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A SENSITIVE ENZYMATIC ASSAY FOR *DEXTRO*- OR *LEVO*-TRANYLCYPROMINE IN BRAIN

JOSÉ A. FUENTES, MARVIN A. OLESHANSKY and NORTON H. NEFF

Laboratory of Preclinical Pharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, DC. 20032, U.S.A.

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Abstract—A sensitive assay for (+)- or (−)-tranylcypromine in brain is described that is based on the enzymatic transfer of ^{14}C-methyl of *S*-adenosyl-L-methionine-^{14}C to tranylcypromine by rabbit lung *N*-methyltransferase. The assay is sensitive enough to measure the drugs in small samples of brain and simple enough so that many assays can be performed in a single day. Studies with amine-containing compounds indicate that other drugs might also be assayed in brain by this procedure.

Tranylcypromine, a monoamine oxidase inhibitor that is used to treat depression [1], is a mixture of (+)- and (−)-trans-2-phenylcyclopropylamine. The (+)-isomer is a more potent inhibitor of monoamine oxidase than the (−)-isomer, and tests in animals suggested that it might be a more potent antidepressant [2]. Recent studies, however, suggest that blockade of catecholamine reuptake by nerve endings might be better correlated with the antidepressant activity of tranylcypromine than blockade of monoamine oxidase [3, 4]. Moreover, a recent clinical study found (−)-tranylcypromine to be more active than (+)-tranylcypromine in depressed patients [5]. The pharmacological action of a drug is usually correlated with the concentration of the drug at its site of action. This communication describes a sensitive assay for (+)- or (−)-tranylcypromine in brain that is based on the enzymatic transfer of the ^{14}C-methyl of *S*-adenosyl-L-methionine-^{14}C (SAM-^{14}C) to tranylcypromine by an *N*-methyltransferase purified from rabbit lung [6]. Axelrod *et al.* [6–9] have demonstrated that *N*-methyltransferases can be successfully used to assay endogenous as well as exogenous amines in animal tissue.

MATERIALS AND METHODS

Preparation of rabbit lung N-methyltransferase. Frozen rabbit lung (Type 1) was purchased from Pel-Freeze Biologicals, Rogers, Ark. The enzyme was prepared as described by Axelrod [6], and the final preparation was divided into aliquots and frozen until used [7]. The enzyme preparation had a protein concentration of 20–30 mg/ml and about 40–60 units/ml using (+)-tranylcypromine as substrate. One unit of enzyme activity is defined as the amount of enzyme that forms 1 nmole product/mg of protein/hr.

Assay of tranylcypromine. Male, Sprague–Dawley rats (Zivic Miller Labs., Allison Park, Pa.), 180–220 g, were injected intraperitoneally with the isomers of tranylcypromine and were killed by exposure of the head, for 2·7 sec, to a focused beam of microwave radiation (model LMMO Microwave Oven, Medical Engineering Consultants, Lexington, Mass.). This procedure denatures protein without destroying the morphology of the brain. Brains were removed and dissected as described by Glowinski and Iversen [10] or homogenized whole 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 assay.

Reactions were carried out in 15-ml screw-cap tubes. The tubes contained: samples of supernatant, 100 μl; rabbit lung *N*-methyltransferase, 100 μl; and SAM-^{14}C (New England Nuclear, Boston, Mass.; 58 mCi/m-mole), 5 μl containing 0·1 μCi of activity. Samples were incubated for 90 min at 37°.

Internal standards of (+)- or (−)-tranylcypromine, 5 nmoles, were added to a separate set of samples. Calculations were based on the differences of radioactivity between internal standards and samples of brain. Tissue blank values were obtained by assaying brain from animals not treated with tranylcypromine or from animals treated 12 hr prior to assay with pargyline (75 mg/kg, i.p.).

The enzymatic reaction was terminated by adding 0·5 ml of 2 N NaOH. A solution containing 0·01 μmole each of *N*-methyltranylcypromine and *N*-dimethyltranylcypromine was added to each tube. The radioactive products were extracted into 6 ml *n*-pentane by mechanically shaking the samples for 10 min. After centrifugation, 5 ml of the organic phase was transferred to another screw-cap tube containing 0·5 ml isoamyl alcohol and 1·0 ml sodium phosphate buffer, 0·25 M (pH 8·0). After shaking for 10 min, followed by centrifugation, 5 ml 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.

Identification of the radioactive reaction products. The final air-dried residue, from animals treated with tranylcypromine or from control reactions where the isomers were added to brain samples of untreated animals, was dissolved in 0·25 ml methanol and then applied to prescored Silica gel G thin-layer chromatographic plates (Analtech, Newark, Del.). The developing solvents were: chloroform–methanol–water (90:10:1) or 1-butanol–acetic acid–water (60:15:25).

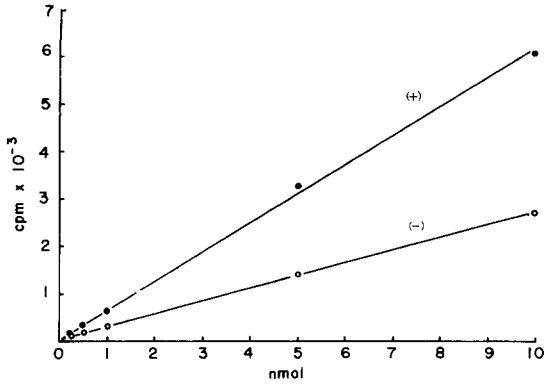

Fig. 1. Linearity of the enzymatic methylation of (+)- and (−)-tranylcypromine by lung *N*-methyltransferase. Rats were killed by microwave irradiation and their brains were homogenized in 2 vol. of sodium phosphate buffer, 0·25 M (pH 8·0). After centrifugation, tranylcypromine was added to samples of supernatant and the assay was conducted as described. Each point is the mean of duplicate determinations.

N-methyltranylcypromine and *N*-dimethyltranylcypromine were visualized by exposing the plates to iodine vapor. Each plate was then divided into 1-cm sections and the Silica gel was scraped into counting vials containing 5 ml Aquasol. Radioactivity was detected in a liquid scintillation counter.

Enzymatic methylation of amines. Various amines were tested (see Table 2) as substrates, using the extraction procedures outlined, to determine the feasibility of developing methods for other compounds. Rats were killed by microwave irradiation and their brains were homogenized with 4 vol of sodium phosphate buffer, 0·25 M (pH 8·0) and then centrifuged at 25,000 *g* for 25 min. Twenty-five nmoles amine were added to 100-μl aliquots of supernatant, and the reaction and extraction were conducted as already described. No attempts were made to identify the product of the reaction.

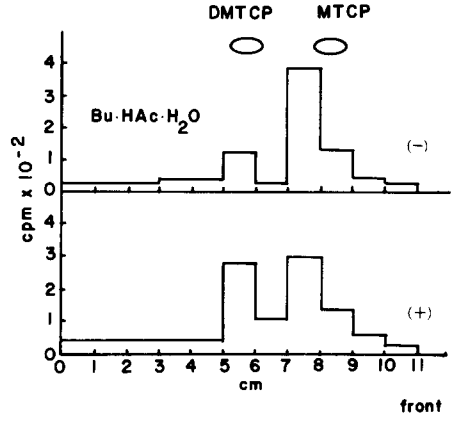

Fig. 2. Chromatographic identification of reaction products. Rats were injected with (+)- or (−)-tranylcypromine (10 mg/kg, i.p.) and sacrificed by microwave irradiation 15 min later. Samples were then prepared as described for assay except that the air-dried residue was dissolved in methanol and applied to Silica gel G thin-layer plates and developed with 1-butanol–acetic acid–water (60:15:25). *N*-dimethyltranylcypromine (DMTCP) and *N*-methyltranylcypromine (MTCP) were added as standards and were localized by exposing the plate to iodine vapor. Each plate was divided into 1-cm sections and counted for radioactivity.

RESULTS

Methylation of tranylcypromine. The rate of methylation of (+)- and (−)-tranylcypromine was linear from 0·25 to 10 nmoles (Fig. 1), and the enzyme was more active toward the (+)-isomer than the (−)-isomer. Background activity varied from 55 to 130 cpm and was directly related to the age of the SAM-^{14}C.

To test for the interference of endogenous amines with the assay of tranylcypromine, pargyline (75 mg/kg, i.p.) was administered 12 hr before assaying brain tissue. Background activity for control and pargyline-treated animals was 88 ± 4 and 87 ± 10 cpm $\pm$ S.E.M. ($N = 5$) respectively.

To evaluate the effect of heat on the stability of tranylcypromine, identical samples of brain were prepared containing 5 nmoles of (+)- or (−)-isomer, and half of the samples were heated in a water bath for 3 min at 98° and then all of the samples were assayed as outlined. Heating had no effect on the outcome of the assay.

Identification of radioactive products. Two radioactive products were detected by chromatography in the two solvent systems, *N*-methyltranylcypromine and *N*-dimethyltrancylcypromine. Both products were found after the administration of either isomer of tranylcypromine (Figs. 2 and 3) and in samples where internal standards were added to brain extract from untreated animals.

Concentration of tranylcypromine in brain parts. The isomers of tranylcypromine were rather uniformly distributed in brain 15 min after administration (10 mg/kg, i.p.); however, the concentration of the (−)-isomer was about 20 per cent higher than the concentration of the (+)-isomer in brain parts (Table 1).

Methylation and extraction of other amines from brain. A variety of amines were evaluated by the methylation and extraction procedure outlined for the potential development of sensitive enzymatic assays (Table 2). Fenfluramine and norfenfluramine were several-fold better as substrates for methylation and

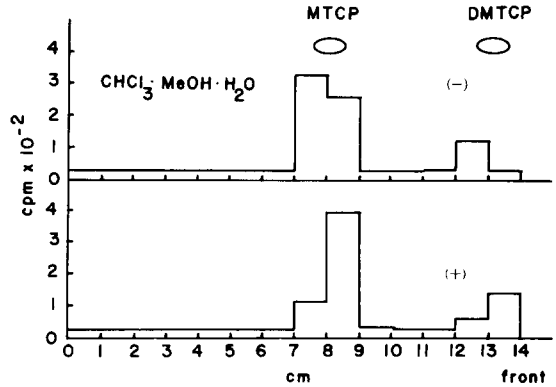

Fig. 3. Chromatographic identification of reaction products. Animals and samples were treated as in Fig. 2. The developing medium was chloroform–methanol–water (90:10:1).

Table 1. Concentration of tranylcypromine in areas of rat brain*

Area	Tranylcypromine (nmoles/g brain ± S. E. M.) (3) (+)	(−)
Midbrain	37 ± 9	46 ± 4
Cerebellum	34 ± 1	45 ± 3
Cortex	39 ± 3	48 ± 3
Hippocampus	44 ± 7	74 ± 5
Medulla oblongata	56 ± 4	67 ± 7
Hypothalamus	56 ± 2	77 ± 6
Striatum	56 ± 9	73 ± 3

* *Dextro-* or *levo*-tranylcypromine was injected (10 mg/kg, 59 μmoles/kg) intraperitoneally, and the animals were killed after 15 min by exposure to microwave radiation. Tranylcypromine was assayed as described.

subsequent extraction by *n*-pentane than any of the other amines tested.

DISCUSSION

The transfer of the ^{14}C-methyl from *S*-adenosyl-L-methionine-^{14}C to various compounds by methyltransferases has been used to develop numerous sensitive enzymatic assays for endogenous constituents as well as administered drugs [6–9]. We have demonstrated that lipophilic drugs, such as tranylcypromine, can be successfully assayed in small areas of brain, without interference from endogenous amines, if a lipophilic organic solvent is used for extraction. Pargyline (75 mg/kg, i.p.) was administered to elevate endogenous amines, but there was no significant difference between background values found for control animals and pargyline-treated animals. Moreover, our procedure, as outlined, could probably be used to assay the concentration of other lipophilic drugs in brain, such as fenfluramine and norfenfluramine.

Table 2. Methylation of amine substrates by rabbit lung *N*-methyltransferase*

Substrate	Activity (cpm/sample)
(+) Amphetamine	1573
(−) Amphetamine	3754
p-Chlorophenylpropylamine	332
Benzylamine	229
Phenylethylamine	303
Norephedrine	201
Ethylenediamine	114
p-Chlorophenylethylamine	1773
Fenfluramine	12,395
Norfenfluramine	56,667
Blank	106

* After microwave irradiation, rat brain was homogenized in 4 vol. of phosphate buffer, 0·25 M (pH 8·0) and then centrifuged at 28,000 *g* for 25 min. Twenty-five nmoles amine were added to 100-μl portions of supernatant, and the assay was performed as outlined in the Methods. Each value is the mean of duplicate determinations.

Methylated products of the reaction with tranylcypromine were identified by chromatography as *N*-methyltranylcypromine and *N*-dimethyltranylcypromine. The reaction products formed were proportional to the concentrations of tranylcypromine from 0·25 to 10 nmoles/sample, the (+)-isomer producing about 2·5 times more radioactivity than the (−)-isomer.

Both isomers of tranylcypromine readily entered the brain. After a dose of 59 μmoles/kg, i.p. (10 mg/kg), concentrations as high as 56 and 77 μmoles/kg were detected after 15 min for the (+)- and (−)-isomer, respectively. The differences in tranylcypromine concentrations found from brain area to brain area are probably due to differences in the circulation of blood. The 20 per cent difference between the concentrations of the (+)- and (−)-isomer in brain may reflect differences in the rate of metabolism of the two drugs.

In conclusion, tranylcypromine can be measured in brain without interference from endogenous amines by a sensitive enzymatic assay. The procedure as outlined can most probably be used to assay other lipophilic drugs as well.

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