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The Role of Gonadal Steroids in Arachidonate-Induced Mortality in Mice[1] (41132)

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Abstract. Female mice are significantly more resistant than male mice to intravenous arachidonate. Estradiol treatment of castrated males, but not females or intact males, provides protection. Testosterone treatment, on the other hand, increased the mortality rate of the intact or castrated males but had no significant effect on the responsiveness to arachidonate in female mice. Progesterone pretreatment did not modify the mortality rate in any of the groups studied. Another steroid, cortisone, is much more protective than the ovarian steroids. In both males and females, intact or castrated, exogenous cortisone greatly increased the ability to withstand an arachidonate injection. These data suggest that gonadal and cortical steroids act at a critical step in the tissue response to arachidonate and alter its ultimate effect on morbidity and mortality.

Studies on the effect of intravenous injection of arachidonate in mice have shown that a thromboembolic response is evoked more rapidly in male than in female animals and that pretreatment with androgens exaggerates this response while estrogens minimize it (1). It has also been observed that in male rats arterial thrombus formation (2) and platelet aggregation (3) take place in a shorter time than in female animals. Prolonged pretreatment with testosterone in either males or females enhances thrombogenesis and platelet aggregability while estradiol produces the opposite effects. Castration ameliorates these damaging effects in males. In a recent paper (4) we reported that intravenous injection of sodium arachidonate (SA) to adult anesthetized mice results in a mortality rate higher in males than in female mice. The cause of death in this model is hypoxia induced by pulmonary platelet aggregation (5). To elucidate this sex difference we studied the effects of pretreatment with gonadal hormones (testosterone, estradiol, and progesterone) and of castration in males and females. Based on our earlier studies involving the role of corticosteroids in the arachidonate response (4), we added another group of normal and castrated mice treated with glucocorticoids.

Methods. Male and Female CD_1 mice weighing 20–30 g were obtained from the Charles River Company, fed *ad libitum*, and housed in a controlled environment with the lighting schedule maintained at 12 hr of light per day. Animals were randomly assigned to the treatments described below. The evening before the experiments the animals were left overnight in the laboratory to avoid the stress of transportation. The next morning the mice were anesthetized with sodium amytal (5% solution) 100 mg/kg by intraperitoneal injection. The anesthetized mice were injected via the jugular vein with a 1% solution of sodium arachidonate at a dose of 50 mg/kg (0.05 ml/10 g body wt) over a period of 10 sec. Preparation of the solution of arachidonate was carried out under nitrogen by adding a molar equivalent of sodium carbonate to an alcoholic solution of arachidonic acid (Nu-Chek) (1). The number of animals which died within 1 hr after arachidonate administration was recorded. Within all groups, 95% of the deaths occurred within 10 min.

Normal or castrated male and female mice (gonadectomized 3 weeks before) were pretreated with Depoestradiol cypionate (Upjohn, 100 μg/kg, sc twice a week for 2 weeks), Depo-testosterone

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cypionate (Upjohn, 10 mg/kg sc, twice a week for 2 weeks), progesterone (Elkim-Sinn, Inc., 10 μg/kg, sc, twice a week for 2 weeks), or cortisone acetate (Upjohn, 100 mg/kg, sc, every day for 4 days). Control animals in these experiments were injected with cottonseed oil (Fisher, 0.2 ml, sc) at the same frequency as the group treated with the sexual hormones and during the last 4 days they were injected daily with the same volume of saline solution (0.9% sc) as the animals treated with cortisone acetate.

Results. As previously observed (4), iv injection of SA (Fig. 1) resulted in a mortality significantly lower in females (10 of 30; 33.3%) than in males (17 of 30; 56.8%). Gonadectomy (9 of 25; 36.0%) did not modify significantly the mortality observed in the intact females. Similarly, the castrated male mice showed no significant change in mortality (20 of 35; 57.1%) when compared to intact male mice.

Treatment with estradiol did not modify significantly the mortality rates of normal male, female, or ovariectomized mice when compared with non-estrogen-treated intact mice. Castrated males were protected by pretreatment with estrogens. The mortality dropped to 28.6% (6 of 21) as compared to the 57.1% mortality seen in the untreated castrates.

Pretreatment with testosterone produced an increase in mortality in the ovariectomized mice (55%, 11 of 20) which was not statistically significantly different from that in normal female mice pretreated (33.3%, 7 of 21) or not treated with testosterone. The same testosterone pretreatment in normal or castrated pretreated males produced a significant increase in mortality (90%, 18 of 20 and 90%, 19 of 21, respectively) compared with nontreated normal or castrated male mice. Pretreatment with progesterone had no effect in any of the groups studied.

Finally, pretreatment with cortisone reduced significantly the mortality in normal female (12.5%, 4 of 32) and male (12.5%, 4 of 32) mice as well as in ovariectomized (15%, 6 of 40) or castrated (31.2%, 10 of 32) animals when compared with the control counterparts.

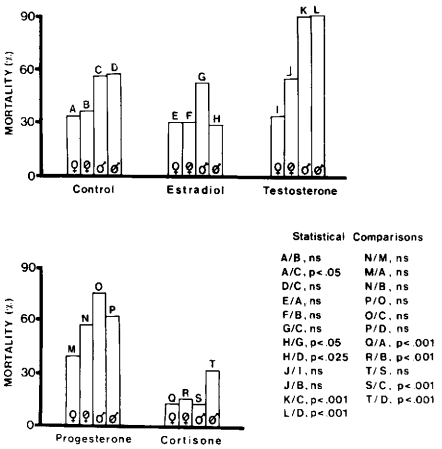

FIG. 1. Percentage of mortality produced by iv injection of sodium arachidonate (50 mg/kg) in normal or gonadectomized adult mice pretreated with steroid hormones. The number of animals per group is given in the text. Statistical comparisons were calculated using the χ^2 method.

Discussion. We have reported previously that female animals are less susceptible than male animals to the damaging effects of arachidonate injection (1). In the experiments reported here, we tested the hypothesis that ovarian steroids protect against the toxicity of large arachidonate infusions while testicular hormones exaggerate the toxicity. We found, however, that intact female mice did not differ significantly from castrated females with regard to mortality rates following exposure to arachidonate. Similarly, although male mice are clearly more vulnerable than females to the toxicity of the prostaglandin metabolites, castration of the male animals did not alter the overall mortality rates. These results suggest that castration of adult male or female mice does not immediately produce animals which are markedly different in their responsiveness to arachidonate. Thus, it is possible that castration in the neonatal stage might lead to the development of a more neutral animal in which the gonadal hormones had not yet established the sex-specific kinetics of the metabolic systems. As another approach, the lapse of a longer time interval after adult castration might also produce measurable changes in the prostaglandin metabolite

system. We are investigating these possibilities.

Our studies did demonstrate, however, that the addition of exogenous gonadal steroids can alter markedly the response to arachidonate as measured by mortality rates. When castrated males were pretreated with estradiol before exposure to arachidonate, their ability to survive this procedure increased significantly over that of estrogen-treated intact or castrated males. Females, gonadectomized or intact, were not helped by giving them more estrogens. This tends to confirm the suggestion that previous exposure to endogenous estrogens induces lasting and optimal protective metabolic pathways. Male mice, on the other hand, without significant previous exposure to endogenous estrogens, seem to benefit from treatment with the exogenous estrogenic hormones while progesterone, unlike estrogens, had no beneficial effects in any of the animals. This is apparently an estrogen-specific response.

In the same models, administration of testosterone to female mice, intact or castrated, did not augment the toxicity of the arachidonate beyond that seen in non-testosterone-treated females. Mortality rates increased significantly in intact or castrated male mice given exogenous testosterone. These data indicate that testosterone has an augmenting effect on arachidonate toxicity in the intact or gonadectomized males. As a counterpart to this, we found that cortisone has the opposite effect in this system. Pretreatment with the glucocorticoid conferred significant protection against arachidonate in all animal groups. Thus, cortisone effectively eliminated or obscured the sex difference described above.

The mechanism of action of all these steroids may be to alter the activities of the enzymes beyond the phospholipase step involved in the biosynthesis and breakdown of prostaglandins and related compounds. Such a mechanism has been previously suggested for glucocorticoids (6, 7). We are investigating this possibility.

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