

Effects of Amino-glutethimide and Diphenylhydantoin Sodium on the Rat Adrenal Cortex

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Abstract

Amino-glutethimide phosphate (AGP) was injected subcutaneously at a dosage level of 100mg/Kg body weight into a group of Charles River strain male rats 21 days of age. Diphenylhydantoin sodium (Dilantin) was administered at the same dosage level to a second group of animals of the same strain. The control animals were injected with physiological saline at a dose of 1ml/Kg body weight. Single daily injections were administered throughout the study.

The study was conducted in three stages. First, a series of 30 successive days of treatment; secondly a week of treatment at which time the test animals were sacrificed; and thirdly, a period like the second trial, but the rats were left a week without treatment before being sacrificed. Histological examination revealed a definite increase in adrenal fat in the AGP-treated rats when compared to the control or Dilantin-treated rat. The cortical fat in the AGP-treated animals was deposited in larger intercellular globules than in the non AGP-treated animals. There was little difference in the adrenal weights between the Dilantin and control animals, but there was an adrenal weight increase found in the AGP-treated animals. However, the body weight was higher in the control groups than in either the Dilantin or AGP-treated groups, by percentage gained. Chromatographic methods indicated a slight increase in cholesterol content in the treated animals versus the controls.

Introduction

Amino-glutethimide phosphate (AGP) and diphenylhydantoin sodium (Dilantin, DPH) are anticonvulsive agents which have been used independently and in combination for the treatment of epilepsy in humans (2, 10). Dilantin administration decreased adrenal response in some epileptic children (3), but this mild effect has not detracted from the use of this drug as an antiepileptic agent. In other studies, Rallison *et al.* (10) reported that AGP induced goiter formation and reduced thyroid activity in children, and Pittman and Brown (9) reported adrenal and ovarian enlargement following administration of this compound in rats. Findings such as these have reduced the clinical use of this drug as an antiepileptic agent. Bonnycastle and Bradley (3) reported that Dilantin probably inhibits functional activity of the pituitary-adrenal axis, whereas Fishman *et al.* (6) found that amino-glutethimide, in large doses, consistently caused an increase in plasma ACTH thus implying that the compound acted peripherally on corticosteroid synthesis rather than through the pituitary. The purpose of the present investigation was to study the effects of these two drugs on the microscopic structure of the adrenal cortex of the male rat and to determine what effect the compounds have on adrenal fat and cholesterol.

Materials and Methods

In this study 47 male rats of the Charles River strain were used. Treatment started at the age of 21 days, at which time experimental

animals were placed in groups of six or eight and maintained on Wayne Laboratory Chow and water *ab libitum*. Litter mates were selected as test animals in attempt to minimize possible genetic differences.

AGP and Dilantin were dissolved in physiological saline and administered in a dose of 100mg/Kg body weight; daily injections were given subcutaneously throughout the study. The control animals were injected with physiological saline in a dose of 0.1ml/100 gms. body weight, and adjustments in dosages were made weekly after body weights were taken.

The study was conducted in three stages. (1) a long term study of 30 days of single daily injections to 31 animals; (2) a short term study of 7 days for 8 animals; (3) another 7-day term of treatment for 8 animals followed by a week of no treatment. Animals were sacrificed by a single blow to the back of the head, the adrenals removed, gently trimmed of excess tissue and carefully weighed to the nearest 0.1 mg on a microbalance. Upon removal the adrenals were frozen in microgel, wrapped in Saran Wrap and stored at -15 degrees centigrade until used. They were then sectioned at 8μ in 80μ intervals using a rotary microtome (International Cryostat) at -15 degrees C. The staining procedures were modified from those described by Lille (8), using Sudan IV stain for fat.

Extracts of adrenal cholesterol were obtained by a method similar to that used by Hechter *et al.* (7). The adrenals, after removal, were rinsed in saline to remove blood and then stored in acetone under refrigeration for 14-48 hours. The acetone layer was poured off and allowed to dry; the residue was then extracted with chloroform and dried again. The tissues were thoroughly minced and extracted four times with chloroform, and the extracted residue from the acetone portion was added to the tissue extract fraction. This mixture was allowed to dry and then dissolved in methanol and spotted on chromatographic strips.

Instant thin layer silica gel chromatographic strips were heated at 110 degrees C for one hour to activate them. Following activation the strips were cooled and spotted with test material. The extracts were run with a known standard cholesterol spot for comparison. The spots were placed 2 cm from the bottom of the strip and run a distance of 16 cm. Each chromatographic run took 20-40 minutes in a Gelman Rapid Chromatographic Chamber. Following the run the strips were dried and then sprayed with phosphoric acid and water (1:1) and heated at 110 degrees C for ten minutes. After heating the strips were sprayed with phosphomolybdic acid and methanol (1:2) and reheated at 110 degrees C for ten minutes. The sprays helped intensify the spots. The solvent used for the ascending technique was 100% benzene.

Results

Treatment did not cause severe adverse effects in the rats, but amino-glutethimide did induce ataxia within five minutes following injection. These animals appeared feeble, especially in the hind legs, and remained this way for 15-30 minutes unless disturbed. If the rats

were stimulated to move, they could do so without difficulty, but most of the animals sat quietly.

In all cases the control groups gained more weight than the treated groups. At the same time, the mean adrenal weight was higher in the AGP-treated animal compared to the Dilantin-treated or control animal, but such differences were not statistically significant (Table 1). Fat infiltration was found to be much greater in the adrenal cortex of the

TABLE 1
Effects of Aminoglutethimide and Dilatin on the Body Weight and Adrenal Weight on the Male Rat

| Treatment | Initial Age (days) | Mean Body Wt. (grams) | | Actual Adrenal Wt. (mg) |
|-------------------------|--------------------|-----------------------|--------|-------------------------|
| | | Init. | % Gain | Combined+S.E. |
| Long Term Group (30)* | | | | |
| AGP (100mg/Kg)** | 21 | 47.3 | 333.2 | 31.8±1.29 |
| (10)*** | | | | |
| Dilantin (100mg/Kg) | 21 | 39.5 | 408.4 | 30.6±0.78 |
| (11) | | | | |
| Control (0.1ml/100 gms) | 21 | 41.1 | 454.3 | 30.6±1.02 |
| (10) | | | | |
| Short Term (7) | | | | |
| AGP | 21 | 41.0 | 69.9 | 22.1±0.40 |
| (3) | | | | |
| Dilantin | 21 | 35.7 | 85.0 | 18.1±2.03 |
| (3) | | | | |
| Control | 21 | 35.0 | 94.2 | 17.8±1.28 |
| (2) | | | | |
| Short Term (7)+ | | | | |
| AGP | 21 | 43.0 | 95.2 | 25.1±0.29 |
| (3) | | | | |
| Dilantin | 21 | 42.7 | 89.1 | 24.3±1.57 |
| (3) | | | | |
| Control | 21 | 41.5 | 102.5 | 23.0±0.33 |
| (2) | | | | |

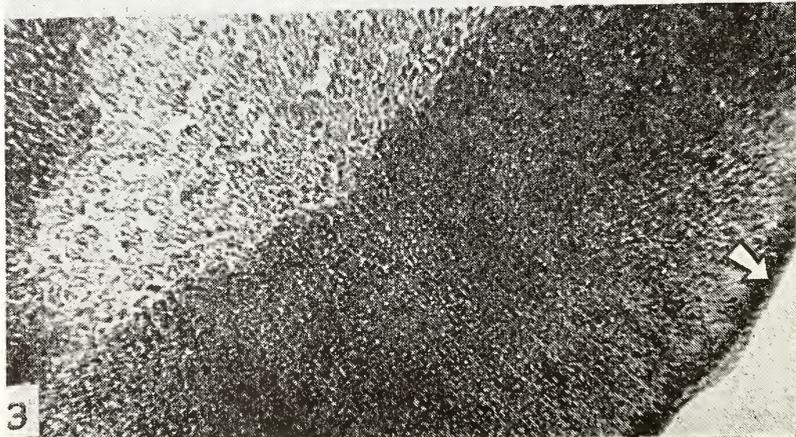
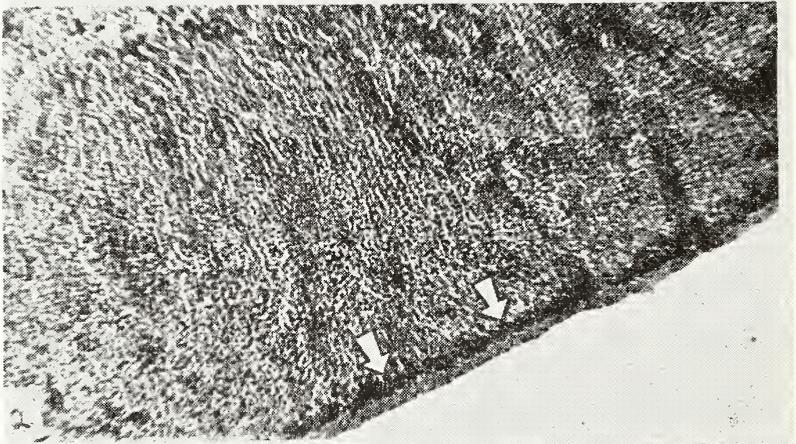
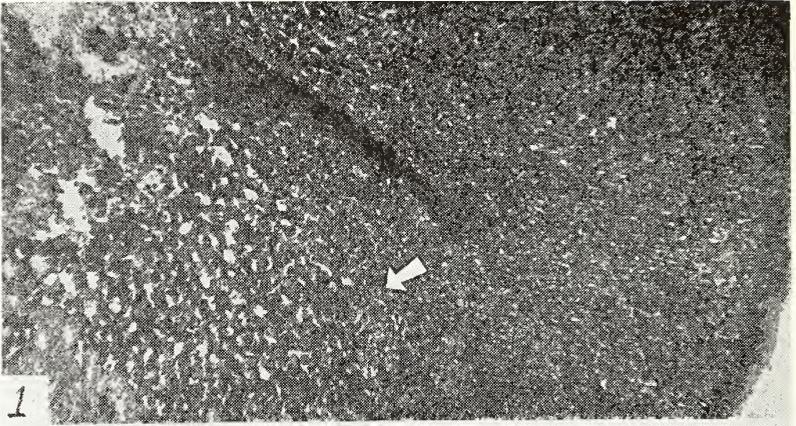
* Number of days treated.

** Dosage levels for respective agents used.

*** Number of animals tested per group.

+ Animals treated for 7 days and left 7 days without treatment.

AGP-treated animal than in either the Dilantin-treated or control animal. The relative amount of fat was estimated by direct observation and found to be approximately 15-20% higher in the AGP-treated animal. There was little difference in the fat deposition between the Dilantin-treated rat and the control rat. These findings correlated closely with the adrenal weights as shown in Table 1. Fat was deposited in intercellular globules which appeared to be larger and more prevalent in the AGP-treated rat than in the other animals studied (Fig. 1). Most of this deposition was found throughout the *zona fasciculata*. A ring of intercellular fat was seen between the capsule and the *zona fasciculata* in the adrenals of all rats studied (Figs. 1, 2, 3). This ring of fat was made up of small globules and accounted for most of the fat observed



in the adrenals of the control and Dilantin-treated animals. In the latter groups there was no indication of fatty infiltration in any of the zones of the adrenal cortex.

Cholesterol was found in all of the chromatographs as evidenced by the consistency of the R_f values. However, the intensities of the spots were so variable that it was difficult to interpret the relative amounts of cholesterol found in the various tests. There seemed to be a tendency for the adrenals of the AGP-treated and Dilantin-treated animals to show an increase in adrenal cholesterol concentration when compared with controls. Confirmation of this apparent increase in cholesterol in the adrenals of treated rats must await further investigation where more refined methods might reveal a clearer picture as to possible influences of these drugs on adrenal cholesterol content.

Discussion

The results of this investigation indicate that amino-glutethimide influences fat deposition in the rat adrenal cortex, where diphenylhydantoin apparently has no effect in this respect. Lack of effect with DPH could be related to low dosage and it would be interesting to determine whether increased amounts modify adrenal morphology. The ataxia observed in this study after AGP treatment has also been reported in human patients receiving large daily dosages (1500 mg) of this compound (1). The sedative action of this compound is a well known effect and it can be modified by replacement or shortening the aliphatic chain on the glutarimide molecule.

Significant adrenal weight increases in the intact rat administered AGP has been reported by Pittman and Brown (9). However these investigators used dosages 2.5-5.0 times larger than those employed here, which probably accounted for the fact that the adrenals of their treated animals were much heavier than those reported in the present investigation. Dexter *et al.* (4) reported that the increase in adrenal weight in AGP-treated rats was associated with a proportional increase in adrenal protein. Although an increase in protein could no doubt account for some increase in adrenal weight, present evidence favors the idea that an increase in fat deposition is a prime factor which should be taken into consideration in contributing to drug-induced adrenal hypertrophy.

Although in this study Dilantin-treated animals showed little evidence of adrenal hypertrophy or fat infiltration, Dill (5) reported adrenal hypertrophy in rats given the same dosage intraperitoneally. His experiment showed a significant increase in adrenal weight over controls

Figure 1. Adrenal section of AGP-treated animal following 30 days treatment; stained with Sudan IV. Note large fat globules (which stained bright orange with Sudan IV) and lack of zonation. About 35x.

Figure 2. Adrenal section of Dilantin-treated animal following 30 days of treatment; stained with Sudan IV. Note dark ring of fat cells near capsule. About 35x.

Figure 3. Adrenal section of a control animal. Note ring of small fat globules near capsule as found in all other sections. About 35x.

and the reason for discrepancies between his results and those reported here may be related to differences in absorption rates.

Present studies revealed a trend toward increased adrenal cholesterol following treatment with either compound but the differences were not striking. Dexter *et al.* (4) reported that amino-glutethimide blocked corticosteroid biosynthesis from cholesterol thus adding support to the possibility of an increase in adrenal cholesterol after AGP treatment. Bonnycastle and Bradley (3), however, observed no significant differences in the cholesterol content of one adrenal following unilateral adrenalectomy in rats administered Dilantin. This observation suggested that the DPH-treated animal has normal cholesterol levels which are not affected by the stress of unilateral adrenalectomy. The investigations of these workers indicated that the initial effect of DPH treatment produced elevated corticosteroid secretion followed by a depression of adrenal response to ACTH treatment. Such findings would appear to indicate fluctuating levels of corticosteroid release without observable changes in adrenal cholesterol. Apparently DPH treatment affects steroid storage and release differently than does AGP, and clarification of specific actions on steroid metabolism must await further detailed investigations.

Summary

1. Animals treated with either Dilantin or AGP gained appreciable weight, although not as much as the non-treated controls. 2. AGP-treatment appeared to result in adrenal enlargement, whereas Dilantin treatment was ineffective in this respect. 3. Fat globules were more abundant in the adrenal cortex of AGP-treated animals, and it is suggested that fat infiltration is in part responsible for apparent adrenal weight increase. 4. The adrenals of treated groups appeared to show a slight increase in cholesterol content, but more detailed studies should be done before definite conclusions are made along these lines.

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