Biochemical Pharmacology, Vol. 25, pp. 801–804. Pergamon Press, 1976. Printed in Great Britain.

COMPARISON OF THE APPARENT ANTIDEPRESSANT ACTIVITY OF (−) AND (+) TRANYLCYPROMINE IN AN ANIMAL MODEL

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(*Received* 20 *February* 1975; *accepted* 18 *July* 1975)

Abstract—The isomers of tranylcypromine (TCP) readily entered the rat brain after intraperitoneal administration and reached peak concentrations within 15 min. Apparently, (−) TCP entered the brain more rapidly and reached somewhat higher concentrations than (+) TCP. After a dose of 2·5 mg/kg of (−) or (+) TCP, there was significantly more drug in brain than has been reported necessary to block the re-uptake of amines by synaptosomes. Both isomers blocked monoamine oxidase *in vivo* and *in vitro*. (+) TCP was between 10 and 60 times more active than (−) TCP, depending on the amine substrate evaluated, and both isomers were better inhibitors of type-B monoamine oxidase activity than type-A activity. The (+) isomer was more active in preventing reserpine-induced sedation in the rat than the (−) isomer. The ability to prevent the reserpine syndrome was apparently related to the ability of the drugs to block monoamine oxidase activity rather than to blockade of amine re-uptake.

Tranylcypromine (TCP), a monoamine oxidase inhibitor that is a potent antidepressant [1], is a mixture of (−) and (+) *trans*-2-phenylcyclopropylamine. The (+) isomer is a more potent inhibitor of monoamine oxidase, while the (−) isomer is a more potent inhibitor of the uptake of catecholamines by nerve endings [2, 3]. Is the antidepressant activity of TCP associated with the (+) or the (−) isomer, that is, with inhibition of monoamine oxidase or inhibition of amine re-uptake? Several reports have implicated blockade of re-uptake as the probable mechanism for the antidepressant activity of TCP [2–4]. A pharmacologic test used to evaluate antidepressant drugs *in vivo* is the prevention or antagonism of reserpine-induced sedation in rat [5]. Drugs that inhibit monoamine oxidase as well as tricyclic compounds of the iminodibenzyl or cycloheptadiene structure are active in this test. Presumably both classes of drugs act by increasing the concentration of the biogenic amines at receptor sites after reserpine treatment. The tricyclic antidepressants prevent the re-uptake of released amines, while monoamine oxidase inhibitors delay the metabolism of the released amines. We present an evaluation of the antidepressant activity of the two isomers of TCP, using the antagonism of reserpine-induced inhibition of spontaneous motor activity, and compare this activity with the concentration of the isomers in brain and with the ability of the drugs to block monoamine oxidase.

MATERIALS AND METHODS

Assay of (+) and (−) tranylcypromine in brain

The assay, described previously,* is based on the enzymatic transfer of [^{14}C]methyl from *S*-adenosyl-L-methionine[^{14}C] to the isomers of TCP. This method probably measures free TCP rather than TCP bound to monoamine oxidase.

Male Sprague–Dawley rats (Zivic-Miller Labs., Allison Park, Pa.) 180–220 g, were injected intraperitoneally with one of the isomers of TCP (doses are expressed as HCl salts) and were killed by exposure of the head to a focused beam of microwave radiation for 2·7 sec (microwave oven, model LMMO, Medical Engineering Consultants, Lexington, Mass.). Brains were removed and homogenized in 2·4 vol of sodium phosphate buffer, 0·25 M (pH 8·0). Homogenates were centrifuged at 28,000 *g* for 25 min and the clear supernatants were used for analysis. Samples of supernatant (100 μl), rabbit lung *N*-methyl-transferase (100 μl) and *S*-adenosyl-L-methionine[^{14}C] (New England Nuclear, 58 mCi/m-mole; 5 μl) containing 0·1 μCi of radioactivity were incubated for 90 min at 37°. Duplicate samples containing internal standards of (+) or (−) TCP were assayed simultaneously. The reaction was stopped by adding 0·5 ml of 2 N NaOH, and *N*-methyltranylcypromine and *N*-dimethyltranylcypromine (0·01 μmole each) were added as carriers. The radioactive products were extracted into *n*-pentane. The organic phase was transferred to another tube and washed once with sodium phosphate buffer, 0·25 M (pH 8·0). A 5-ml portion of the organic phase was transferred to a counting vial and dried under a stream of air. The residue was dissolved in Aquasol (New England Nuclear) and counted in a Beckman LS-250 liquid scintillation counter with automatic quench correction.

Assay of monoamine oxidase activity

Studies in vitro. Rats were decapitated and their brains were homogenized in 20 vol. of 67 mM phosphate buffer (pH 7·2). Monoamine oxidase activity

* J. A. Fuentes, M. A. Oleshansky and N. H. Neff, *Biochem. Pharmac.* **24**, 1971 (1975).

was measured as described previously with radioactive tyramine[1-^{14}C] (2·1 mM), serotonin[2-^{14}C] (1·2 mM) or 2-phenylethylylamine[1-^{14}C] (0·2 mM) (New England Nuclear) as substrates [6, 7]. The isomers of TCP were preincubated with brain homogenate for 15 min at 20° before adding substrate, after which the incubation was continued at 37° for 30 min (serotonin 20 min). The specific radioactivity of the substrates was adjusted so that 5·10 × 10^3 c.p.m. of product was formed per sample of homogenate during the incubation of untreated samples. Serotonin is a specific substrate for type-A monoamine oxidase, 2-phenylethylamine is a specific substrate for type-B enzyme and tyramine is a common substrate, that is, a substrate for both types of enzyme [8].

Studies in vivo. The isomers of TCP were injected intraperitoneally and the animals were killed 15 min later by decapitation. TCP can be considered as an irreversible inhibitor; however, it is possible to partially restore enzyme activity by prolonged dialysis [9].

Antagonism of reserpine-induced sedation

Dextro- or *levo*-TCP, dissolved in water, was administered intraperitoneally to groups of five rats each, and after 15 min reserpine (2 mg/kg) was administered intravenously. Reserpine, 8 mg, was dissolved in 0·1 ml of acetic acid and diluted to 10 ml with water. Control animals were treated with solvents. Thirty min after the injection of reserpine, rats were placed in an infrared motility monitor (type 160FC, Motor Products, Sweden) and activity was recorded for 15 min.

RESULTS

Tranylcypromine concentrations in brain

The isomers of TCP readily entered brain after the intraperitoneal injection of 10 mg/kg (59 μmoles/kg) and reached peak concentrations in about 15 min (Fig. 1). Five min after injection, the concentration of (−) TCP was 60 per cent higher, and at 15 min it was 20 per cent higher than that of the (+) isomer. The (−) isomer may have entered brain more rapidly than the (+) isomer, or it is possible that (+) TCP was more readily incorporated into tissue components than the (−) isomer, resulting in higher concentrations of free (−) TCP in brain. At about 60 min the concentrations of the isomers were similar. When animals were killed 15 min after injecting increasing doses of the drugs, higher concentrations of the (−) isomer were found after 2·5, 5 and 10 mg/kg, but not after 20 mg/kg (Table 1).

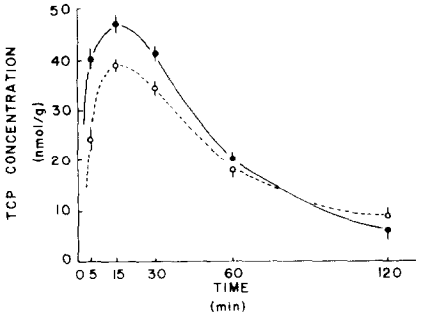

Fig. 1. Concentration of TCP isomers in rat brain following the administration of 10 mg/kg, i.p. (59 μmoles/kg). Values shown are the mean for five rats ± S.E.M.: (+) TCP (○) and (−) TCP (●).

Table 1. Concentration of (+) and (−) TCP in rat brain after various doses*

Dose [mg/kg (μmoles/kg)]	TCP concentrations (nmoles/g ± S.E.M.) (+)	(−)
20 (118)	102 ± 5	102 ± 7
10 (59)	39 ± 4	48 ± 2†
5 (29·5)	10 ± 2	16 ± 2†
2·5 (14·7)	4·9 ± 0·2	8·1 ± 0·9†

* The isomers of TCP were administered intraperitoneally to four to six rats which were killed 15 min later by exposure to microwave radiation.

† $P < 0·05$ when compared with (+) TCP-treated animals.

Inhibition of monoamine oxidase

In vitro, 10–60 times higher concentrations of (−) TCP were required to block enzyme activity by 50 per cent, depending on the amine substrate, when compared with (+) TCP (Fig. 2). Moreover, both isomers inhibited the deamination of 2-phenylethylamine at lower concentrations than were required to inhibit serotonin deamination. *In vitro*, the (+) isomer was about ten times more active than the (−) isomer when compared at 50 per cent inhibition of enzyme activity (Fig. 3), and the drugs blocked 2-phenylethylamine deamination more readily than serotonin deamination. Furthermore, the slopes of the percentage inhibition of enzyme activity curves were greater after (+) TCP than after (−) TCP. After high doses of the TCP isomers, inhibition of enzyme activity approached similar values.

Antagonism of reserpine-induced sedation

(+) TCP at doses of 2·5 or 5 mg/kg inhibited motor activity, while (−) TCP was without effect (Fig. 4).

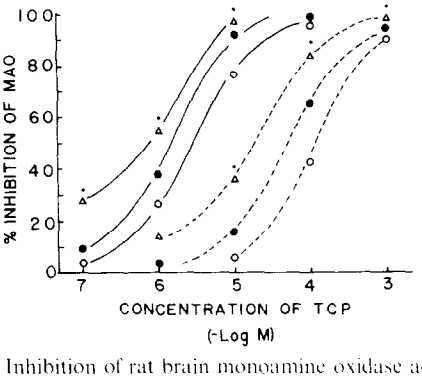

Fig. 2. Inhibition of rat brain monoamine oxidase activity *in vitro* by (+) TCP (solid line) and (−) TCP (dashed line) using three amine substrates. Substrates were 2-phenylethylamine (△), tyramine (●) and serotonin (○). Assays were performed as described in Materials and Methods. Each point represents the mean value for three homogenates. An asterisk indicates a significant difference ($P < 0·05$) between the inhibition of serotonin and 2-phenylethylamine deamination.

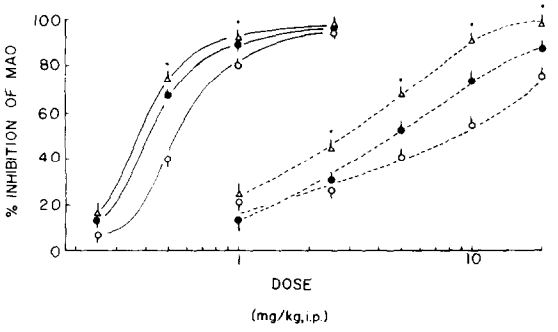

Fig. 3. Inhibition of rat brain monoamine oxidase activity *in vivo* by (+) TCP (solid line) and (−) TCP (dashed line) using three amine substrates. Substrates were 2-phenylethylamine (△), tyramine (●) and serotonin. (○). Assays were performed as described in Materials and Methods. Data are presented as mean ± S.E.M. for four to six animals. An asterisk indicates a significant difference ($P < 0.05$) between the inhibition of serotonin and 2-phenylethylamine deamination.

Reserpine (2 mg/kg, i.v.) virtually abolished activity. Pretreatment with the (+) isomer of TCP resulted in motor responses that were significantly greater than control value in rats treated with reserpine. In contrast, a dose of 2·5 mg/kg of (−) TCP had no effect on the activity of reserpine-treated rats, while the activity returned to about normal after 5 mg/kg of (−) TCP. Doses greater than 5 mg/kg of (+) or (−) TCP resulted in significant mortality when the animals were treated with reserpine.

DISCUSSION

The isomers of TCP readily passed into brain and reached peak concentrations within about 15 min.

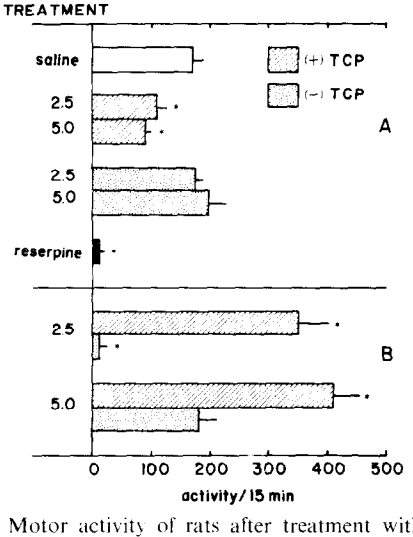

Fig. 4. Motor activity of rats after treatment with TCP or reserpine alone or in combination. (A) Activity was measured 45 min after treatment with TCP (doses in mg/kg, i.p. shown to the left), or saline alone, or 30 min after reserpine (2 mg/kg, i.v.) alone. (B) Motor activity after treatment with TCP and reserpine. TCP and reserpine were administered 45 and 30 min, respectively, before assaying motor activity. Data are presented as mean ± S.E.M. for five to ten animals. An asterisk indicates $P < 0.01$ when compared with saline-treated rats.

There was significantly more of the (−) isomer than of the (+) isomer in brain after all but the highest dose evaluated. Apparently the (−) isomer entered the circulation more rapidly or it was metabolized more slowly during passage through the liver than the (+) isomer. The greater difference between the concentrations of TCP in brain after low doses than after high doses may indicate that metabolic pathways in the periphery were approaching saturation as the dosage was increased. This, however, remains to be studied.

(+) TCP *in vitro* as well as *in vivo*, was a more effective inhibitor of monoamine oxidase than (−) TCP [10]. (+) TCP *in vitro*, was between 10 and 60 times more potent than (−) TCP when compared at 50 per cent blockade of enzyme activity. The degree of inhibition depended on the amine substrate. Apparently both (+) and (−) TCP are better inhibitors of type-B monoamine oxidase (blockade of 2-phenylethylamine deamination) than of type-A monoamine oxidase (blockade of serotonin deamination). This is not surprising, since TCP is similar in structure to the type-B enzyme inhibitor, deprenyl. *In vivo*, (+) TCP was about 10-fold more potent than (−) TCP when compared at 50 per cent inhibition of enzyme activity and both drugs were better inhibitors of type-B than of type-A enzyme.

The inhibition of monoamine oxidase found for the study *in vivo* (Fig. 3) was consistent with the expected inhibition (Fig. 2) for the concentration of drug found in brain (Table 1). For example, a dose of 2·5 mg/kg of the isomers produced concentrations in brain of about 4 and 8 μM for the (+) and (−) isomers respectively (Table 1). The expected inhibition of tyramine deamination for these concentrations from Fig. 2 would be about 80 and 15 per cent for (+) and (−) TCP respectively. The actual inhibition was about 95 per cent for (+) TCP and 30 per cent for (−) TCP (Fig. 3). The inhibition found in homogenates from injected rats may be somewhat different from the true inhibition *in vivo* if homogenization exposed uninhibited enzyme to TCP.

Reserpine in high doses produces depression in man [11]. The depression of spontaneous motor activity seen after reserpine in animals is used as a pharmacological model for the naturally occurring disorders [5]. However, reserpine has a multitude of pharmacological actions; thus it is doubtful that it is a good model for human depression. Nevertheless, many drugs that alleviate depression in man reverse the depression induced by reserpine in animals. These drugs include the monoamine oxidase inhibitors and the tricyclic compounds that block the re-uptake of released amines by nerve endings [5]. Both classes of drugs are thought to act by increasing the concentration of amines at receptor sites. Recently, however, the mechanism of action of the tricyclic compounds has been challenged. They are now postulated by some investigators [12] to act as monoamine oxidase inhibitors and not solely as inhibitors of amine re-uptake.

The (+) isomer of TCP is a more potent inhibitor of monoamine oxidase, while the (−) isomer is a more potent inhibitor of catecholamine re-uptake [2, 3]. When administered alone, (+) TCP inhibited normal motor activity while (−) TCP had no effect.

Horn and Snyder [2] postulated that if the antidepressant action of TCP involved monoamine oxidase inhibition the (−) isomer should be the less potent antidepressant, whereas if inhibition of catecholamine uptake is a major factor it should be the more active of the two isomers. We found that (+) TCP, 2·5 mg/kg, enhanced motor activity in reserpine-treated animals while the same dose of (−) TCP was without effect. Reserpine was administered when the concentration of free TCP was assumed to be the highest. After this dose, monoamine oxidase was blocked by about 95 per cent in the (+) TCP-treated animals and by about 30 per cent in the (−) TCP-treated animals. Although it is difficult to evaluate blockade of re-uptake of endogenous catecholamines by TCP within the synaptic junction, we can compare the concentration of drug in brain with the concentration of drug known to block re-uptake by synaptosomes. The concentration of (−) TCP in brain, 8 μM (Table 1), was about 15 times greater than that required to block norepinephrine, and about 8 times greater than that required to block dopamine re-uptake by synaptosomes by 50 per cent [2]. When the dose of (−) TCP was increased by 5 mg/kg, the reserpine syndrome was prevented concomitant with blockade of monoamine oxidase by about 50 per cent. We conclude, therefore, that in our animal model prevention of motor depression after reserpine is better correlated with inhibition of monoamine oxidase than with blockade of amine re-uptake.

A recent clinical report [4] found (−) TCP to be more effective than (+) TCP in depressed patients. There was no statistical difference in the side effects. The authors stated that their findings support the idea that inhibition of re-uptake of released amines appears to be a major mechanism for the antidepressant action of TCP. Their conclusion, however, lacks experimental support. Both TCP isomers block monoamine oxidase. The degree of enzyme inhibition is dependent on the concentration of drug in brain, which is related to dose as well as to metabolism of the isomers. Future clinical studies should include a measure of enzyme inhibition after both drugs, such as blockade of platelet enzyme, which has been shown to be related to blockade of brain enzyme [13], to evaluate the mechanism of action of the isomers. Moreover, the possibility should be considered that the isomers of TCP mimic some of the actions of amphetamine such as release of catecholamines [14, 15], which could also explain, in part, the antidepressant activity and the side effects of TCP.

Acknowledgements We thank Mr. J. Rubenstein for his expert technical assistance. The isomers of tranylcypromine were generously supplied by Dr. A. Misher of Smith, Kline & French Laboratories.

REFERENCES

1. A. Pletscher, in *Psychopharmacology: A Review of Progress* (Ed. D. H. Efron), p. 649. U.S. Government Printing Office, Washington, D.C. (1968).
2. A. S. Horn and S. H. Snyder, *J. Pharmac. exp. Ther.* **180,** 523 (1972).
3. E. D. Hendley and S. H. Snyder, *Nature, Lond.* **220,** 1330 (1968).
4. J. I. Escobar, B. C. Schiele and R. Zimmermann, *Am. J. Psychiat.* **131,** 1025 (1974).
5. E. B. Sigg, in *Psychopharmacology: A Review of Progress* (Ed. D. H. Efron), p. 655. U.S. Government Printing Office, Washington, D.C. (1968).
6. C. Goridis and N. H. Neff, *Br. J. Pharmac.* **43,** 814 (1971).
7. H.-Y. T. Yang and N. H. Neff, *J. Pharmac. exp. Ther.* **187,** 365 (1973).
8. N. H. Neff and H.-Y. T. Yang, *Life Sci.* **14,** 2061 (1974).
9. L. Hellerman and V. G. Erwin, *J. biol. Chem.* **243,** 5234 (1968).
10. C. L. Zirkle, C. Kaiser, D. H. Tedeschi, R. E. Tedeschi and A. Burger, *J. med. Chem.* **5,** 1265 (1962).
11. R. W. Achor, N. O. Hanson and R. W. Gifford, *J. Am. med. Ass.* **159,** 159 (1955).
12. J. A. Roth and C. N. Gillis, *Biochem. Pharmac.* **23,** 2537 (1974).
13. D. S. Robinson, W. Lovenberg, H. Keiser and A. Sjoerdsma, *Biochem. Pharmac.* **17,** 109 (1968).
14. J. J. Schildkraut, *Am. J. Psychiat.* **126,** 925 (1970).
15. T. G. Reigle, P. A. Platz, J. Avni and J. J. Schildkraut, *Fedn Proc.* **33,** 245 (1974).