Possible Alternate Routes in the Metabolism of Betahistine in the Rabbit (36679)

FAYE J. BOWMAN, EDWARD R. BOWMAN, AND HERBERT MCKENNIS, JR.
Medical College of Virginia, Department of Pharmacology, Richmond, Virginia 23219

In recent years the orally active histamine-like drug betahistine (2-(2-methylaminoethyl)pyridine dihydrochloride) has been used (1–3) in the treatment of Meniere's disease and other conditions presumed to be or actually accompanied by poor microcirculation. Interest in the mechanisms (4, 5) through which vascular resistance is decreased by betahistine led us (6) to compare vascular responses to betahistine and related compounds. Betahistine and its *N*-demethyl derivative on a molar basis were equipotent in decreasing vascular resistance in the forelimb of the dog, while one of the acidic metabolites of betahistine, 2-pyridylacetate (6, 7), produced no noticeable effect.

From available data on the metabolism of betahistine and related compounds (8, 9), it was considered (6) that demethylbetahistine (2-(2-aminoethyl)pyridine) and 2-(2-hydroxyethyl)pyridine might both participate as intermediates in the metabolism of betahistine to 2-pyridylacetate. Preliminary experiments have shown (10) that the 12000 x g supernatant from rabbit liver is rich in ability to oxidize betahistine to 2-pyridylacetate. The present study provides evidence for the conversion of betahistine, demethylbetahistine, and 2-(2-hydroxyethyl)pyridine to 2-pyridylacetate in the rabbit and the conjugation of some of this 2-pyridylacetate with glycine to form *N*-2-pyridylacetylglycine.

Materials and Methods. Betahistine base (2-(2-methylaminoethyl)pyridine, demethylbetahistine base (2-(2-aminoethyl)pyridine), 2-(2-hydroxyethyl)pyridine, and 2-pyridylacetic acid hydrochloride were obtained from the Aldrich Chemical Co. Thin-layer chromatograms were prepared on silica gel plates (E. Merck) with solvent K (11) and on Whatman No. 1 paper in solvent C (12). The dried chromatograms were sprayed with 2% *p*-aminobenzoic acid, redried, and exposed to cyanogen bromide to locate Koenig-positive zones. Ultraviolet absorption spectra were obtained with a Cary model 11 PM recording spectrophotometer. Gas chromatography was carried out at 165° on a stainless steel column (6 ft × 1/8 in.) packed with 3% SE-30 on 80–90 mesh Anakrom ABS; 50 ml/min helium carrier; injector at 200°, and detector at 210°.

N-2-Pyridylacetylglycine was prepared by a modification of the method of Burns, Duncan and Scales (9). The ethyl ester was prepared by refluxing a mixture of 30 mg of *N*-2-pyridylacetylglycine in 4 ml of absolute ethanol and 0.01 ml concentrated sulfuric acid for 16 hr. The cooled solution was treated with 10 ml of methylene chloride followed by 5 g of crushed ice. The aqueous phase was adjusted to pH 9 by addition of 10% sodium hydroxide. The mixture was shaken vigorously and the methylene chloride extract was removed. The aqueous phase was reextracted twice with 10 ml of methylene chloride. The combined extracts were dried with anhydrous magnesium sulfate. After filtration the filtrate was concentrated to yield 34 mg of ethyl *N*-2-pyridylacetylglycine. The light yellow oil was dried over KOH at 1 mm (RT 9 min; R_f 0.62 solvent K; R_f 0.71 solvent C), and stored in the desiccator for use as a primary standard.

Commercial 2-pyridylacetic acid hydrochloride (2.0 g), which had been recently recrystalized from absolute ethanol, was dissolved in 10 ml of water. The solution was placed upon a column (1.5 × 14 cm) of Dowex 21 K (OH^-). The effluent, which gave a faint positive Koenig test, was dis-

carded, and the column was washed with water. The column was then treated with 60 ml of 2 *N* acetic acid. The acetic acid eluate was concentrated to dryness (1.5 g, or 94%) at room temperature and finally recrystallized from absolute alcohol. The vacuum dried product melted at 100° (dec) in good agreement with the literature values (13, 14).

Metabolism of betahistine and related compounds in the rabbit. Betahistine base (or related compound) in aqueous solution (0.35 mmole/ml) was administered orally (0.7 mmole/kg) to female Dutch rabbits (1.6–3.0 kg). Urine from the individually housed animals was collected as run-off from the metabolism cages with sodium fluoride as a preservative. The animals were allowed food and water *ad libitum* during the collection period. Pooled urine, which was separated from feces by a screen in the metabolism cages, was filtered through diatomaceous earth and placed upon a column of Dowex 50 (H^+) (500 ml). The effluent and a water wash from the column were put aside for further possible investigation; the column was then treated with 1400 ml of 5 *N* ammonium hydroxide. The ammoniacal eluate (A) was then placed upon a column of Dowex 21 K (OH^-) (200 ml). The effluent and water wash were put aside. The column was then treated with 600 ml of 2 *N* acetic acid. The acetic acid eluate (B), which showed the presence of pyridylacetate by the qualitative methods previously employed (6), was then examined in the absorption spectrophotometer.

Gas chromatographic estimation of 2-pyridylacetylglycine. The acetic acid eluate (B) from the Dowex 21 K (OH^-) column above was concentrated under diminished pressure at 40° to a brown residue, which was triturated with 50 ml or absolute ethanol. After removal of the ethanol under diminished pressure, the mixture was heated at reflux temperature for 16 hr with 15 ml of absolute ethanol and 0.6 ml of concentrated sulfuric acid. After addition of crushed ice (20 g) and methylene chloride (15 ml), the aqueous layer was adjusted to pH 9 by careful addition of 10% sodium hydroxide. The methylene chloride extract was combined with two additional extractions (15 ml each) and dried over anhydrous magnesium sulfate. The residue from evaporation of the solvent was dissolved in alcohol for gas chromatographic investigation. The ethyl *N*-2-pyridylacetylglycinate concentration was estimated from peak heights in comparison with those obtained from primary standard ester.

Stability of 2-pyridylacetate and its glycine conjugate. Solutions of 2-pyridylacetic acid and of *N*-2-pyridylacetylglycine were prepared in 5 ml of water, and in 5 ml of pH 7.4 phosphate buffer (0.2 *M* sodium–potassium) and heated in sealed tubes at 120° for 16 hr. The 2-pyridylacetate solutions showed upon chromatography in all cases a single Koenig-positive zone corresponding to 2-picoline (R_f 0.52, solvent K), and the *N*-2-pyridylacetylglycine solutions showed evidence only for unchanged material (R_f 0.08, solvent K; R_f 0.28 solvent C).

Results. The urine of rabbits that received betahistine or the related pyridine compounds, demethylbetahistine and 2-(2-hydroxyethyl)pyridine, showed upon thin-layer chromatography a number of Koenig-positive compounds, which served to suggest common metabolic routes. After processing on the resin, the ultraviolet absorption spectra of acidic fraction of the urines (obtained with 2 *N* acetic acid from Dowex 21 K (OH^-)) showed maxima in the vicinity of 262 nm, as depicted for the betahistine-treated animals in Fig. 1. A calculated measure of the 2-pyridylacetate excretions is indicated in Table I. *N*-2-Pyridylacetylglycine in 2 *N* acetic acid solution shows a similar absorption spectrum with a maximum at 262 nm, and other 2-substituted pyridine compounds, such as the glucuronide of 2-pyridylethanol, could present themselves in the fraction and show no outstanding absorption difference.

N-2-Pyridylacetylglycine, previously reported as a metabolite of 2-(2-methoxyethyl)pyridine (9), chromatographed essentially with the same R_f value (0.08, solvent K) as 2-pyridylacetic acid, and the two consistently overlapped each other when chromatographed together on paper or silica gel. The Koenig reaction of 2-pyridylacetate pro-

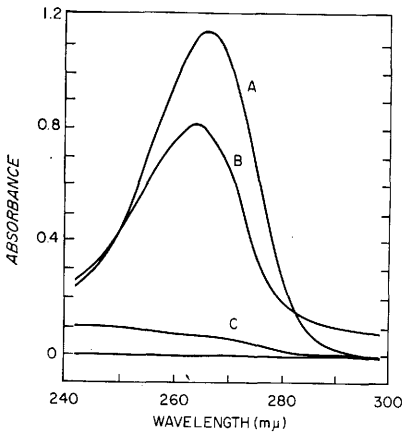

FIG. 1. Comparative ultraviolet absorption spectra of control and experimental urine fractions [eluted from Dowex 21 K (OH^-)] with 2 *N* acetic acid. (A) Urine fraction from betahistine-treated rabbits; (B) 2-pyridylacetic acid in 2 *N* acetic acid; (C) urine fraction from untreated rabbits.

vided a greyish color in contrast to the orange-like color of *N*-2-pyridylacetylglycine. Evidence for the presence of this conjugate was achieved through the ethanol–sulfuric acid esterification of the processed urine of one group of betahistine-treated animals. Gas chromatography of the ethyl ester fraction provided evidence for ethyl pyridylacetate

TABLE I. Forty-eight-hour Urinary Excretion of Apparent 2-Pyridylacetate by Female Dutch Rabbit Groups After Oral Administration of Betahistine and Related Compounds.

Compound administered[a] (0.7 mmole/kg)	Percentage of dose excreted as 2-pyridylacetate[b]		
	I	II	III
Betahistine base	62[c]	94[d]	59
Demethylbetahistine base	67	49	55
2-(2-Hydroxyethyl)pyridine	91	79	
2-Pyridylacetic acid hydrochloride	86[e]		

[a] Mean weight of groups ranged from 1.8–2.4 kg; individual animals weighed 1.6–3.0 kg.

[b] Calculated value based on absorption of Dowex 21 K (OH^-) eluate at 262 nm.

[c] $N = 4$.

[d] $N = 5$.

[e] $N = 3$; all other groups contained six animals.

(R_f 0.62, solvent K) and ethyl *N*-2-pyridylacetylglycine accounted for 1.36% of the administered dose. Only indications of trace amounts (less than 0.1%) were obtained from the urine of a group that received 2-(2-hydroxyethyl)pyridine.

Esterification of *N*-2-pyridylacetylglycine under acidic conditions such as those employed in this study provide a compound of sufficient volatility for estimation by gas chromatography. 2-Pyridylacetic acid on the other hand is a compound noted for its instability under many conditions (13–16), decarboxylated during the esterification procedure with acid and alcohol to give some picoline (R_f 0.50, solvent K) along with the expected ester, ethyl 2-pyridylacetate (R_f 0.62, solvent K; R_f 0.71, solvent C).

Discussion. Prior to the present study, Burns, Duncan and Scales (9) and Duncan and Scales (8) studied the metabolism of methyridine (2-(2-methyoxyethyl)pyridine) in a variety of animals, *viz.*, sheep, calves, rabbits, mice and rats. From their studies it was concluded that the major urinary metabolite of methyridine, accounting for 80–90% of the administered dose, was 2-pyridylacetate. Little methyridine (0.2–0.5% of the administered dose) was excreted unchanged. 2-(2-Hydroxyethyl)pyridine was excreted to the extent of 0.1% in sheep that had received methyridine. 2-(2-Hydroxyethyl)pyridine, in the light of its importance as a metabolic intermediate, was administered (9) to rats and 82% of the administered dose appeared in the urine as 2-pyridylacetate within 2 days.

In the present studies in the rabbit using betahistine, demethylbetahistine, and 2-(2-hydroxyethyl)pyridine the urinary pattern of excretion of the administered 2-pyridyl derivatives resembles markedly those observed in the methyridine studies and is similar to those in previous studies (6) on the metabolism of betahistine in the dog where the 2-pyridylacetate fraction of urine accounted for a calculated 80% of the administered dose. In the present studies on the metabolism of betahistine in the rabbit, the acidic metabolic fraction was shown in one group to contain *N*-2-pyridylacetylglycine, equivalent to 1.36%

of the administered betahistine. Koenig-positive urinary material, other than 2-pyridylacetate and its glycine conjugate, was not investigated in this study.

In the studies of methyridine-^{14}C (2-(2-methoxyethyl)pyridine-2-^{14}C), traces of radioactivity were found (8) to remain in the animal long after the major fraction of radioactivity had been eliminated by way of the urine. Since the residual ^{14}C was eliminated at a rate comparable to that expected for labeled protein, it was suggested that this radioactivity resulted from a general labeling of the carbon dioxide pool, via the decarboxylation of 2-pyridylacetic acid (to yield carbon dioxide and 2-picoline). During the processing of urine in the current study on betahistine, chromatographic evidence for 2-picoline was encountered. Stability studies on 2-pyridylacetic acid indicate that spontaneous decarboxylation of 2-pyridylacetic acid in the physiological pH range can lead to the production of 2-picoline. Previous authors (15) have studied the photodecomposition of 2-pyridylacetate. In order to minimize the possible production of 2-picoline as a photochemical artifact in processing the urine, our operations were conducted in subdued light. The Dowex 21 K (OH^-) columns were stripped of pyridine compounds by treatment with 2 *N* acetic acid without delay, although control columns were found to retain 2-pyridylacetate without evident decomposition for as long as 2 weeks when stored in the darkness at 2°. Although much data exists (16–18) on the instability of 2-pyridylacetate, no studies show whether or not the decarboxylation of 2-pyridylacetic acid *in vivo* is subject to enzymatic control.

While data now available point to the probability of alternate pathways in the metabolism of betahistine to 2-pyridylacetate, one via 2-pyridylacetaldehyde directly and one via demethylbetahistine with subsequent formation of pyridylacetaldehyde, additional knowledge of the factors required in the enzymatic oxidation of 2-(2-hydroxyethyl) pyridine to pyridylacetate are currently needed. The failure of an active dehydrogenase preparation to produce (9) 2-pyridylacetaldehyde or 2-pyridylacetate from 2-(2-hydroxy ethyl)pyridine *in vitro* has been reported. Oxidation of 2-(2-hydroxyethyl)pyridine took place, however, and many additional pathways for the metabolism of betahistine and its congeners may be forecast from the studies.

Summary. The metabolism of betahistine, demethylbetahistine and 2-(2-hydroxyethyl)pyridine in the rabbit leads to the urinary excretion of acidic metabolities, including 2-pyridylacetate, which is excreted in part as *N*-2-pyridylacetylglycine. Other unidentified Koenig-positive compounds were in the acidic fraction.

1. Horton, B. T., Mayo Clin. Proc. **37**, 713 (1962).

2. Godlowski, Z., Angiology **16**, 644 (1965).

3. Elia, J. C., J. Amer. Med. **196**, 187 (1966).

4. Baez, S., *in* "5th European Conference on Microcirculation, Gothenburg 1968" (H. Harders, ed.), p. 340. Karger, Basel (1969).

5. Anderson, W. D., and Kubicek, W. G., Stroke **2**, 409 (1971).

6. Konzett, H., Bost, R. G., Bowman, F. J., Bowman, E. R., and McKennis, H., Jr., J. Pharmacol. Exp. Ther. **178**, 122 (1971).

7. Bowman, F. J., Bowman, E. R., and McKennis, H., Jr., Va. J. Sci. **21**, 143 (1970).

8. Duncan, W. A. M., and Scales, B., Biochem. J. **80**, 1P (1961).

9. Burns, J., Duncan, W. A. M., and Scales, B., Biochem. Pharmacol. **16**, 463 (1967).

10. Bowman, F. J., Bowman, E. R., and McKennis, H., Jr., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **30**, 336 (1971).

11. McKennis, H., Jr., Turnbull, L. B., and Bowman, E. R., J. Biol. Chem. **239**, 1215 (1964).

12. McKennis, H., Jr., Bowman, E. R., and Dar, M. S., Va. J. Sci. **18**, 13 (1967).

13. Pattison, D. B., and Carmack, M., J. Amer. Chem. Soc. **68**, 2033 (1946).

14. Oparina, M. P., Khim. Prom. **4**, 12 (1934), Chem. Abstr. **29**, 1820 (1935).

15. Stermitz, F. R., and Huang, W. H., J. Amer. Chem. Soc. **93**, 3427 (1971).

16. White, W. N., and Lazdins, D., J. Org. Chem. **34**, 2756 (1969).

17. Stermitz, F. R., and Huang, W. H., J. Amer. Chem. Soc. **92**, 1446 (1970).

18. Doering, W. von E., and Pasternak, V. Z., J. Amer. Chem. Soc. **72**, 143 (1950).

Received May 4, 1972. P.S.E.B.M., 1972, Vol. 140.