BETAHISTINE, ITS METABOLITES AND VASCULAR RESPONSES IN THE FORELIMB OF THE DOG1, 2

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ABSTRACT

KONZETT, HERIBERT, ROBERT G. BOST, FAYE J. BOWMAN, EDWARD R. BOWMAN AND HERBERT MCKENNIJS, JR.: Betahistine, its metabolites and vascular responses in the forelimb of the dog. J. Pharmacol. Exp. Ther. 178: 122-129, 1971. Betahistine, and two of its putative metabolites, 2-(2-aminoethyl)pyridine and 2-(2-hydroxyethyl)pyridine have been compared for effects (at constant blood-flow rate) on vascular resistance in the forelimb of the dog. Betahistine and its N-demethyl derivative, 2-(2-aminoethyl)pyridine, were of equal or similar potency in decreasing vascular resistance at a dose range of 0.22 to 2.2 µmol/min. In contrast, 2-(2-hydroxyethyl)pyridine at doses up to 22 µmol/min and 2-pyridylacetic acid, a urinary metabolite of betahistine in the dog, at doses up to 22 µmol/min produced no demonstrable change in vascular resistance. After p.o. administration of betahistine (15 mg/kg b.wt. for eight days) the urine of male mongrel dogs contained acidic metabolites in amounts corresponding by spectrophotometric estimate to 45% of betahistine administered. The presence of 2-pyridylacetate in the acidic metabolic fraction was demonstrated by chemical preparation of methyl 2-pyridylacetate picrate, which was compared with an authentic sample of synthetic compound.

Betahistine [2-(2-methylaminoethyl)pyridine], originally synthesized by Löffler (1904), was studied by Walter et al. (1941) and by Hunt and Fossebinder (1942) in comparison with a number of pyridylalkylamines for possible sympathomimetic activity. The authors found in their studies a lack of sympathomimetic activity, and reported numerous histamine-like effects, including an activity that approached that of histamine on the isolated guinea-pig uterine and intestine. In the intact animal vascular effects of betahistine resembled those of histamine.


Despite extensive use of betahistine in a variety of vascular deficiencies (Esser and Reis, 1968), and in the pretransplant perfusion of organs (Groenewald et al., 1971), little is known about the metabolism of betahistine and the events responsible for initiating and terminating the pharmacologic response to the compound. This report deals, therefore, with a preliminary investigation of hypothetical routes in the metabolism of betahistine, the identification of pyridylacetate as a metabolite of betahistine and a study of effects of betahistine and some related compounds on vascular resistance in the forelimb of the dog.

MATERIALS AND METHODS. Reagents. All reagents were analytical grade, or U.S.P. grade, unless other-
wise noted. 2-Pyridylacetic acid hydrochloride, 2-(2-aminooxyethyl)pyridine and 2-(2-hydroxyethyl)pyridine were obtained from the Aldrich Chemical Company, Inc. (Cedar Knolls, N.J.).

Chromatography. Thin-layer chromatography was conducted on silica gel-coated glass plates (Brinkmann Instruments, Inc., Westbury, N.Y.) with solvent K (McKennia et al., 1964). Koenig-positive zones were disclosed by spraying with 2% p-aminobenzoic acid in absolute alcohol, drying and subsequent exposure to cyanogen bromide vapor. Paper chromatography was conducted on Whatman no. 1 paper with solvents B and C (McKennia et al., 1964).

Preparation of methyl 2-pyridylacetate. To a mixture of 1 g of 2-pyridylacetic acid hydrochloride and 25 ml of methanol were added 2 ml of concentrated sulfuric acid. The solution was heated under reflux for 16 hours and then cooled. Chloroform (50 ml) and 10 g of crushed ice were then added. After adjustment of the aqueous phase to pH 9 by addition of 10% sodium hydroxide, the mixture was shaken thoroughly and the organic layer was removed. The aqueous phase was then treated with two fresh portions (50 ml each) of chloroform. The combined chloroform extracts were treated with anhydrous magnesium sulfate and then filtered. The filtrate was concentrated to an oily residue (500 mg). The oil was dissolved in 10 ml of ethanol and then treated with a saturated solution of picric acid (10% H2O) in absolute ethanol until precipitation was complete. The crystalline picrate (950 mg) was recrystallized from acetone and dried at 60°C over KOH. The melting point was determined by the capillary method (McKennia et al., 1964). Preparation of methyl 2-pyridylacetate. Similar cochromatography was achieved with solvents B and C on Whatman no. 1 paper. The sample was dried at 60°C and 1 mm Hg over KOH, and infrared spectra of the metoxic and the authentic methyl ester picrate were compared.

Examination of dog urine for acidic metabolites of betahistine. Two male mongrel dogs (15.5 and 13.7 kg b.w.t.) received by gavage 15 mg of betahistine base per kg daily for eight days in aqueous solution (10 mg/ml). The urine was collected in sodium fluoride as a run-off from the metabolism cages, and frozen daily for subsequent processing. The urines from the treatment period and the following day were combined, filtered, and then placed upon a column (6 × 60 cm) of Dowex 50 W (H+). The effluent and water wash (1000 ml) were saved for further investigation.

The column was then washed with 2 liters of 5 N ammonium hydroxide. The eluate was examined in 100-ml fractions for absorption at 261 μm in a 1-cm cell. Those fractions absorbing at 261 μm were combined and placed upon a column (4 × 40 cm) of Dowex 21 K (OH−). The column was washed with distilled water until the effluent was neutral (pH 7 with pHydron paper) and then treated with 25 liters of 2 N acetic acid. The fractions of the eluate that showed absorption at 261 μm were combined. Control processing of 2-pyridylacetic acid by this procedure on the two resin columns gave a virtually quantitative recovery of the acid, as adjudged by absorption at 261 μm.

The combined 261-μm absorbing fractions were evaporated on the water bath under diminished pressure. After addition of 180 ml of water to the oily residue, the mixture was again evaporated to an oily residue. Benzene (100 ml) was added, and the evaporation was repeated. Methanol (300 ml) and 11 ml of sulfuric acid were then added. The mixture was refluxed under protection of calcium chloride for 19 hours. The solution was then concentrated to approximately 100 ml. The solution was cooled in the ice bath and was then adjusted to pH 8.0 to 8.5 (pHydrion paper) by addition of cold 50% sodium hydroxide. After addition of 50 ml of cold water, the mixture was extracted with 100 ml of chloroform; the aqueous phase was reextracted with chloroform (180 ml). The combined chloroform solutions were concentrated to an oil, which was dissolved in methanol and then treated with decolorizing carbon. After filtration, the solution was concentrated to a volume of approximately 3 ml. A saturated solution of picric acid (10% water) in methanol (4 ml) was added. After cooling and scratching, a yellow crystalline precipitate (m.p. 144–146°C) formed. The picric acid salt upon thin-layer chromatography (silica gel) gave a single Koenig-positive zone at Rf 0.85 (solvent K), corresponding in Rf value to authentic methyl 2-pyridylacetate. Similar cochromatography was achieved with solvents B and C on Whatman no. 1 paper. The sample was dried at 60°C and 1 mm Hg over KOH, and infrared spectra of the metabolic material and the authentic methyl ester picrate were compared.

Perfusion of the dog forelimb. By a modification of the procedure of Haddy (1960), vascular resistance of the forelimb was measured in male and female mongrel dogs (12–23 kg) under pentobarbital anesthesia (30 mg/kg). With the animals usually under artificial respiration, the right femoral artery was cannulated with Intramedic polyethylene tubing (0.085 × 0.128-inch diameter), which was connected with Tygon tubing (3/16 × 5/16-inch diameter) for passage of blood at constant flow rate through a Harvard peristaltic pump. Blood from the pump passed through a coil thermostatically controlled at 37°C and thence to the cannulated brachial artery of the right forelimb with tie-offs as indicated in figure 1. Pressures in the ulnar artery (which did not differ from those in the inflow catheter when simultaneously measured), close and proximal to the carpus, in
and saline solutions of the test substances were administered at the rate of 0.46 ml/min and administrations of the test substances were as follows: betahistine base (0.22, 0.67, 2.2, 6.7 and 22 µmol/min), histamine base (0.011, 0.033, 0.11 and 0.33 µmol/min), 2-(2-aminoethyl)pyridine (0.22, 0.67 and 22 µmol/min), 2-(2-hydroxyethyl)pyridine (2.2, 6.7 and 22 µmol/min), 2-pyridylacetic acid hydrochloride (2.2, 6.7 and 22 µmol/min), adjusted to pH 7 by addition of 1 N sodium hydroxide), atropine sulfate (2.0 µmol/min) and nicotinic acid (37.4 µmol/min). Changes in ulnar artery pressures were taken as a measure of change in vascular resistance. After infusion of betahistine, histamine or 2-(2-aminoethyl)pyridine, a period of five minutes or more (for confirmed return of ulnar artery pressure to the preinfusion level) always elapsed before administration of additional compound(s).

RESULTS. Isolation of 2-pyridylacetate as a urinary metabolite of betahistine. Concurrent with the processing of the urine from dogs that received betahistine, control urine was similarly processed on Dowex 50W (H+) and Dowex 21 K (OH-). The eluate obtained from treatment of the Dowex 21 K (OH-) column with acetic acid showed no significant absorption at 261 mµ in the case of the control dogs. The comparable eluate from processing urine of the dogs that received betahistine p.o. showed a ultraviolet absorption spectrum resembling closely that of 2-pyridylacetate with characteristic absorption maxima at 261 mµ. The optical density at this wavelength corresponded to a calculated 2-pyridylacetate acid content of 1.58 g or 45.1 mole percent of the administered betahistine. Upon thin-layer chromatography the solution gave a single Koenig-positive zone of Rf 0.19, corresponding in Rf value to authentic 2-pyridylacetate acid. Confirmation of identity of 2-pyridylacetate acid was obtained by conversion to the methyl ester picrate which showed no depression of melting point upon admixture with an authentic sample. The infrared spectra of authentic and metabolically derived samples in KBr pellets were in close agreement (fig. 2).

Effect of betahistine and related compounds on vascular resistance in the forelimb of the dog. Betahistine, infused into the brachial artery, produced a dose-dependent fall in perfusion pressure of the ulnar artery of the surgically prepared dog. The response to three increasing doses (0.22, 0.67 and 2.2 µmol/min over two minutes) is shown in figure 3A. The pressure in
the superficial vein of the forepaw and the general blood pressure in the carotid artery remained unchanged. When the experiments were conducted with the dermis and epidermis of the forepaw removed, no essential differences in response to betahistine, 2-(2-aminoethyl)pyridine or histamine were noted.

The possible metabolite 2-(2-aminoethyl)-pyridine infused i.a. produced a dose-dependent fall in perfusion pressure of the ulnar artery, which was much the same as that produced by betahistine (fig. 4). Here again, the pressure in a superficial vein and the general blood pressure in the carotid artery remained unchanged. The dose-response curves of betahistine ($N = 10$ for each dose level) and of 2-(2-aminoethyl)-pyridine ($N = 5$ for each dose level) with the standard deviation are shown in figure 4. There is no significant difference of action between the two compounds at the 95% confidence level. 2-(2-Hydroxyethyl)pyridine, an additional putative metabolite of betahistine, when administered into the brachial artery at dose levels up to 22 $\mu$mol/min produced no significant changes in pressures at the ulnar artery, accessory cephalic vein or carotid artery. 2-Pyridylacetic acid, a metabolite of betahistine, was similarly without effect on the measured pressures at doses up to 22 $\mu$mol/min. Betahistine and 2-(2-aminoethyl)pyridine were given to the same animal in some experiments. The measured vascular response to one compound was not altered significantly by prior administration of the other compound. The responses at the dose levels of 0.22, 0.67 and 22 $\mu$mol/min fell within one standard deviation of the mean (fig. 4). Repeated (up to three times) doses of betahistine at a dose level of 0.67 $\mu$mol/min did not produce tachyphylaxis.

Histamine ($N = 4$ for each dose level) was approximately 200 times more active (fig. 4) than the two synthetic pyridylalkylamines, betahistine and 2-(2-aminoethyl)pyridine in decreasing vascular resistance. In contrast to the responses from the synthetic compounds where return to pretreatment ulnar arterial pressure was rapid and occurred at termination of infusion, or within one minute thereafter (fig. 3, A and B), the return to pretreatment pressures after infusion of histamine was slower and at the higher doses (0.11–0.22 $\mu$mol/min) required as much as 10 minutes. The relationships between
FIG. 3. Recording (A) of ulnar arterial blood pressure in the surgically prepared forelimb of the dog during a two-minute infusion of betahistine into the brachial artery, and (B) during a two-minute infusion of 2-(2-aminoethyl)pyridine.

FIG. 4. Dose-response curves showing mean percent fall of ulnar arterial blood pressure, taken from maxima during two-minute infusion of histamine, betahistine and 2-(2-aminoethyl)pyridine into the brachial artery of the surgically prepared forelimb of the dog. Standard deviations are shown by the bars.

Histamine, betahistine and demethylbetahistine held after removal of the dermis and epidermis from the surgically prepared forelimb and were not modified in those preparations when an atropine pretreatment (11-12 minutes before test dose) was employed.

Discussion. A wide variety of mechanisms, including sympathetic, parasympathetic and histamine responses (Zimmerman, 1966; Beck, 1961 and others) can be evoked to produce changes in vascular resistance. Hunt and Fossbinder (1942) emphasized that despite the
structural resemblance between epinephrine and betahistine or its demethyl derivative, no evidence for adrenergic activity of the two pyridylalkylamines appeared in their investigations. With the finding that betahistine and demethylbetahistine had histamine-like effects, they were struck by the relationship of structure of histamine to these pyridylalkylamines, which was shown two-dimensionally and is even more striking by comparison of molecular models. Baes and co-workers (1969a,b, 1970) have recently compared betahistine and histamine with respect to their ability to block the constrictor effects of norepinephrine on the microcirculation. Werle and Palm (1953) found betahistine was somewhat more efficacious than demethylbetahistine in its ability to contract the isolated guinea-pig gut. The action of both pyridylalkylamines was suppressed by the antihistamine antazoline. Konzett and Bost (1970) noted that chlorpheniramine diminished the effects of histamine and betahistine on vascular resistance in the perfused forelimb of the dog.

Ariëns and Simons (1960) investigated betahistine and demethylbetahistine on the isolated ileum and found the specific intrinsic activities and receptor affinities of the two histaminomimetics to be substantially identical. These and related findings have been subjected to additional study and review (Lee and Jones, 1949; van Rossum and Van den Brink, 1963; Kost et al., 1967; Van den Brink, 1969) Horton and von Leden (1962) found demethylbetahistine active and betahistine the preferred drug in the treatment of a number of vasomotor disorders. Extension of this work to the long-term treatment of Meniere's disease by many led Anderson and Kubicek (1971) to compare the effects of betahistine, histamine and nicotinic acid, which has a questionable validity as an agent in treating Meniere's disease, on the flow of blood in the basilar artery of the dog. Their experiments indicated that betahistine hydrochloride (1 mg/40 pounds b.wt.) produced an increase of 51%, whereas histamine phosphate (0.25-0.50 mg/40 pounds b.wt.) produced a 45% increase and nicotinic acid (20 mg/40 pounds b.wt.) a 9% increase in the blood flow through the basilar artery.

In the present investigations on vascular resistance in the forelimb of the dog, betahistine and nicotinic acid were also compared. Nicotinic acid at the dose levels employed, on a molar basis up to 20 times those of betahistine, produced no significant changes in the vascular resistance. Consistent with the many other published observations of the pharmacologic activity of demethylbetahistine on various preparations in vitro, this compound produced in the forelimb of the dog an apparent histamine-like effect, a decrease in vascular resistance virtually identical to those resulting from equimolar doses of betahistine. Histamine was the most potent of the substances tested and had a duration of action longer than that of the two synthetic pyridylalkylamines.

Although demethylbetahistine, which hypothetically arises from betahistine as indicated in figure 5, has never been identified as a metabolite of betahistine in the dog, chromatographic evidence for its presence and that of 2-pyridylacetate has been found after ingestion of betahistine by man (H. Holmen-Christiansen, private communication). 2-(2-Hydroxyethyl)pyridine (O'Leary et al., 1951), an additional hypothetical metabolite of betahistine, was devoid of pharmacologic activity by criteria of the present study, and 2-pyridylacetic acid, identified as a metabolite of betahistine in the dog, showed a similar lack of activity.

The present finding that 2-pyridylacetic acid is excreted as a urinary metabolite is not without precedent since Duncan and Scales (1961) reported the metabolic oxidation of 2-(2-methoxyethyl)pyridine to 2-pyridylacetate. Further studies are required to establish the pathways leading from betahistine to 2-pyridylacetate. Both betahistine and demethylbetahistine [2-(2-aminoethyl)pyridine] presumably could serve as precursors of 2-pyridylacetaldehyde, the putative immediate precursor of 2-pyridylacetate. Only limited studies on the effect of amine oxidases on 2-(2-methylaminoethyl)pyridine appear in the literature. Werle and Palm (1953) found that 2-(2-methylaminoethyl)pyridine strongly inhibited the oxidative degradation of histamine by a diamine oxidase (histaminase) of plant origin. A similar inhibitory effect was observed with 2-(2-aminoethyl)pyridine, and virtually no oxidation of the two inhibitors was apparent. Lindell and Westling (1957) reported that 2-(2-aminoethyl)pyridine was not oxidized by histaminase preparations from pig kidney and from cat kidney. These findings were considered to be in general agreement with the observations
Fig. 5. Abridged hypothetical scheme for the metabolism of the methylaminoethyl chain of betahistine.

of Arumalakshana et al. (1954), who used pig kidney cortex for their histaminase preparations.

In contrast to the reported inactivity of various histaminase preparations in the oxidation of 2-(2-aminomethyl)pyridine and 2-(2-methylaminoethyl)pyridine, Lindell and Westling (1957) found that dialyzed preparations of both guinea-pig liver and rabbit liver, virtually devoid of histaminase, were active in the oxidation of 2-(2-aminomethyl)pyridine. From this it may be inferred that some of the 2-pyridylacetate from the metabolism of betahistine may arise from 2-(2-aminomethyl)pyridine as indicated in the hypothetical route in figure 5.

Additionally, it is considered that the hypothetical intermediate 2-pyridylacetaldihyde, in addition to oxidation, may undergo reduction to yield 2-(2-hydroxyethyl)pyridine, which could undergo reoxidation to 2-pyridylacetate. Some satisfying evidence for the possibilities of such a shunt may be found in the experiments of Duncan and Scales (1961). These investigators administered 2-(2-methoxyethyl)pyridine to five species of animals and reported that a maximum of 1.5% of the administered dose could be considered unchanged after 48 hours. After injection of 2-(2-methoxyethyl)pyridine into a calf, the 48-hour urine yielded a substantial quantity of 2-pyridylacetate, and no significant increase in urinary glucuronides or ethereal sulfates was found. This serves to suggest (Burn et al., 1967) that the major part of the methoxyethyl compound was converted to 2-(2-hydroxyethyl)pyridine and thence via 2-pyridylacetaldihyde to 2-pyridylacetate. In this connection, it is interesting to note that the urine of animals after administration of 2-(2-methylaminoethyl)pyridine in the present study showed only one acidic Koenig-positive zone upon paper chromatography and thin-layer chromatography, corresponding in Rf value to 2-pyridylacetate. It must be considered however, that the glucuronide and the sulfate ester of 2-(2-hydroxyethyl)pyridine could be present in amounts too small to disclose themselves readily by the methods employed in the studies of Duncan and Scales and in our own work.

Additional investigation is required to determine the definite structure of intermediates in the metabolism of betahistine to 2-pyridylacetate. The present investigations show, however, that formation of this acidic metabolite contributes materially to elimination of betahistine and termination of its vascular activity.
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REFERENCES


