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ACKNOWLEDGMENTS AND ADDRESSES

Received March 26, 1976, from the *College of Pharmacy, Butler University, Indianapolis, IN 46208.*

Accepted for publication August 26, 1976.

The authors thank the Riley Tar and Chemical Co., Indianapolis, Ind., for the gift of methylpyridines and dimethylpyridines.

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Determination of Dextrorphan in Plasma and Evaluation of Bioavailability of Dextromethorphan Hydrobromide in Humans

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Abstract □ A method is described for the estimation of dextrorphan, a metabolite of dextromethorphan, in plasma. The bioavailability of dextromethorphan hydrobromide after 30 mg po, as measured by the concentration of total (free and conjugated) dextrorphan in the plasma, was determined in six human volunteers with this procedure.

Keyphrases □ Dextrorphan—fluorometric analysis, human plasma □ Dextromethorphan hydrobromide—bioavailability evaluated using fluorometric analysis of dextrorphan in human plasma □ Fluorometry—analysis, dextrorphan in human plasma □ Bioavailability—dextromethorphan hydrobromide evaluated using fluorometric analysis of dextrorphan in human plasma □ Antitussives—dextromethorphan hydrobromide, bioavailability evaluated using fluorometric analysis of dextrorphan in human plasma

Dextromethorphan hydrobromide, a widely accepted, nonnarcotic antitussive agent, is a common ingredient in many cough–cold preparations. Despite its clinical use for over two decades, a method for estimating its plasma levels in humans has not been published. Presently available analytical methods lack the sensitivity needed to determine its plasma levels after oral administration of the maximum permissible over-the-counter dose of 30 mg[1] (1).

The metabolism of dextromethorphan has been studied in several species (2–6). Following oral administration, this drug is rapidly metabolized; the principal metabolites are the *O*-demethylated product dextrorphan and its glucuronide and sulfate ester conjugates. In humans, dextrorphan [(+)-3-hydroxy-*N*-methylmorphinan], (+)-3-hydroxymorphinan, and traces of the unmetabolized drug were found in urine after oral administration of the drug (6). Enzymatic hydrolysis of urines from patients administered dextromethorphan yielded 40–50% of the drug and metabolites, mostly in the form of glucuronide and sulfate conjugates[1].

Because the blood levels of unmetabolized dextromethorphan were low, another approach was to measure the levels of the major metabolites. Dextrorphan was estimated in plasma and urine by paper chromatography (7), colorimetric determination of the methyl orange complex (8), and radiotracer techniques (9). These methods suffer from inherent disadvantages such as a lack of sensitivity and cumbersome operation. This report describes a sensitive and more convenient method for the determination of plasma levels of dextrorphan and its conjugates. Its applicability to bioavailability studies in humans also is demonstrated.

EXPERIMENTAL

Plasma Levels of Dextrorphan and Its Conjugates—Plasma (3.0 ml) was transferred into a glass-stoppered erlenmeyer flask (25 ml). The pH was adjusted to 5.5 with 3.0 *N* acetic acid, and an enzyme solution[2] (0.2 ml) containing 30,000 units of β-glucuronidase and 10,000 units of arylsulfatase was added. The flask was stoppered and incubated at 37° for 2 hr. The incubate was brought to room temperature, and the pH of the contents was adjusted to 9.5 by the addition of a saturated solution of sodium carbonate (0.6 ml).

Spectroscopically pure ethyl acetate (15 ml) was then pipetted into the flask, and the contents were shaken for 20 min on a mechanical shaker. The mixture was transferred quantitatively into a 30-ml centrifuge tube, and the extract was separated by centrifuging at 3000 rpm for 15 min. Then an aliquot (12 ml) of the supernate was transferred into a stoppered 50-ml erlenmeyer flask containing 3.0 ml of 1.0 *N* HCl, and

[1] B. A. Koechlin and F. Rubio, work cited in Ref. 1.

[2] Glusulase, Endo Laboratories, Garden City, NY 11530.

the drug and its metabolites were extracted into the acid by shaking the mixture for 20 min.

The aqueous layer was separated using a 50-ml separator, and its fluorescence was measured in a fluorescence spectrophotometer[3]. The fluorescence and excitation wavelengths were 310 and 280 nm, respectively, and the slit dials were set to give a bandpass of 8 nm for excitation and of 5 nm for fluorescence. The concentrations of the drug and its metabolites in the sample were read from a calibration curve whose validity was determined by including three or four known concentrations of dextrorphan added to normal human plasma with each series of unknowns.

Bioavailability Studies in Humans—Six healthy male volunteers, 27–32 years old, received 30 mg po of dextromethorphan hydrobromide in a syrup (10 ml) after an overnight fast. Blood samples were drawn from the antecubetal vein into heparinized tubes immediately prior to and at 15 and 30 min and 1, 2, and 4 hr after drug administration. Plasma was separated by centrifuging at 3000 rpm for 20 min, and an aliquot was assayed by the described procedure.

RESULTS AND DISCUSSION

A calibration curve was constructed by plotting the fluorescence intensity against the concentration of dextrorphan added to drug-free plasma. Regression analysis of the least-squares line fitting the data points provided an equation for the curve as $y = 0.2658x + 3.728$ ($r = 0.9956$), where y represents fluorescence units and x is nanograms of dextrorphan per milliliter of plasma. The recovery of known amounts of dextrorphan added to human plasma was 70%. To determine the precision of the method, eight plasma samples spiked with 400 ng of dextrorphan/ml and four samples containing 600 ng of dextrorphan/ml were analyzed in duplicate over 6 months. The coefficients of variation at these two concentrations were 8 and 4%, respectively.

The fluorescence characteristics of dextrorphan and dextromethorphan in 1.0 *N* hydrochloric acid are rather similar. The fluorescence and excitation wavelengths of dextrorphan are 310 and 280 nm, respectively; for dextromethorphan, they are 305 and 270 nm, respectively.

The fluorescence spectrum of dextrorphan extracted from plasma was identical with that of dextrorphan in 1.0 *N* HCl. Some interference by the unmetabolized drug and possibly other minor metabolites could be expected in the measurement of dextrorphan levels. However, the contribution to fluorescence by the unmetabolized drug would be negligible in this method because extracts from plasma of humans administered dextromethorphan hydrobromide exhibited fluorescence spectra characteristic of dextrorphan but not dextromethorphan. Furthermore, since dextromethorphan is metabolized rapidly and extensively upon oral administration, the amount present in the extracts would be very low.

Plasma Levels in Humans—The average plasma levels ($\pm SE$) of dextrorphan and its conjugates following 30 mg po of dextromethorphan hydrobromide to six volunteers at 15, 30, and 60 min were 21.3 ± 6.7, 107.3 ± 44.6, and 368.0 ± 71.2 ng/ml, respectively. The peak level was 381.3 ± 56.9 ng/ml at 2 hr and it declined thereafter, reaching 262.7 ± 43.3 ng/ml at 4 hr. Almost all of the dextrorphan was present in the form of conjugates. This finding was confirmed by analyzing the plasma samples from one of the six volunteers, with and without enzymatic hydrolysis. Whereas hydrolysis gave rise to concentrations ranging from 26 to 380 ng/ml, none of the unhydrolyzed samples contained measurable levels of the unconjugated drug or metabolites.

It may be concluded that this method is applicable to the determination of the bioavailability of dextromethorphan hydrobromide in humans.

[3] Perkin-Elmer model MPF-3.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 10, 1974, from the *Warner–Lambert Research Institute, Morris Plains, NJ 07950.*

Accepted for publication August 18, 1976.

Presented at the 57th Annual Meeting of the Federation of the American Societies for Experimental Biology, Atlantic City, N.J., April 1973.

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Effects of 3,4-Dimethoxyphenethylamine Derivatives on Monoamine Oxidase

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Abstract □ The cactus alkaloid 3,4-dimethoxyphenethylamine and its naturally occurring *N*-methylated homologs inhibited the deamination of tyramine and tryptamine by rat brain monoamine oxidase. In contrast, the β-hydroxylated derivatives of this series failed to inhibit the action of monoamine oxidase on both tyramine and tryptamine.

Keyphrases □ 3,4-Dimethoxyphenethylamine and *N*-methyl homologs—effect on monoamine oxidase-catalyzed deamination of tyramine and tryptamine □ Monoamine oxidase—deamination of tyramine and tryptamine, effect of 3,4-dimethoxyphenethylamine and *N*-methyl homologs □ Tyramine—monoamine oxidase-catalyzed oxidative deamination, effect of 3,4-dimethoxyphenethylamine and *N*-methyl homologs □ Tryptamine—monoamine oxidase-catalyzed deamination, effect of 3,4-dimethoxyphenethylamine and *N*-methyl homologs □ Enzymes—monoamine oxidase, deamination of tyramine and tryptamine, effect of 3,4-dimethoxyphenethylamine and *N*-methyl homologs □ Structure–activity relationships—3,4-dimethoxyphenethylamine and *N*-methyl homologs, effect on monoamine oxidase-catalyzed deamination of tyramine and tryptamine

The present study was directed at observing the effects of 3,4-dimethoxyphenethylamine (I) and its *N*-methyl homologs on rat brain monoamine oxidase, using both tyramine and tryptamine as substrates. The results of these experiments were contrasted with those obtained with the β-hydroxy derivatives of the same series. The al-