Effect of Exogenous Steroids and Inhibitors of Steroidogenesis on Endotoxin Shock (37088)

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Endotoxin shock, still a serious clinical problem, is poorly understood and has no definitive therapy, but glucocorticoids administered in large doses generally afford some protection against endotoxin shock. In experimental animals such treatment is highly effective when initiated about the time the endotoxin is given.

By contrast, elimination of the major supply of steroids by removal of the adrenal glands results in animals far more susceptible to endotoxins by a factor approximating as much as a thousandfold (6). However, not only is the major source of steroids removed by this manipulation, but other important substances; notably the catecholamines. It is essentially impossible in mice to remove only the cortex of the adrenals, but it is now possible to inhibit cortical activities chemically.

In the past few years several drugs known to interfere with the formation of steroids have been developed. Amphenone B (3,3-bis-(*p*-aminophenyl)-butan-2-one) has a relatively nonspecific effect, inhibiting 11-, 17- and 21-hydroxylations in the steroid nucleus. Metyrapone (2-methyl-1,2-bis-(3-pyridyl)-propan-1-one), a relatively specific agent, affects the synthesis of cortisol and corticosterone by interfering with the 11-hydroxylation (3, 4). Aminoglutethimide (α-(*p*-aminophenyl)-α-ethylglutarimide) and its *o*-aminophenyl derivative act at the level of the cholesterol desmolase system, thereby preventing the entry of the steroid nucleus into the pathways to produce the adrenal cortical steroids (7–9).

Therefore, the availability of compounds which inhibit adrenal steroid synthesis make it possible to achieve decreased blood corticosteroid levels without depriving the animal of medullary function. Changes in the susceptibility to endotoxin and in metabolic responses can be determined in mice with corticosteroids eliminated or decreased by these drugs. The response of inhibitor treated mice to endotoxin shock after replacement of the steroids has been compared with the response of the untreated animals.

Materials and Methods. Endotoxin. The endotoxin was prepared by the method of Boivin (2) from washed *Salmonella enteritidis* grown on Brain Heart Infusion (Difco Labs.) solidified with 2% agar. The lyophilized endotoxin was dissolved in sterile nonpyrogenic distilled water immediately before use.

Inhibitors. Amphenone B, graciously supplied by the Upjohn Co., was dissolved in saline and 5-mg doses administered subcutaneously at various intervals from 24 hr before the endotoxin to 4 hr following the endotoxin. Metyrapone (Metopirone, CIBA Pharmaceutical Co.) was diluted in saline and 1.5 mg injected subcutaneously two hr before and after the endotoxin.

Aminoglutethimide (kindly supplied by J. J. Chart, CIBA as "Elipten" phosphate) was dissolved in saline and 2.5 mg given subcutaneously from 24 hr before to 2 hr after the endotoxin. The ortho-aminophenyl derivative of aminoglutethimide was dissolved in saline and 7.5 mg administered subcutaneously 2 hr before, 2 hr after and simultaneously with endotoxin.

Steroids. Pregnenolone, progesterone, 17-α-hydroxyprogesterone, deoxycorticosterone, corticosterone-21-acetate, hydrocortisone and androstendione (Sigma Chemical Co., St. Louis, Mo.) were suspended in saline containing 2.5% of Tween 80 (1) and 5 mg (0.2 ml) administered intraperitoneally immediately following the endotoxin.

TABLE I. The Effect of 2.5 mg Aminoglutethimide, Administered at the Times Indicated, on the LD_{50} of Endotoxin in Mice.

Treatment	LD_{50} (μg)
Endotoxin control	244 (2)[a]
Aminoglutethimide (2.5 mg)	
2 hr pre	27.3 (6)
2 hr post	22.5 (2)
24 hr pre	67.3 (1)
24 hr and 2 hr pre	35.8 (3)

[a] Number of determinations.

Mice. The mice, 20–22 g male, strain ICR, obtained from the Rawley Farms, Plymouth, Michigan, were housed on wire mesh in metal cages and were maintained in the laboratory for one week before use. Food and water were freely available to the animals at all times. For the LD_{50} studies, groups of six mice were selected at random for each of the five doses of endotoxin. The LD_{50} was calculated by the method of Reed and Muench (13).

Results. Amphenone B, the first inhibitor studied, was administered from 24 hr before to 4 hr following endotoxin. When the amphenone B was given 24 hr or 1 hr before 250 μg of endotoxin 14 of the 24 test animals died, as was also observed for the endotoxin controls. However, when amphenone B was given at the same time as the endotoxin the toxicity was enhanced so no mice survived at 24 hr. A delay in administration of the amphenone B for 1, 2, or 4 hr enhanced the effect of the endotoxin so that 19 of 24 mice died. By contrast amphenone B administered 2 hr before endotoxin conferred appreciable protection, as only 5 of 24 animals died. These effects were further demonstrated by the 20% decrease in the LD_{50} of endotoxin observed when the amphenone B was given 2½ hr after endotoxin or the 20% larger dose required when the amphenone B was given 4 hr before the endotoxin. The second inhibitor, metyrapone, administered 2 hr prior to endotoxin had no apparent effect, but if administered 2 hr after the endotoxin the LD_{50} was decreased by 20%.

The third agent, aminoglutethimide, was highly effective in enhancing the susceptibility of the host to endotoxin. A 2.5-mg dose of aminoglutethimide given within reasonable proximity, reduced the LD_{50} dose of endotoxin by about 90% (Table I). Even when aminoglutethimide was given as much as 24 hr prior to the endotoxin the LD_{50} was 67.3 μg, only about three times the value (27.3 μg) obtained when the inhibitor was given close to the endotoxin, but still well below the 244 μg determined for the control LD_{50}. Mice given two doses of aminoglutethimide, one at 24 hr and the second 2 hr before endotoxin, had susceptibility intermediate to that of animals given a single dose either 24 hr before endotoxin or 2 hr before endotoxin. The effect of aminoglutethimide is dose dependent, and the LD_{50} for this drug in animals receiving a standard 175-μg dose of endotoxin was 920 μg. The ortho-derivative of aminoglutethimide had less effect on the susceptibility of mice to endotoxin than was observed with the para-derivative. A standard dose of 225 μg of endotoxin caused only 37% of the test mice to die, but when 7.5 mg of the ortho-aminoglutethimide was given at the same time as or 2 hr following the endotoxin, 73% of the mice died. Administration of the same dose of this inhibitor 2 hr before the endotoxin was slightly less effective as only 64% of the mice died.

Cortisone acetate was the first steroid used to neutralize the effect of aminoglutethimide on endotoxin shock in mice. In these experiments the LD_{50} of the endotoxin was increased to in excess of 350 μg in both the control animals and in the aminoglutethimide-treated animals. The initial steroid of the synthetic chain, pregnenolone, was not as effective as cortisone but essentially obviated the increased susceptibility of aminoglutethimide-treated mice to endotoxin (Table II). In the aminoglutethimide treated mice the LD_{50} of the endotoxin was 202 μg after treatment with pregnenolone. A similar though not as marked response was obtained with progesterone which maintained the LD_{50} near 165 μg. These compounds, formed early in the steroid pathway, returned the aminoglutethimide-treated animals to essentially normal animals, although a slight increase in susceptibility was noted. These two steroids, given in a single dose immediately after the en-

TABLE II. The Effect of Various Steroids (5 mg) on the Mean (Six Determinations) LD_{50} of Endotoxin in Aminoglutethimide (2.5 mg) Treated and Untreated Mice.

Treatment	Endotoxin control		Endotoxin and aminoglutethimide		
	LD_{50} (μg) Mean ± SD	p^a	LD_{50} (μg) Mean ± SD	p^a	p^b
Control	229.7 ± 15.8		17.5 ± 4.7	<0.001	—
Pregnenolone	243.5 ± 16.2	N.S.[c]	202.3 ± 43.0	N.S.	<0.001
Progesterone	240.2 ± 14.1	N.S.	165.6 ± 50.7	<0.02	<0.001
Deoxycorticosterone	259.8 ± 24.1	<0.05	163.2 ± 49.8	<0.01	<0.001
17-α-Hydroxyprogesterone	231.3 ± 29.7	N.S.	26.3 ± 6.2	<0.001	<0.05
Corticosterone	316.3 ± 12.3	<0.001	310.9 ± 11.3	<0.001	<0.001
Hydrocortisone	367.8 ± 12.4	<0.001	352.0 ± 26.6	<0.001	<0.001
Androstenedione	175.5 ± 16.2	<0.001	9.4 ± 4.5	<0.001	<0.05

[a] Statistical significance relative to endotoxin control by Student *t*.
[b] Statistical significance relative to endotoxin and aminoglutethimide control by Student *t*.
[c] Not significantly different.

dotoxin, also afforded a slight increase in the LD_{50} of the endotoxin. Deoxycorticosterone yielded greater protection than pregnenolone and progesterone in endotoxin treated mice (Table II), but in the endotoxin-aminoglutethimide treated animals deoxycorticosterone conferred about the same degree of protection as progesterone.

Corticosterone was found highly effective in protecting both the endotoxin-treated mice as well as the animals given both aminoglutethimide and endotoxin (Table II). The LD_{50} in both groups was increased by about one half that of the control intoxicated mice. These results approach those determined for cortisone except that the LD_{50} with that steroid was not exceeded at 350 μg of endotoxin even in the animals pretreated with aminoglutethimide. Hydrocortisone also increased the resistance of both the endotoxin treated and the endotoxin-aminoglutethimide-treated mice. The LD_{50} of the endotoxin determined in these cases approached those obtained after cortisone.

In contrast to the above findings, 17-α-hydroxyprogesterone afforded essentially no protection against endotoxin (Table II), but in the aminoglutethimide pretreated animal the LD_{50} of the endotoxin was increased about 48%. The final steroid employed in these studies, androstenedione, seemed to be toxic for the mice at the dose level used, and in mice given this steroid the LD_{50} of the endotoxin was approximately 75% of that determined in the endotoxin control. The LD_{50}, when androstenedione was used in adjunct with aminoglutethimide, was 50% of that for the aminoglutethimide-endotoxin control.

Discussion. The importance of corticosteroids in the response of animals to endotoxin is well established (1, 14) and interference with availability of these substances seriously affects the ability of the host to contend with the effects of endotoxemia. The loss of catecholamine secretion in the absence of the adrenal medulla may also account for a part of the increased susceptibility of adrenalectomized mice (6) to endotoxin shock.

Circulating steroid levels can be decreased by several drugs which interfere with biosynthesis of adrenal cortical steroids (4, 5, 7, 9). These compounds which specifically inhibit adrenal steroidogenesis furnish a way to evaluate the role of individual steroids in the response of the host to endotoxin. Amphenone B and metyrapone are of limited value because of a short life in the host. Metyrapone, employed to evaluate pituitary activity, allows a marked rebound in circulating steroid level soon after the treatment is discontinued (12). The increase in adrenal activity which occurs following withdrawal of the drugs may explain the increased resistance observed when metyrapone and amphenone B were administered in advance of the endotoxin.

The increased susceptibility observed in mice treated with metyrapone supports the

observation of Plager *et al.* (11) that dogs infused with metyrapone were more susceptible to endotoxin. In that study metyrapone was administered continuously for seven hours before the endotoxin was administered. Berry (personal communication) also noted the protection afforded by amphenone B when the drug was given to mice 2 hr or more before the endotoxin.

Aminoglutethimide, on the other hand, has prolonged effectiveness as susceptibility to endotoxin was increased for at least 24 hr after injection. This drug has been shown to inhibit the cholesterol desmolase system (8, 9) by inhibition of the hydroxylation of the carbon-20 of cholesterol (7) the step at which ACTH is believed to exert its effect. The LD_{50} of the endotoxin administered to the aminoglutethimide-treated mice was increased by 10- to 20-fold by most of the steroids used in this study, demonstrating the importance of a continuing supply in protection of the host against endotoxin shock. Administration of the initial corticoids, pregnenolone or progesterone, concurrently with endotoxin to aminoglutethimide-treated mice resulted in an LD_{50} similar to that of the endotoxin control. Corticosterone and hydrocortisone were the most effective of the steroids as the LD_{50} of the endotoxin was increased to about one and a half times of that determined in the control mice.

By contrast, androstenedione appeared to cause increased susceptibility of mice to endotoxin whether in the control or aminoglutethimide-treated animals. A similar effect was reported to occur in rats treated with estrogens at least 4 hr before the endotoxin was administered (10).

Drugs which inhibit steroid synthesis furnish a valuable aid in the study of the effect of steroids upon a variety of metabolic and physiologic activities. The results reported in this study show that the position of the steroid in the synthetic pathway determines the degree of protection against endotoxin afforded the host.

Summary. Suppression of corticosteroid production by inhibitors effected a tenfold increase in susceptibility of mice to death from endotoxin shock. Amphenone B and metyrapone caused some increase in susceptibility, but if given more than 1 hr before the endotoxin afforded protection to the test animals. Aminoglutethimide exerted a deleterious effect for at least 24 hr. The effect of the aminoglutethimide was reversed by pregnenolone, progesterone and deoxycorticosterone, although the LD_{50} remained lower than in the control animals. Corticosterone, cortisone and hydrocortisone afforded marked protection in both the endotoxin treated mice as well as the endotoxin-inhibitor treated mice. Androstenedione caused a decrease in the LD_{50} value in both the control and aminoglutethimide-treated mice. By contrast 17-α-hydroxyprogesterone afforded no protection against endotoxin in the control mice and a significant, but miniscule, degree of protection in the inhibitor-treated mice.

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