Isolation of Naloxone-3-glucuronide from Human Urine[1] (34465)

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Naloxone (Fig. 1) is a potent opioid antagonist in animals (1) and in man (2) and has been used recently to treat heroin dependence (3). The purpose of the present study was to isolate a metabolite of naloxone from human urine and compare this metabolite to those already isolated from the urine of the rabbit and chicken (4). Although the formation of naloxone-3-glucuronide as occurs in the rabbit may be a foregone conclusion, the formation of *N*-allyl-14-hydroxy-7, 8-dihydronormorphine-3-glucuronide (where the 6 keto group in naloxone is reduced to an OH) in the chicken immediately raised the question as to what the corresponding metabolite may be in man. Because the spectrum of pharmacologic activity of naloxone is different from its reduced surrogate, EN 2265 (5), identification of this metabolite in man is important.

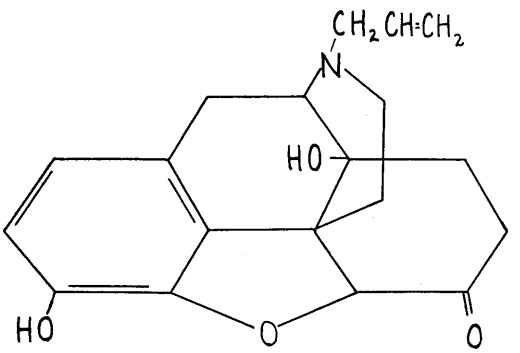

FIG. 1. Formula of naloxone.

Materials and Methods. Five gallons of pooled urine from patients were sent to this laboratory by Drs. Max Fink and Arthur Zaks of the New York Medical College. The patients were participants in a study of naloxone in the treatment of opiate dependence and were receiving between 1.0 and 1.8 g of naloxone daily in single oral doses. The dosages used were higher than those previously reported (3) and were administered to determine if higher dosages would extend the duration of effective antagonism to heroin. The procedure for isolating the naloxone metabolite from human urine was a modification derived from the experience accrued in isolating not only naloxone metabolites from the rabbit and chicken (4) but also metabolites of nalorphine (6) and morphine (7, 8) from various sources. The essential details and the modification will be described.

To a 1 × 12-in. (bed size) column of Amberlite XAD-2, 150 ml of urine were applied. This was followed with 150 ml of distilled water and in turn with about 300 ml of absolute methanol. The urine and the water effluents were discarded. The initial 10 ml of the methanol effluent which was recognizable by the appearance of highly colored urinary pigments were also discarded. The subsequent 250 ml of methanol effluent were collected. Six such columns were run. The 6 × 250 ml of methanol fraction were combined; the methanol was removed by evaporation. To the tar-like residue in the flask, 100 ml each of upper and lower phases of the countercurrent solvent system were added. The solvent system consisted of *n*-butanol, glacial acetic acid, water (100:1:100; v/v). A four-tube countercurrent distribution was performed manually using 100 ml for each solvent phase in reagent bottles. The aqueous phases were transferred with a syringe and long needle. The aqueous, mobile phase was transferred 8 times; the procedure thus entailed single withdrawal of the butanol phase. The butanol removed about two-thirds of the colored material from the aqueous phase. The aqueous phases of the first 3 tubes were combined. The volume was reduced under vacuum to about 15 ml. An approximately

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equal volume of ethanol was added. This mixture was applied to a silica gel column (¾ × 11-in. bed size), prepared earlier by pouring a slurry of silica gel (Woelm, for partition chromatography) in 95% ethanol into the column. The silica gel was packed by allowing the gel to settle as new ethanol was added. The column was eluted with ethanol. All initial fractions including 50 ml of eluate collected after the first colored fraction were discarded. The next 300 ml which was slightly colored contained the metabolite. The 300 ml were reduced under vacuum to about 15 ml and a precipitate resulted. Addition of 200 ml of acetone led to a clear solution. The flask was placed in the cold room for 1 hr and yielded 70 mg of precipitated tan powder, P_1. The acetone in the filtrate was removed by vacuum evaporation and 150 ml of water were added. A precipitate formed and was filtered and discarded. The volume of the filtrate was reduced to about 70 ml and acetone was added to make about 200 ml. The flask was placed in the cold room overnight; crystals formed. The crystalline material was filtered, washed with acetone, and dried in a desiccator. It weighted 152 mg (P_2). The filtrate still contained some metabolite. The purification process was monitored by thin-layer chromatography of the materials spotted on Gelman type SG fiber glass sheets. The solvent system was *n*-butanol, acetic acid, water (35:3:10; v/v). Alkaloids were detected with iodoplatinate spray; organic materials, both metabolite and impurities, were detected by charring after spraying with sulfuric acid.

Results and Discussion. One recrystallization was attempted with P_1, and yielded 36 mg of the tan material again with apparently no improvement in physical form. The IR curve of the latter was identical to the naloxone-3-glucuronide isolated previously from the urine of the rabbit (4). The IR spectrum for P_2 was also identical to the naloxone-3-glucuronide. From the 900 ml of human urine, 188 mg of naloxone-3-glucuronide were isolated. This yield was a minimal figure since the supernatant from the final crystallization steps still contained the metabolite and other losses occurred during purification.

The metabolite samples P_1 and P_2 were hydrolyzed by adding several drops of concentrated hydrochloric acid to about 0.5 ml of aqueous solutions in test tubes and boiling these mixtures in a pressure cooker at 15 lb pressure for 1 hr. Thin-layer chromatography of these hydrolysates was performed in chloroform saturated with ammonium hydroxide. The alkaloids in the two samples had the same R_f as naloxone (0.57) and cochromatographed with naloxone. Nothing corresponding to EN 2265 (R_f = 0.16) was detected. These results ascertain the presence of naloxone and not EN 2265 in the human metabolite and confirm the interpretation from IR spectral data.

Dayton and Blumberg (5) reported that in animals EN 2265 has more agonist activity than naloxone. It would be an attractive hypothesis to suggest that naloxone shows agonist activity in animals where EN 2265 is formed. Perhaps in the chicken (4, 9) and the pigeon (10), this may be the case. Although the present experiments do not entirely rule out the formation of EN 2265 in man, application of this hypothesis to man may be complicated by the fact that in pathological pain, 2 mg of naloxone produced analgesia whereas 8 mg produced little analgesia (11). Thus, more work is necessary to test the hypothesis.

Summary. Using column chromatography with Amberlite XAD-2 and silica gel and countercurrent analysis, a metabolite of naloxone was isolated from human urine. The metabolite was identical to naloxone-3-glucuronide isolated earlier from rabbit urine.

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1. Blumberg, H., Dayton, H. B., and Wolf, P. S., Proc. Soc. Exptl. Biol. Med. **123**, 755 (1966).

2. Foldes, F. F., Med. Clin. N. Am. **48**, 421 (1964).

3. Fink, M., Zaks, A. Sharoff, R., Mora, A., Bruner, A., Levit, S., and Freedman, A. M., Clin. Pharmacol. Therap. **9**, 568 (1968).

4. Fujimoto, J. M., J. Pharmacol. Exptl. Therap. **168**, 180 (1969).

5. Dayton, H. B. and Blumberg, H., Federation Proc. **28**, 736 (1969).

6. Fujimoto, J. M., Watrous, W. M., and Haarstad, V. B., Proc. Soc. Exptl. Biol. Med. **130**, 546 (1969).

7. Fujimoto, J. M. and Haarstad, V. B., J. Pharmacol. Exptl. Therap. **165**, 45 (1969).

8. Fujimoto, J. M. and Way, E. L., J. Pharmacol. Exptl. Therap. **121**, 340 (1957).

9. Sparber, S. B. and Wachter, J., Pharmacologist **10**, 305 (1968).

10. McMillan, D. E., Federation Proc. **28**, 736 (1969).

11. Lasagna, L., Proc. Roy. Soc. Med. **58**, 978 (1965).

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