ratio of (+)-TCP to (−)-TCP in brain was slightly lower in the IPR/TCP group than in the SAL/TCP group at both 1 and 3 hr, these differences were not evident in liver (see Table 1). Although we have assumed that the elevation of TCP was the result of the known effects of iprindole on aromatic ring hydroxylation [23–25], a possible direct effect of iprindole on the clearance of TCP cannot be ruled out at this time. It should be emphasized that the results presented here represent findings at only two time intervals, and detailed studies on the kinetics of the interaction of IPR and TCP should now be conducted.

In summary, a rapid gas chromatographic procedure has been developed for the simultaneous analysis of both enantiomers of TCP. This method is readily applicable to body fluids and tissues from rats and should be useful in future studies on the pharmacodynamics and pharmacokinetics of (+)- and (−)-TCP.

Acknowledgements—Operating funds for this project were provided by the Medical Research Council of Canada. L. Aspeslet is an Alberta Mental Health Research Fund Scholar. The authors are grateful to Ms. S. Omura for typing this manuscript.

Neurochemical Research Unit
Department of Psychiatry and
Faculty of Pharmacy and
Pharmaceutical Sciences
University of Alberta
Edmonton, Alberta
Canada T6G 2B7

LAUNA J. ASPESLET
GLEN B. BAKER*
RONALD T. COUTTS
DARRELL D. MOUSSEAU

REFERENCES

1. Coutts RT and Baker GB, Implications of chirality and geometric isomerism in some psychoactive drugs and their metabolites. *Chirality* **1**: 99–120, 1989.
2. Jamali F, Mehvar R and Pasutto FM, Enantioselective aspects of drug action and disposition: Therapeutic pitfalls. *J Pharm Sci* **78**: 695–715, 1989.
3. Horn AS and Snyder SH, Steric requirements for catecholamine uptake by rat brain synaptosomes: Studies with rigid analogs of amphetamine. *J Pharmacol Exp Ther* **180**: 523–530, 1972.
4. Fuentes JA, Oleshansky MA and Neff NH, Comparison of the apparent antidepressant activity of (−) and (+) tranylcypromine in an animal model. *Biochem Pharmacol* **25**: 801–804, 1976.

* Corresponding author: Dr. Glen B. Baker, Neurochemical Research Unit, Department of Psychiatry, University of Alberta, Edmonton, Alberta, Canada T6G 2B7. Tel. (403) 492-6591; FAX (403) 492-6841.

5. Reigle TG, Orsulak PJ, Avni J, Platz PA and Schildkraut JJ, The effects of tranylcypromine isomers on norepinephrine-H^3 metabolism in rat brain. *Psychopharmacology* **69**: 193–199, 1980.
6. Reynolds GP, Rausch W-D and Riederer P, Effects of tranylcypromine stereoisomers on monoamine oxidation in man. *Br J Clin Pharmacol* **9**: 521–523, 1980.
7. Hampson DR, Baker GB and Coutts RT, A comparison of the neurochemical properties of the stereoisomers of tranylcypromine in the central nervous system. *Cell Mol Biol* **32**: 593–599, 1986.
8. Tuomisto J and Smith DF, Effects of tranylcypromine enantiomers on monoamine uptake and release and imipramine binding. *J Neural Transm* **65**: 135–145, 1986.
9. Smith DF and Petersen HN, Stereoselective effect of tranylcypromine enantiomers on brain serotonin. *Life Sci* **31**: 2455–2461, 1982.
10. Fuentes JA, Oleshansky MA and Neff NH, A sensitive enzymatic assay for *dextro-* or *levo*-tranylcypromine in brain. *Biochem Pharmacol* **24**: 1971–1973, 1975.
11. Lang VA, Geissler HE and Mutschler E, Bestimmung und Vergleich der Plasma und Urinkonzentrationen nach Gabe von (+)- und (−)-Tranylcypromin. *Arzneimittel Forschung/Drug Res* **29**: 154–157, 1979.
12. Youdim MBH, Aronson JK, Blau K, Green AR and Grahame-Smith DG, Tranylcypromine ("Parnate") overdose: Measurement of tranylcypromine concentrations and MAO inhibitory activity and identification of amphetamines in plasma. *Psychol Med* **9**: 377–382, 1979.
13. Bailey E and Barron EJ, Determination of tranylcypromine in human plasma and urine using high-resolution gas–liquid chromatography with nitrogen-sensitive detection. *J Chromatogr Biomed Appl* **183**: 25–31, 1980.
14. Calverley DG, Baker GB, Coutts RT and Dewhurst WG, A method for measurement of tranylcypromine in rat brain regions using gas chromatography with electron capture detection. *Biochem Pharmacol* **30**: 861–867, 1981.
15. Edwards DJ, Mallinger AG, Knopf S and Himmelhoch JM, Determination of tranylcypromine in plasma using gas chromatography–chemical-ionization mass spectrometry. *J Chromatogr Biomed Appl* **344**: 356–361, 1985.
16. Rao TS, Baker GB and Coutts RT, Pentafluorobenzenesulfonyl chloride as a sensitive reagent for the rapid gas chromatographic analysis of tranylcypromine in tissues and body fluids. *Biochem Pharmacol* **35**: 1925–1928, 1986.
17. Mutschler E, Gietl Y, Krauss D, Martin E, Pflugmann G and Weber H, Stereospecific analysis and human pharmacokinetics of the enantiomers of drugs administered as racemates. In: *Chirality and Biological Activity* (Eds. Holmstedt B, Frank H and Testa B), pp. 199–219. Alan R. Liss, New York, 1990.
18. Hampson DR, Baker GB, Nazarali AJ and Coutts RT, A rapid and sensitive electron-capture gas chromatographic method for the analysis of tranylcypromine in brain tissue using acetylation and pentafluorobenzoylation. *J Biochem Biophys Methods* **9**: 85–87, 1984.
19. Keck PE, Carter WP, Nierenberg AA, Cooper TB, Potter WZ and Rothschild AJ, Acute cardiovascular effects of tranylcypromine: Correlation with plasma drug, metabolite, norepinephrine, and MHPG levels. *J Clin Psychiatry* **52**: 250–254, 1991.
20. Amsterdam JD and Berwish NJ, High dose tranylcypromine therapy for refractory depression. *Pharmacopsychiatry* **22**: 21–25, 1989.
21. Baker GB, Hampson DR, Coutts RT, Micetich RG, Hall TW and Rao TS, Detection and quantitation of a ring-hydroxylated metabolite of the antidepressant drug tranylcypromine. *J Neural Transm* **65**: 233–243, 1986.
22. Nazarali AJ, Baker GB, Coutts RT and Greenshaw AJ, Para-hydroxytranylcypromine: Presence in rat brain and heart following administration of tranylcypromine and an N-cyanoethyl analogue. *Eur J Drug Metab Pharmacokinet* **12**: 207–214, 1987.
23. Freeman JJ and Sulser F, Iprindole–amphetamine interactions in the rat: The role of aromatic hydroxylation of amphetamine in its mode of action. *J Pharmacol Exp Ther* **183**: 307–315, 1972.
24. Fuller R and Hemrick-Luecke S, Long-lasting depletion of striatal dopamine by a single injection of amphetamine in iprindole-treated rats. *Science* **209**: 305–307, 1980.
25. Steranka LR, Long-term decreases in striatal dopamine, 3,4-dihydroxyphenylacetic acid, and homovanillic acid after a single injection of amphetamine in iprindole-treated rats: Time course and time-dependent interactions with amfonelic acid. *Brain Res* **234**: 123–136, 1982.