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Nasal absorption of 17α-ethinyloestradiol in the rat

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Abstract—The bioavailability of 17α-ethinyloestradiol was determined after intravenous, nasal, and intraduodenal, administration in the rat. The results showed that the bioavailability of nasally-administered and intraduodenally-administered drug was 80–85, and 15–25%, respectively, of that following intravenous administration. The study demonstrates that the nasal route for the administration of 17α-ethinyloestradiol is superior to the oral route in this species, and may thus provide an alternative for the administration of this drug at lower doses.

Although the synthetic contraceptive steroid 17α-ethinyloestradiol was designed as an orally active oestrogen because of its lower susceptibility to biotransformation than the natural steroids, it has subsequently been shown that the drug undergoes significant metabolism in several animal species (Bolt et al 1977; Bolt 1979; Hirai et al 1981; Maggs et al 1982; Schmid et al 1983; Purba et al 1987). It is extensively metabolized when given orally; 40% of the administered dose is metabolized by the gut wall and 79% of the unchanged drug in the portal vein is metabolized by the liver to either oxidation products or sulphate conjugates (Hirai et al 1981). Some glucuronidation also occurs (Bolt et al 1973), but not to the extent of the natural oestrogens.

It has recently been shown that the bioavailability of the steroidal drugs progesterone and 17β-oestradiol when given via the nasal route in rats is greatly superior to that via the oral route (Bawarshi-Nassar et al 1988). The purpose of this study, therefore, was to determine if increased bioavailability of 17α-ethinyloestradiol could be achieved when this steroidal drug is administered via the nasal route in rats.

Materials and methods

Compounds. [6,7-^{3}H]-17α-ethinyloestradiol (spec. act. 56 mCi mmol^{-1}) was obtained from New England Nuclear, Boston, MA. 17α-Ethinyloestradiol was purchased from the Sigma Chemical Company, St. Louis, MO. Heptan-1-ol was purchased from the Fisher Scientific Company, Fairlawn, NJ. Polysorbate 80 (Tween 80) was purchased from Atlas Chemical Industries, Inc., Wilmington, DE. All other reagents and chemicals were obtained from Aldrich Chemicals, Milwaukee, WI.

Animal studies. For the i.v., nasal and intraduodenal routes of administration, doses of 1·75, 3·5 and 7 μg kg^{-1} of 17α-ethinyloestradiol in 0·1 mL saline (0·9% NaCl) were used. The amount of radioactivity administered to the rats was the same in each dose, i.e. ~35 μCi/rat. The administered solutions were prepared in the following way: for the 1·75 μg kg^{-1} dose, 35 μL of [6,7-^{3}H]-17α-ethinyloestradiol stock solution in benzene, 7·8 μL of 17α-ethinyloestradiol (35 μg mL^{-1} in toluene) and 1 mg polysorbate 80 were mixed. The solvents were removed under a stream of nitrogen and 0·1 mL of saline was added to the residue. The 3·5 and 7 μg kg^{-1} doses were prepared by adding 17α-ethinyloestradiol (30 and 100 μL of 35 mg mL^{-1} in toluene, respectively) to 35 μL of [^{3}H]-17α-ethinyloestradiol. To the above, 1 mg polysorbate 80 was added and the samples were treated as described previously.

Groups of 3 or 4 Sprague-Dawley male rats (270 ± 23 g) were used. The surgical procedures carried out for the i.v., nasal and intraduodenal administrations were similar to those described previously for progesterone (Bawarshi-Nassar et al 1988). Blood was sampled from the femoral artery at the following times: 2, 5, 10, 15, 20, 30, 45 min, 1. 1·5, 2, 3, 4 and 6 h, in all experiments.

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Analytical methodology. To determine intact 17α-ethinyloestradiol in whole blood, a specific method of analysis was developed. The solvent system, benzene–heptane (2:3 v/v), was found to extract only ethinyloestradiol without any of its metabolites, based on the following procedure. A dose of 3·5 μg kg^{-1} of 17α-ethinyloestradiol (prepared as mentioned previously) was administered i.v. to a rat. After 5 min, the total blood of the animal was collected by arterial puncture. Fifty mL of a solution of 17α-ethinyloestradiol (10 mg in ethanol) were added to polypropylene tubes and evaporated to dryness. To each of the tubes, 0·2 mL of whole blood, 1 mL of 0·01 M HCl and 6 mL of benzene–heptane (2:3 v/v) were added. After the tubes had been shaken and centrifuged, the aqueous phase was frozen in dry ice-acetone mixture and the organic solvent was decanted into another tube and evaporated to dryness. Fifty mL of toluene was added to the residue and 40 μL of the toluene solution spotted onto TLC plates (Uniplates, Analtech, Inc., Newark, DE). After being developed in three solvent systems (i.e. benzene–ethanol, 9:1 v/v; benzene–ethyl acetate, 4:1 v/v; and cyclohexane–acetone, 4:1 v/v), the plates were air-dried and separated into 10 divisions. Each division was then scraped into a scintillation vial, scintillation cocktail was added, and the radioactivity determined. Only one spot of radioactivity corresponding to unchanged ethinyloestradiol was found, indicating that the solvent system, benzene–heptane (2:3 v/v), extracted intact ethinyloestradiol without its metabolites. Based on the above, the following method of analysis was used to assay for unchanged ethinyloestradiol in the blood.

In polypropylene tubes, 0·2 mL of whole blood withdrawn from the treated animals was added to 1 mL of 0·01 M HCl and extracted with 6 mL of benzene–heptane (2:3 v/v). After the tubes had been shaken and centrifuged, the aqueous layer was frozen in a dry ice-acetone mixture. The organic phase was decanted into a scintillation vial and evaporated to dryness. Ten mL of scintillation cocktail were then added and radioactivity was counted. To calculate the percent recovery of 17α-ethinyloestradiol using the analytical method described above, a standard solution of [6,7-^{3}H]-17α-ethinyloestradiol in ethanol was prepared to give approximately 3500 counts 0·1 mL min^{-1}. The percent recovery was calculated as described for progesterone (Bawarshi-Nassar et al 1988) and was found to be about 80%.

The unchanged 17α-ethinyloestradiol blood levels were calculated according to the equation:

$$\text{Concentration (ng mL}^{-1}) = \frac{\text{counts min}^{-1}}{\text{efficiency}} \times \frac{1}{\text{recovery}} \times \frac{1\text{ mL}}{0{\cdot}2\text{ mL}} \times \frac{\text{ng}}{\text{d min}^{-1}}$$

The ng/d min^{-1} ratio was calculated as described for progesterone (Bawarshi-Nassar et al 1988). The area under the blood level curve was calculated by the trapezoidal rule.

Results and discussion

Fig. 1A–C show the mean blood concentrations of unchanged 17α-ethinyloestradiol in rats after the i.v., nasal and intraduodenal administration of 1·75, 3·5 and 7 μg kg^{-1} of 17α-ethinyloestradiol, respectively. The area under the blood level curve for unchanged 17α-ethinyloestradiol as a function of the dose for the three routes of administration is given in Fig. 2; as can be seen,

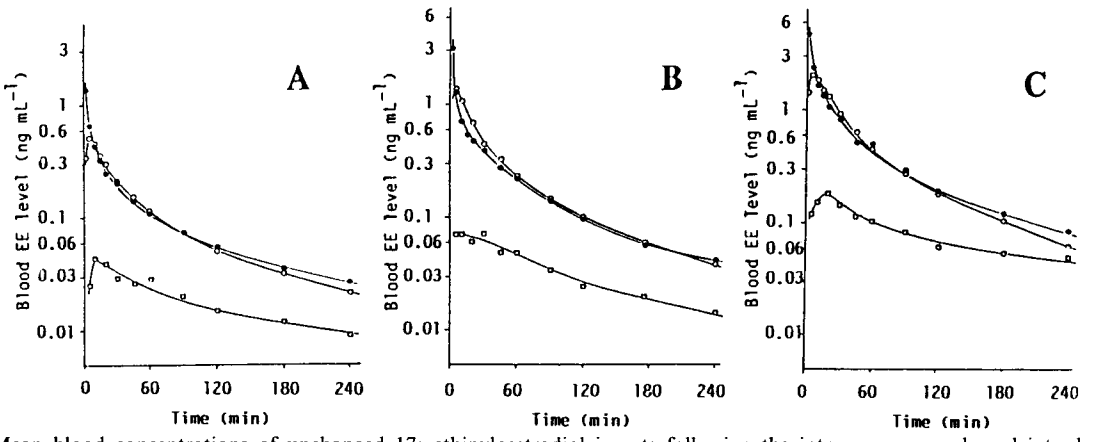

FIG. 1. Mean blood concentrations of unchanged 17α-ethinyloestradiol in rats following the intravenous, nasal, and intraduodenal administration of 1·75 (A), 3·5 (B), and 7·0 (C) μg kg^{-1} of 17α-ethinyloestradiol. Key: ●, intravenous route; ○, nasal, route; □, intraduodenal route.

the area under the blood level curve obtained after nasal administration was directly proportional to the dose administered within the dose range studied.

The data in Table 1 shows the bioavailability and the degree of "first pass metabolism" for the nasal and intraduodenal routes of administration at the three doses used. It is clear from these data that the blood levels of intact 17α-ethinyloestradiol after nasal administration are similar to those after i.v. administration, with the peak blood levels occurring at 5 min after administration, thus indicating rapid absorption.

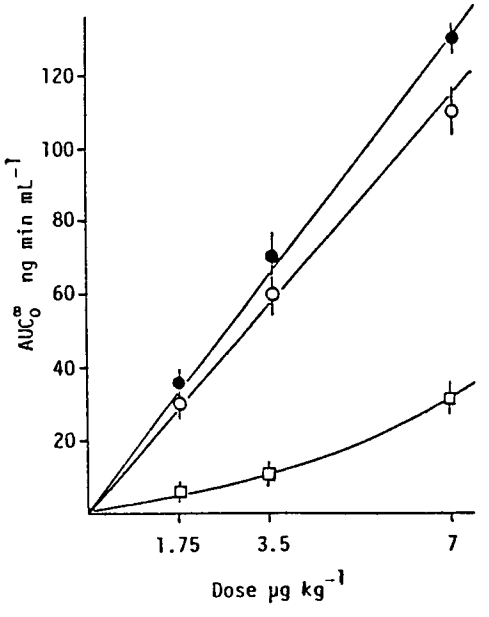

FIG. 2. Area under the blood level curve (AUC) for unchanged 17α-ethinyloestradiol as a function of the dose administered for the intravenous (●), nasal (○), and intraduodenal (□) routes of administration in the rat.

Table 1. Comparative bioavailability and degree of first pass metabolism of 17α-ethinyloestradiol in rats for the nasal and intraduodenal routes of administration at three dose levels

Dose, μg kg^{-1}	Route	Bioavailability[a]	Degree of first pass metabolism
1·75	Nasal	0·80	0·20
	Intraduodenal	0·18	0·82
3·5	Nasal	0·85	0·15
	Intraduodenal	0·15	0·85
7	Nasal	0·84	0·16
	Intraduodenal	0·25	0·75

[a] Compared with bioavailability = 1·0 via the i.v. route.

The bioavailability of intact 17α-ethinyloestradiol after nasal administration was 80 to 85% of that following i.v. administration for the three doses used, whereas, equivalent intraduodenal doses resulted in only 15 to 25% bioavailability, as is shown in Table 1. The 20% difference in bioavailability of 17α-ethinyloestradiol via the nasal route compared with the i.v. route suggests that the synthetic steroid may undergo metabolism in the nasal mucosa. However, the degree of "first pass metabolism" after nasal administration was smaller than that after intraduodenal, and did not vary with the dose in the dose range studied (see Table 1). In this respect, it has been shown previously (Bawarshi-Nassar et al 1988) that 17β-oestradiol is metabolized in the nasal mucosa via conjugation and oxidation, however, the extent of these two metabolic pathways was smaller following nasal administration than that after intraduodenal administration.

In conclusion, the results of this study demonstrate that the nasal route for the administration of 17α-ethinyloestradiol is superior to the oral route, and may thus provide an alternative for the administration of this synthetic steroid at lower doses.

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