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Short Communication

Isolation and identification of β-hydroxyethylaprophen: a urinary metabolite of aprophen in rats

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ABSTRACT

The metabolism of the anticholinergic drug aprophen was studied in rats after oral administration via stomach intubation. β-Hydroxyethylaprophen, a major urinary metabolite of aprophen, was isolated and identified by normal-phase high-performance liquid chromatography and electron ionization mass spectrometry. More than 22% of the parent drug was recovered and quantified over a 72-h collection period. Results show that 2,2-diphenylpropionic acid, another major metabolite of aprophen which lacks anticholinergic properties, was also isolated and identified in this study. Experiments are currently underway to synthesize and test the anticholinergic properties of β-hydroxyethylaprophen in mammals.

INTRODUCTION

Previously, we have shown that desethylaprophen (2-ethylaminoethyl 2,2-diphenylpropionate), a principal metabolite of aprophen in serum after intravenous administration, exhibits significant antimuscarinic activities [1]. Furthermore, there are no apparent pharmacological differences between aprophen and desethylaprophen, after the loss of the ethyl group during the biotransformational process.

In this report, β-hydroxyethylaprophen [N-ethyl-N-(β-hydroxyethyl)aminoethyl 2,2-diphenylpropionate] (Fig. 1) was isolated and characterized as a ma-

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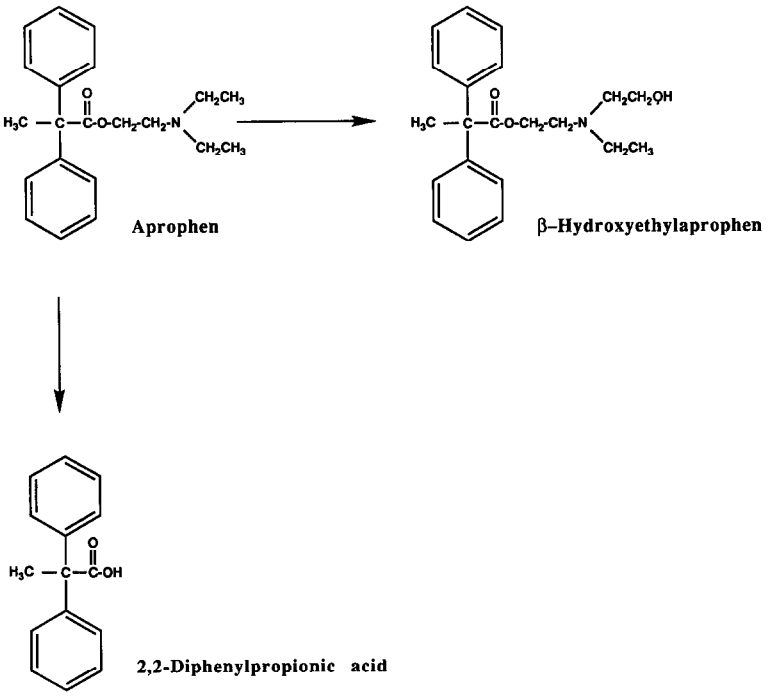

Fig. 1. Major metabolic pathway for the metabolism of aprophen in rats, after oral administration.

jor metabolite of the anticholinergic drug, aprophen, from the urine of rats after oral administration via stomach intubation. While aprophen has been shown to be a potent cholinolytic and antispasmodic agent [2–4], the pharmacological actions of this newly isolated metabolite remain to be defined.

β-Hydroxyethylaprophen was isolated from rat urine, using a combination of organic solvent extractions and liquid column chromatography. Normal-phase high-performance liquid chromatography (HPLC) was employed to purify the extracted compounds; electron ionization mass spectrometry was then used to identify β-hydroxyethylaprophen. In addition, we report here the quantitative percentage recovery of this metabolite in urine during the 72-h collection period.

EXPERIMENTAL[a]

Aprophen hydrochloride was synthesized in our laboratory using the method of Zuagg and Horrom [5]. Its identification was confirmed by its melting point (161–162°C), by infrared and nuclear magnetic resonance spectroscopic proper-

[a] The manufacturers' names and products are given as scientific information only and do not constitute an endorsement by the United States Government.

ties and by chemical ionization mass spectrometry. 2,2-Diphenylpropionic acid was obtained from Pfaltz and Bauer (Stamford, CT, U.S.A.).

Metabolism of aprophen in rats

Animals were cared for in accordance with the principles outlined in the "Guide for Care and Use of Laboratory Animals", prepared by the Institute of Laboratory Resources, National Academy of Science/National Research Council.

Three male Sprague–Dawley rats (250–350 g) were used for the metabolic study. Each rat was placed into a glass metabolic cage 24 h prior to the experiment and allowed food and water ad libitum. Urine samples were collected during this conditioning period (pre-dose).

Immediately preceding the acclimation period, each rat was administered a 2-ml aqueous slurry of aprophen hydrochloride (mean dose of 225 mg) via a stomach tube. A 1-ml bolus was given at the beginning of each experiment, followed by the second dose 30 min later. Urine samples (24 h) were collected during the 72-h experimental period.

Extraction procedure

Urine samples, including the pre-dose, control specimens, were mixed with equal volumes of 0.067 *M* dibasic sodium phosphate (pH 8.5) and extracted directly into four volumes of diethyl ether. The organic phase, containing the unmetabolized aprophen and the metabolites was removed after shaking for 2 min and evaporated to dryness. The extraction procedure was performed three times for each urine sample. The dried residue was dissolved in HPLC-grade acetonitrile to the original urine volume. Samples were then ready for analysis.

Apparatus

A Waters Assoc. (Milford, MA, U.S.A.) Model ALC/GPC-204 liquid chromatograph was used to complete this study. The system consisted of two Model 6000A high-pressure pumps, a Model 660 solvent programmer, a U6K loop injector, a Model 481 LC spectrophotometer set at 254 nm and a Hitachi D-2000 Chromato-integrator.

HPLC separation

A 100 mm × 8 mm I.D. Radial-Pak, Type B, 10-μm silica cartridge (Waters Assoc.) was employed to chromatograph all compounds separated in this study. The mobile phase consisted of methanol–acetonitrile–triethylamine (60:40:0.01, v/v). The prepared solution was isocratically pumped through the cartridge at a flow-rate of 1 ml/min. Column pressures ranged between 35 and 42 bar. All separations were performed at ambient temperatures (18–20°C). A 50-μl volume of the acetonitrile extract was injected onto the column through a continuous-flow loop injector. Peak areas were measured by an on-line computing integrator.

Peak collection

Aliquots of peak 2 (Fig. 2A) containing β-hydroxyethylaprophen were collected after multiple injections of the urine extract. High peak "cuts" of the ascending and descending slopes of the peak were collected to assure the purity of β-hydroxyethylaprophen (Fig. 2B). Mass spectrometric analyses were performed on the acetonitrile extracts of the pooled peaks.

Mass spectrometry

Electron ionization mass spectra were scanned from samples introduced directly into the ion source via the direct insertion probe, which was initially set at 40°C for a 2-min isothermal period, and then elevated at 20°C/min to a final temperature of 200°C. The ion source temperature was 250°C, the ionizing potential was 70 eV, and the ionizing current was 220 μA. Mass spectra were recorded with a Hewlett Packard 5995 mass spectrometer, equipped with a 59970C MS Chem Station data system.

RESULTS AND DISCUSSION

After 50 μl of a urine extract were injected onto the HPLC column, three major peaks were observed for each injection (Fig. 2A). None of the peaks seen in the chromatogram were observed in the pre-dose urine specimens (Fig. 2C). A comparison of the retention times of the HPLC peaks for the urine extract and

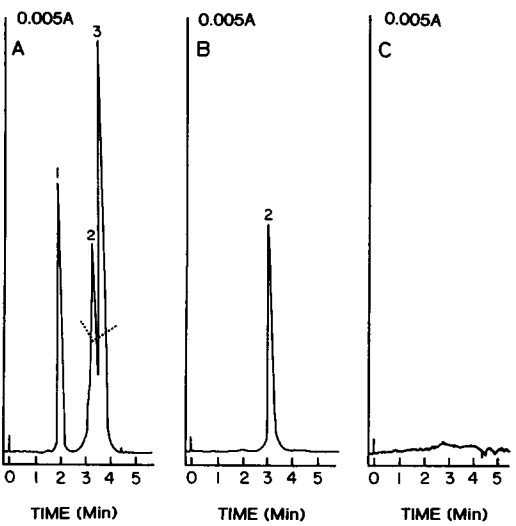

Fig. 2. Chromatograms of (A) 24-h urine extract from an aprophen-treated rat, showing peaks (1) 2,2-diphenylpropionic acid, (2) β-hydroxyethylaprophen and (3) unmetabolized aprophen, (B) pooled fractions from peak 2 and (C) pre-dosed rat urine extract (control). Conditions: column, Radial-Pak, Type B, 10-μm silica cartridge; mobile phase, methanol–acetonitrile–triethylamine (60:4;:0.01, v/v); flow-rate, 1 ml/min; column temperature, ambient.

neat standards showed that peaks 1 and 3 were similar to the standards representing 2,2-diphenylpropionic acid and aprophen, respectively.

The pooled samples for peak 2 (retention time = 3.55 min) were lyophilized to dryness, dissolved in acetonitrile to 50% of the original urine volume and re-injected onto the column. One peak was observed, having a similar retention time as peak 2, as seen in the original HPLC run. For the three rats receiving 0.3 LD_{50} (mean dose of 225 mg), approximately 10% (23 mg) of the parent compound was metabolized to β-hydroxyethylaprophen during the first 24 h. During the 72-h experimental period, 22% of the aprophen was converted to β-hydroxyethylaprophen. The electron ionization mass spectrum (Fig. 3) shows the molecular radical cation (*m/z* 341), along with expected fragments for the analyzed peak, thus comparing favorably with the chemical structure of β-hydroxyethylaprophen.

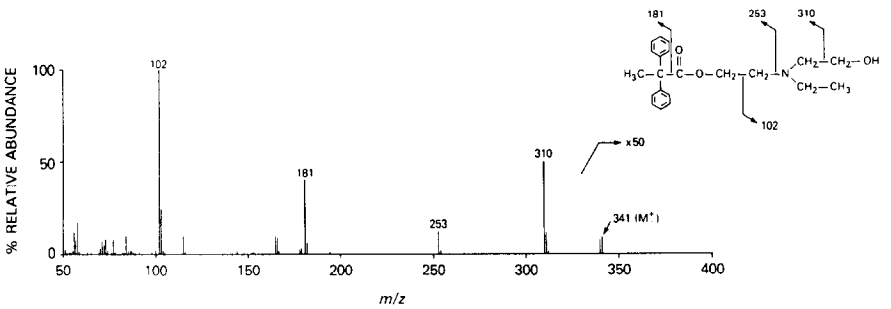

Fig. 3. Electron ionization mass spectrum of the molecular radical cation, *m/z* 341 of peak 2 (β-hydroxyethylaprophen). Sample introduced directly into ion source.

It was not surprising that the metabolism for aprophen should not be different from any other compound having a diethylamino terminal group. However, β-hydroxyethylaprophen has never been isolated or identified before. The biotransformation of aprophen to a substituted N-β-hydroxyethyl analogue was a metabolic event similar to what has been observed with other drugs possessing the end terminal diethylamino functional group. Chloroquine is a prime example of this biotransformational process occurring in man, where β-hydroxyethylchloroquine is the principal metabolite excreted in urine. Although the antimalarial properties of β-hydroxychloroquine are not attenuated by the biotransformation, a different mode of action has been observed in human subjects with connective tissue disorders. Apparently, β-hydroxyethylchloroquine becomes an excellent anti-rheumatoid arthritis compound [6]. The possibility of β-hydroxyethylaprophen of having unaltered anticholinergic properties or other pharmacological properties are important aspects to be explored. Further studies are planned to synthesize β-hydroxyethylaprophen and to test the pharmacological properties of this compound in various biological systems.

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