Effects of Sex and Route of Administration on Water Diuresis in Elipten-Treated Rats

W. J. Eversole
Department of Life Sciences
Indiana State University, Terre Haute, Indiana 47809

Abstract

Results from water diuresis studies in rats treated with amino-glutethimide phosphate (Elipten, AGP) indicated that the drug was less effective by the oral route than when administered subcutaneously and females were more sensitive to the compound than males. There was a graded dose-response relationship to the drug in both sexes over the range of 50 to 200 milligrams per kilogram of body weight. These findings emphasize the importance of considering dosage, route of administration, and sex of the animals in studies dealing with responses to Elipten.

Introduction

The compound aminogluthethimide phosphate (Elipten-CIBA) elicits a broad spectrum of endocrine effects and is known to be an inhibitor of steriod hormone synthesis (2, 3, 5, 8, 9). Cash *et al.* (2) reported that Elipten treatment of a patient with Cushing's syndrome, due to metastatic adrenal carcinoma, resulted in an episode of frank adrenal insufficiency after only 5 days of treatment. It is well established that in adrenal insufficiency administered water is excreted at a very slow rate and retention of large amounts of water leads to water intoxication (4, 6). Since aminoglutethimide phosphate (AGP) inhibits adrenal steroidogenesis and, in some instances at least, causes frank adrenal insufficiency it was postulated that it would depress the diuretic response to water in intact animals. Preliminary tests indicated that AGP indeed would depress the renal excretion of administered water and this response was used as a simple testing procedure to determine the effects of the compound when administered by various routes to the two sexes. The question of sex and route as possible modifying factors arose from divergent unpublished findings on the effects of Elipten on water intake and excretion in the laboratories at Indiana State University and those at the CIBA Corporation in Summit, New Jersey (R. Gaunt, personal communication). At Indiana State females and subcutaneous injections had been used, whereas at CIBA males and oral administration had been employed.

Since sex and differences in route of administration conceivably contribute to modifications in Elipten's biological effectiveness it seemed necessary to investigate these two parameters to determine whether these factors would be important ones to control in further experimentation with this compound.

Methods and Materials

A total of 240 unbred rats of the Charles River strain, weighing 225 ± 50g, were given a two-dose water diuresis test. The animals were separated into 20 groups with 12 rats per group: 10 groups were males

and the other 10 groups were females. Forty-eight males received a watery solution of Elipten by stomach tube and one group of 12 untreated controls were stomach-tubed with the same volume of water; 60 females were similarly treated. For the animals injected subcutaneously with Elipten the grouping and treatment followed the same pattern as above, the only difference being the route of administration. The grouping design and dosages used are shown in Figure 1.

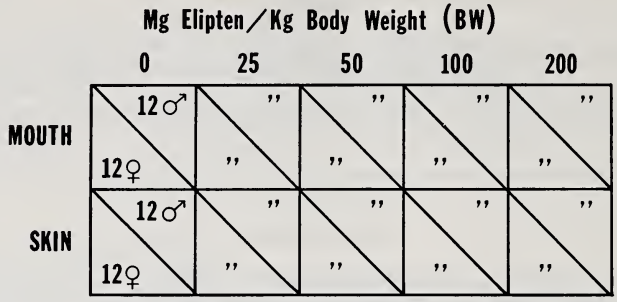

FIGURE 1. *Experimental design for grouping of rats treated with Elipten.*

The water diuresis experiments were conducted as follows:

1) Animals were fasted for 18-24 hours prior to testing but were allowed access to drinking water.
2) All tests were conducted in air conditioned rooms kept at 72° ± 2° F and 50 ± 10% relative humidity.
3) Each animal was placed in an individual metabolism cage equipped with a wire mesh bottom, a funnel, and 100 ml graduate cylinder for collecting urine. Six animals were accomodated for each run.
4) Elipten or water vehicle (control) was given at 0 hour followed immediately by a dose of distilled water (98°F) equal to 5% of the body weight administered with a long blunt-tipped hypodermic needle serving as a stomach tube.
5) A second dose of water was given 30 min later.
6) Measurements of cumulative urine volumes were taken at 1, 2 and 3-hour intervals.
7) The volume of urine excreted was expressed as percentage of water administered in arriving at the rate of urinary excretion of administered water.
8) Analysis of variance was conducted on excretion rates at 2 and 3 hours for effects of dosage, route of administration, and sex. Since the volume of urine at 1 hour was scanty and highly variable it was deemed unnecessary to analyze the data statistically for this time interval.
9) Only the 3 hour excretion values are tabulated here. However, both the 2 hour and 3 hour-mean values of treated rats were significantly different from the controls and from each other.

Results

The results obtained for mean percentage water excreted 3 hours after the first water load and Elipten treatment are summarized in Table 1. The data show decreasing water excretion with increasing dosage, particularly over the ranges of 50 to 200/kg BW. There was no effect with 25 mg/kg in males with oral or subcutaneous administration, but females showed reduction in water excretion at this dosage regardless of route of administration. It would appear that 25 mg/kg is at or near the minimum effective dose for females. Rats of either sex receiving 50 mg/kg of Elipten by subcutaneous or oral administration excreted significantly less water than untreated controls, and there were significant differences ($<1\%$) between 50, 100, and 200 mg/kg dosage levels.

TABLE 1. *The effects of Elipten on water excretion at three hours.*

Route of Administration of Elipten	Dose mg/kg	% Excretion (Mean±S.D.) Male	Female
Mouth	0	85.8± 7.0	87.5± 6.6
"	25	88.3± 6.3	75.1±13.3
"	50	83.0± 5.5	70.0± 5.0
"	100	77.6±10.0	59.8± 5.0
"	200	47.7±10.9	10.4± 5.0
Skin	0	81.1±13.2	84.9± 2.4
"	25	88.0± 7.4	78.4± 7.2
"	50	67.5± 9.5	65.1± 7.9
"	100	55.2±11.6	49.3±11.7
"	200	26.0±14.9	12.9± 9.0

*Each mean was derived from excretion values for 12 rats.

The studies on effects of sex on water excretion show that at each dosage the females excreted less of the administered water than males (Table 1). In other words, the drug was more antidiuretic in females.

Results on the effects of route of administration showed at each dosage level that subcutaneous injection of Eilpten was more effective in depressing water excretion than oral administration (Table 1).

Discussion

My results demonstrate that aminogluthethimide phosphate Elipten, AGP), in doses ranging from 50 to 200 mg/kg BW, suppresses the renal excretion of administered water regardless of sex and mode of administration. A dosage of 25 mg/kg BW depresses water excretion in females but is ineffective in males. Reasons for the antidiuretic effects are unknown but it is quite likely that one factor involved is the inhibition of adrenal cortical function and lowering of circulating adrenal cortical steroids. Such an hypothesis is in line with the reports of several investigators who have shown that aminoglutethimide inhibits steroidogenesis in the adrenal cortex and that there is sluggish excretion

of administered water associated with adrenal cortical insufficiency (2-4, 5, 6). Elipten does have a generalized depressing effect on body functions, particularly at high dosage levels, and is an anticonvulsant (1, 7). It is quite possible that the effects on water excretion are mediated *via* neurohumoral mechanisms involving both neuromuscular and endocrine systems. The author has observed a delayed emptying time of the stomach after Elipten treatment and it appears likely that there is slowing of the action of smooth muscle in the gastrointestinal tract as well as depression of skeletal muscle function.

Reasons why the female is more sensitive to Elipten than the male are also obscure but here again steroidogenesis is probably involved. The cyclic hypothalamico-pituitary-gonadal axis of the female is probably more responsive to the depressing effects of AGP than the noncyclic corresponding axis in the male. Preliminary work by the author and his students have indicated that the miscroscopic structure of steroid producing tissue in the ovary is subject to greater modification with Elipten than similar tissue in the testis. Further work along these lines would help elucidate the reasons why Elipten is more effective in females than in males.

It is likely that the reasons why the oral route is inferior to subcutaneous injection is because of modification of the Elipten molecule by digestive enzymes and/or liver action. It would be interesting and informative to study the effects of Elipten pellets implanted into a portal drainage site. Results from such an experiment should help indicate whether the liver or the gut is involved in depressing Elipten's actions.

The major significance of this study is that it emphasizes the necessity of considering dosage, route of administration, and sex in experimental models designed to study the effects of Elipten on biological processes.

Acknowledgment

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