Biochemical Pharmacology, Vol. 29, pp. 1669–1672.

BIOCHEM MS. 0006-2952/80/0615-1669 $02.00/0

EFFECTS OF DANTROLENE ON ADRENAL CORTICAL FUNCTION

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(*Received* 2 *November* 1979; *accepted* 14 *January* 1980)

Abstract—Adverse effects of dantrolene include abnormal liver function tests, hepatic injury, and alteration of the hepatic mixed function oxidase cytochrome P-450 system. The following study conducted in rats indicates that the effective dose of dantrolene that has been shown to decrease hepatic cytochrome P-450 also causes adrenal enlargement with a marked reduction in serum glucocorticoids. The ratio of adrenal wet weight/body weight was significantly ($P < 0.05$) increased following 5 days of injection with a minimum dose of 25 mg dantrolene/kg body weight. The minimum effective dose of dantrolene that significantly ($P < 0.05$) lowered serum glucocorticoids was 50 mg/kg body weight. Serum glucocorticoids were reduced by half following 5 days of treatment with 100 mg dantrolene/kg body weight. The levels of serum glucocorticoids were 80 per cent of control serum levels and the adrenal wet weight/body weight ratio was 85 per cent of control values following 3 days of recovery after 5 days of pre-treatment with 100 mg/kg of dantrolene. It appears that dantrolene acts in a manner similar to diphenylhydantoin to reduce adrenal glucocorticoid secretion, with a possible alteration or reduction in the negative feedback response to the pituitary and resultant enlargement of the adrenals.

Dantrolene sodium (1-{[5-(*p*-nitrophenyl) furfurylidene]amino}hydantoin sodium) is a muscle relaxant which acts by depressing the contractility of skeletal muscle. The drug is used for treatment of spasticity [1–3]. Its site of action is within the muscle, where it inhibits the release of calcium from the sarcoplasmic reticulum and thereby uncouples excitation–contraction coupling [4–6]. Putney and Bianchi [7] have reported that dantrolene inhibits the inward movement of the calcium pulse across the membrane, which triggers the release of calcium from the sarcoplasmic reticulum.

Adverse effects of dantrolene include abnormal liver function tests, hepatic injury, and even death [8]. Recently, Francis and Hamrick [9] have shown that dantrolene, in addition, depletes the quantity of cytochrome P-450 of the hepatic mixed function oxidase (MFO) system and thereby alters specific drug metabolism. The adrenal cortex, which produces steroid hormones, is also known to contain a number of P-450 cytochromes which function in oxygen activation of the hydroxylation of steroids [10–12]. Because of the depressive effect of dantrolene on the hepatic mixed function oxidase system and drug metabolism, it was hypothesized that dantrolene would, in the same way, alter the adrenal cortical hormone synthesis with a resultant decrease in steroid secretion.

METHODS

Male Sprague–Dawley rats, weighing 160–250 g, were obtained from Southern Animal Farms in Prattville, AL. Rats utilized in the study of the dose–response effect of dantrolene on adrenocortical function were injected for 5 days with 25, 50 or 100 mg/kg of dantrolene suspended in corn oil. This range of dosages was chosen because 25 mg/kg of dantrolene administered orally is the minimum dose that produces skeletal muscle relaxation in rats [5], plus the fact that previous studies [9] indicate that this range of dosages severely affects the hepatic mixed function oxidase system. All rats received a daily intraperitoneal (i.p.) injection of dantrolene; controls received equal volumes of corn oil. Twenty-four hr after the last injection both control and treated rats were anesthesized lightly with ether and a blood sample was obtained, by heart puncture, for glucocorticoid and glucose estimation. The rat was then killed and the adrenal glands were removed and weighed. Because of the diurnal variation in blood glucocorticoids [13], all rats were maintained in controlled lighting of 12 hr light/12 hr dark, with the lights on from 6:00 a.m. to 6:00 p.m. All blood samples were obtained at the peak period of glucocorticoid secretion (4:00 p.m.). The mild stress associated with light ether anesthesia did not appear to alter serum glucocorticoid levels. Control values recorded in this study agree closely with previous recorded literature values [13–15].

Rats, used to determine the effect of repeated high doses of dantrolene on adrenal cortical function, were injected i.p. with 100 mg/kg of dantrolene for 5 days. Each rat served as its own control. Twenty-four hours after each daily injection, a small sample of blood was obtained by heart puncture for glucocorticoid and glucose determination.

In experiments investigating the reversibility of the effect of dantrolene on adrenocortical function, rats were pre-treated with 100 mg/kg of dantrolene, i.p., in corn oil for 5 days. A blood sample was obtained by heart puncture and the adrenal weight was obtained on days 3, 5 and 7 post-dantrolene treatment.

Alterations of 17-ketosteroid excretion were studied during 5 days of dantrolene treatment and the succeeding 5 days following dantrolene treatment. Rats were injected for 5 days with 100 mg/kg of dantrolene in corn oil and were placed in metabolism cages. Twenty-four-hour urine samples were collected on days 3 and 5 of dantrolene treatment and on days 3 and 5 post-injection. Controls received similar volumes of corn oil.

Rats, used to study the effect of dantrolene on testosterone secretion by the male reproductive system, were injected i.p. with 100 mg/kg of dantrolene in corn oil. Controls received an equal volume of corn oil. Twenty-four hours after the last injection, a blood sample was obtained by heart puncture, the rats were killed and the testes and seminal vesicles were removed and weighed. Because of the diurnal variation in serum levels of testosterone, animals were killed during peak levels (8:00 a.m.) of serum testosterone [16].

For histological examination, paraffin sections of the adrenal gland were prepared and stained with hematoxylin and eosin.

Analysis. Glucocorticoids were assayed by radioimmunoassay according to the procedure of Rolleri *et al.* [17] and Catt and Tregear [18]. The primary glucocorticoid measured by this procedure was corticosterone; however, the cross reactivity of the antiserum used was 50 per cent with cortisol. Hence, glucocorticoid was used as a more inclusive term. Serum glucose was assayed using the *O*-toluidine method [19]. Urinary 17-ketosteroids were assayed using the Zimmermann reaction as described by Sobel *et al.* [20]. Serum levels of testosterone were assayed by radioimmunoassay [21].

Statistical analysis. Where appropriate, statistical comparisons of independent sample means were made using Student '*t*'-test or paired '*t*'-test at the 95 per cent level of confidence.

RESULTS

Figure 1 shows that the administration of 25 mg/kg of dantrolene for 5 days reduced serum glucocorticoids 21 per cent and increased the ratio of adrenal wet weight/body weight by 15 per cent in comparison to corn oil-treated rats. The administration of 50 mg/kg of dantrolene for 5 days significantly ($P < 0.05$) reduced serum glucocorticoids by 33 per cent and increased the ratio of adrenal wet weight/body weight by 14 per cent ($P < 0.05$); however, the level of serum glucose was not altered in comparison to corn oil-treated rats. The adrenal glands from rats treated with 100 mg/kg of dantrolene for 5 days were 31 per cent heavier on a wet-weight basis and 48 per cent heavier on a dry-weight basis than those from rats receiving only corn oil. Similarly, the ratio of adrenal wet weight/body weight was significantly ($P < 0.01$) increased following injection for 5 days with 100 mg/kg of dantrolene. The increase in this ration may be attributed partially to the 10 per cent decrease in body weight of rats receiving the dantrolene. Correspondingly, the levels of serum glucocorticoids and serum glucose were significantly ($P < 0.001$ and < 0.05, respectively) reduced after 5 days of pretreatment with dantrolene.

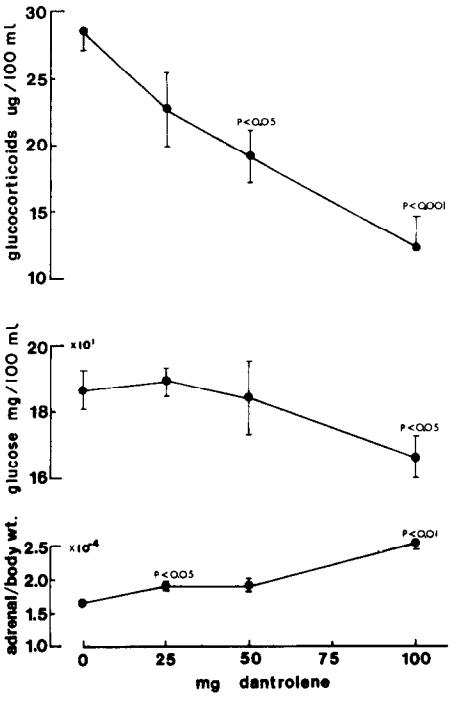

Fig. 1. Effects of 5 days of pretreatment of 25, 50 or 100 mg/kg of dantrolene, i.p., in corn oil on serum levels of glucocorticoids, glucose and the ratio of wet adrenal weight to body weight in male rats. Controls received an equal volume of corn oil. All values are means ± S.E. of eight animals.

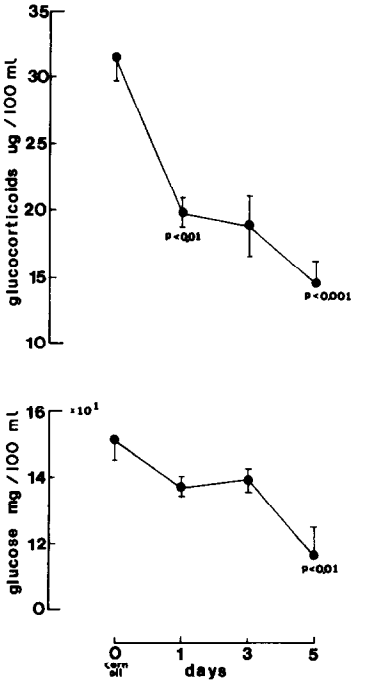

Fig. 2. Effects of 1, 3 and 5 days of treatment with dantrolene on serum levels of glucocorticoids and serum glucose in male rats. Dantrolene was administered in a dose of 100 mg/kg, i.p., in corn oil. Controls received an equal volume of corn oil. All values are means ± S.E. of eight animals.

Figure 2 shows that, after only one injection of 100 mg/kg dantrolene, serum glucocorticoids were reduced 37 per cent ($P < 0.01$) in comparison to pre-injection levels. Three days of dantrolene treatment (100 mg/kg for 3 days) reduced the serum glucocorticoids by 40 per cent ($P < 0.01$) and 5 days of injection (100 mg/kg for 5 days) reduced serum glucocorticoids 54 per cent ($P < 0.001$) in comparison to pre-injection levels. Similarly, the level of serum glucose exhibited a trend similar to that of serum glucocorticoids during the treatment with dantrolene. Serum glucose decreased 10 per cent after one injection of 100 mg/kg of dantrolene and 23 per cent ($P < 0.01$) after injection for 5 days of 100 mg/kg of dantrolene (100 mg/kg for 5 days) when compared to pre-injection levels. However, the level of serum glucocorticoids was 80 per cent of control serum levels and the adrenal wet weight/body weight ratio was 85 per cent of control values following 3 days of recovery after treatment for 5 days with 100 mg/kg of dantrolene (Fig. 3).

Figure 4 shows that 3 days of treatment with 100 mg/kg of dantrolene significantly ($P < 0.05$) reduced urinary excretion of 17-ketosteroids (17-KS) when compared to corn oil-treated rats. Five days of injection with 100 mg/kg of dantrolene resulted in a 64 per cent ($P < 0.01$) reduction in urinary 17-KS. However, 3 days after terminating the dantrolene treatment, the urinary level of 17-KS returned to pre-injection levels.

Histological analysis of adrenal gland. The increase in the ratio of wet and dry adrenal weight/body

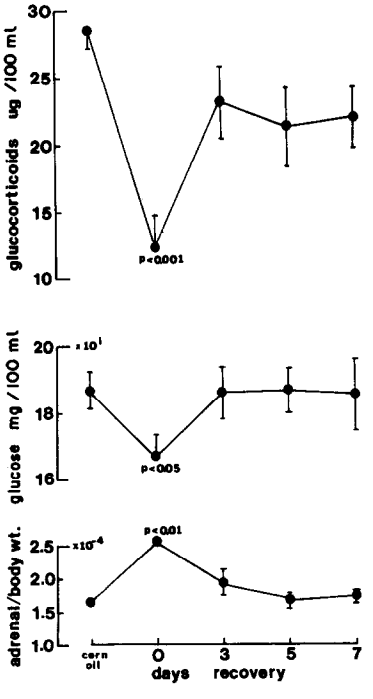

Fig. 3. Reversible inhibition of adrenocortical function by dantrolene in male rats. Dantrolene was administered in a dose of 100 mg/kg, i.p., in corn oil for 5 days. Controls received an equal volume of corn oil. All values are means ± S.E. of eight animals.

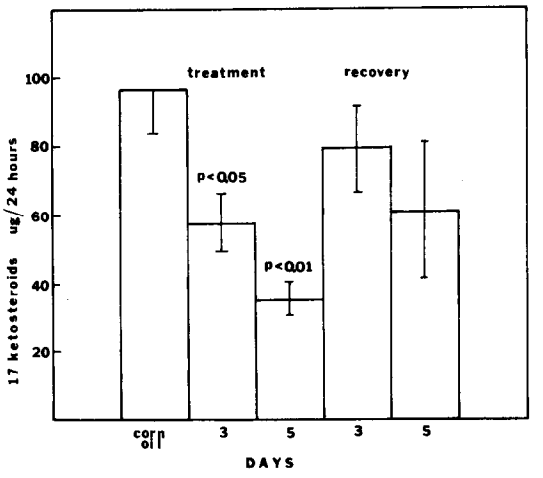

Fig. 4. Twenty-four-hour urinary excretion of 17-ketosteroids in male rats. Dantrolene was administered in a dose of 100 mg/kg, i.p., in corn oil for 5 days. Controls received an equal volume of corn oil. All values are means ± S.E. of eight animals.

weight that occurred during dantrolene treatment was due to hyperplasia of the adrenal cortex. Hyperemia and vacuolization of the adrenal cortex were observed in rats treated with as little as 25 mg/kg for 5 days. In addition to the hyperemia and vacuolization that occurred with the smaller doses of dantrolene, cell necrosis was evident with the higher doses of dantrolene (100 mg/kg for 5 days). Hyperemia and vacuolization were still evident 5 days after cessation of this higher dose.

Dantrolene treatment (100 mg/kg for 5 days) and serum testosterone. Five days of treatment with 100 mg/kg of dantrolene resulted in no change in the ratio of wet or dry seminal vesicle weight/body weight or wet or dry testis weight/body weight when compared to corn oil-treated rats (Table 1). Similarly, there were no significant changes in the serum levels of testosterone in the dantrolene-treated rats compared to corn oil-treated animals.

DISCUSSION

The minimum effective oral dose of dantrolene in rats for producing muscle relaxation is 25 mg/kg [5]. Ellis and Carpenter [5] have reported that an i.p. injection of dantrolene (10–1600 mg/kg) to rats caused a dose-dependent skeletal muscle relaxation with no evidence of toxicity. However, previous studies [9] on the effects of dantrolene on the hepatic MFO system and the present studies of the effect of dantrolene on adrenal cortical function indicate that pre-treatment with as little as 25 mg/kg of dantrolene for 5 days prolongs pentobarbital sleep times, aminopyrine *N*-demethylation, and lowers serum glucocorticoids and serum glucose.

It is interesting that dantrolene not only inhibits the hepatic MFO system in a dose-related manner [9] but also appears to inhibit adrenal cortical function in a similar dose-related manner (Figs. 1 and 2).

The parallel decreases in urinary 17-ketosteroid

Table 1. Effects of dantrolene treatment on the ratio of wet and dry seminal vesicle and wet and dry testis weight to body weight and serum levels of testosterone*

	Seminal vesicle ratio		Testis		
	Wet wt/Body wt $\times 10^{-3}$	Dry wt/Body wt $\times 10^{-4}$	Wet wt/Body wt $\times 10^{-2}$	Dry wt/Body wt $\times 10^{-3}$	Testosterone (ng/ml)
Control	2.73 ± 0.37	5.85 ± 0.81	0.97 ± 0.06	1.25 ± 0.07	1.39 ± 0.35
Dantrolene	2.15 ± 0.19	4.24 ± 0.28	1.10 ± 0.03	1.34 ± 0.03	1.13 ± 0.31

* Rats received 100 mg/kg of dantrolene, i.p., in corn oil for 5 days. Controls received an equal volume of corn oil. Each value represents the mean ± S.E. of eight rats.

secretion and serum glucocorticoids after 5 days of injection with dantrolene (100 mg/kg for 5 days) and the subsequent recovery of both variables 3 days after cessation of dantrolene treatment indicate that the decrease in serum glucocorticoids is probably due to a decrease in adrenal secretion. Although the metabolic clearance of glucocorticoids was not measured, the conclusion that adrenal steroid synthesis was inhibited is substantiated by the histological observation that the adrenal cortex was damaged by dantrolene and by the fact that inhibition of the hepatic mixed function oxidase system would decrease the rate of glucocorticoid metabolism. The observation that dantrolene affects adrenocortical function without affecting testosterone (Table 1) indicates that a possible site for inhibition of glucocorticoids occurs at some step past 17-hydroxyprogesterone synthesis. A likely site in the inhibitory process could be the 11-hydroxylation of 11-desoxycorticosterone. The exact site of inhibition must await further studies.

It now appears that dantrolene, 'a hydantoin derivative', might act like the widely used drug diphenylhydantoin (DPH). DPH administration has been shown to cause adrenal hypertrophy [22] and adrenal cortical degenerative lesions [23]. Bonnycastle and Bradley [24] have indicated an inhibition of the pituitary–adrenal secretion after chronic DPH treatment of mice and rats. Costa *et al.* [25] have reported that DPH also decreases urinary 17-ketosteroid secretion. It is possible that dantrolene acts in a similar manner to reduce adrenal cortical function with a possible alteration or reduction in the negative feedback response to the pituitary with resultant enlargement of the adrenals, decrease in serum glucocorticoids, and decrease in urinary 17-ketosteroid excretion.

Acknowledgements—Dantrolene was a gift from Eaton Laboratories. This work was supported, in part, by a grant from the Medical Rehabilitation Research and Training Center, University of Alabama in Birmingham, Project R-68.

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