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Effect of Estrogen on Lysosomal Enzyme Activities in Rat Heart (41968)

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Abstract. The activities per microgram DNA of five lysosomal enzymes [cathepsin D, cathepsin B, β-*N*-acetylglucosaminidase (β-NAG), β-glucuronidase, and acid phosphatase] were measured in homogenates of female and male rat (Sprague–Dawley) hearts. Female rats were studied during stages of the estrous cycle and at 3 weeks after ovariectomy. Three-week-postovariectomized female rats and intact male rats were injected subcutaneously with 17β-estradiol-3-benzoate. Lysosomal enzyme activities in the male rat heart were more responsive to exogenous estradiol than were activities in the female rat heart. Cathepsin B, β-NAG, and β-glucuronidase were increased dramatically in the male rat heart upon short-term administration of estrogen (4 days). In both female and male rat hearts, activities of two lysosomal proteinases, cathepsins B and D, were reduced significantly (~50%) by extended administration of estrogen for 10 days.

Receptors for sex steroids have been found in the hearts and major arteries of male and female animals (1–5). Thus, circulating estrogens and androgens could have direct effects on the cardiovascular system which result in normal and/or pathologic sex differences. Koenig *et al.* (6) have demonstrated that endogenous androgens can regulate activity of four lysosomal hydrolases in mouse and rat ventricular myocardium. We had preliminary evidence indicating that the specific activities of two lysosomal hydrolases, a glycosidase (β-glucuronidase) and a proteinase (cathepsin D), can be altered in female rat hearts by estrogen withdrawal and replacement (7). In the present study we examined the effects of estrogen (endogenous in female rats and exogenous in female and male rats) on activities of five lysosomal enzymes in the heart.

Materials and Methods. *Animals.* Adult Sprague–Dawley rats (200–250 g) were purchased from Spartan Research Animals (Haslett, Mich.) and maintained on Purina Lab Chow and water *ad libitum*. The effects of endogenous and exogenous estrogen were evaluated in virgin female rats under the following conditions: (A) during stages of the estrous cycle determined by daily vaginal smears, (B) at 3 weeks after bilateral ovariectomy, and (C) after daily sc administration of 17β-estradiol-3-benzoate (E_2B; Sigma Chemical Co., St. Louis, Mo.) to rats which had been ovariectomized for 3 weeks. In male rats the effects of exogenous estrogen were evaluated under the following conditions: (A) in normal control male rats and (B) after daily sc administration of E_2B to intact male rats. E_2B (1 μg/day) was administered in peanut oil for 4 or 10 days. These hormone treatments have been shown to produce vaginal cornification (7) and to restore plasma levels of hormones to physiological ranges (8) in ovariectomized female rats. Rats were sacrificed by cervical dislocation and their hearts quickly excised, cut open, rinsed in ice-cold 250 m*M* sucrose plus 1 m*M* EDTA (pH 7.4) and weighed.

Homogenization. Heart minces (5%, w/v) were homogenized in 250 m*M* sucrose plus 1 m*M* EDTA (pH 7.4) at 4°C using two 5-second bursts of a Tekmar Tissumizer (Cincinnati, Ohio) at maximum speed separated by a >2-min cooling period in an ice slurry. Triton X-100, 0.1% (v/v), was added to the homogenates to disrupt the membranes.

Biochemical assays. Heart homogenates were assayed fluorometrically as previously described for lysosomal enzymes, protein, and DNA (7). Cathepsin D (EC 3.4.2.3.5) was assayed at pH 3.8 by a modification of the Anson (9) method using 2.5% (w/v) acid-denatured hemoglobin as substrate. TCA soluble reaction products were measured using fluorescamine (10). Cathepsin B (EC 3.4.22.1) was assayed at pH 6.2 using benzyloxycarbonyl-arginyl-arginine-methoxy-2-naphthylamine (NNapOMe; Enzyme Systems Prod-

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ucts, Livermore, Calif.) as substrate (11). β-*N*-Acetylglucosaminidase (β-NAG; EC 3.2.1.30), β-glucuronidase (EC 3.2.1.31), and acid phosphatase (AcPase; EC 3.1.3.2) were assayed at pH 4.8 with 4-methylumbelliferyl (OMec) substrates (12) obtained from Research Products International (Elk Grove Village, Ill.). All enzyme assays were standardized to ensure linearity. In order to control for nonspecific fluorescence, enzyme blanks were run in the proteinase assays and substrate blanks were run in the remaining assays. Concentrations of reaction products, of protein, and of DNA were derived by linear regression analysis from standard curves run with each assay.

Statistics. Data were analyzed by Student's two-tailed *t* test for unpaired data. Differences were considered significant when $P \leq 0.05$.

Results. The activities of five lysosomal enzymes (cathepsins B and D, β-NAG, β-glucuronidase, and AcPase) were measured in homogenates of female and male rat hearts and expressed on the basis of DNA. The changes in lysosomal enzyme activities described in this paper did not seem to merely reflect anabolic or catabolic changes in the heart, but were more complex. In the female rats estrogen withdrawal resulted in a 10% reduction in the ratio of heart weight to body weight (Table I). Estrogen administration for 10 days to ovariectomized female rats partially restored the heart weight to body weight ratio. Short-term estrogen induced anabolism as reflected in an increased (16%) protein/DNA ratio in the heart (Table I). Estrogen administration in intact males resulted in catabolism in the heart: the ratio of heart weight to body weight was reduced 11% and the ratio of protein to DNA in the heart was decreased 17% (Table I).

Females. Lysosomal enzyme activities in the female rat heart were not affected by the stages of the estrous cycle; therefore, data for normally cycling females were pooled and are designated as N in Fig. 1. The activities per microgram DNA of the two lysosomal proteinases measured, the cysteine proteinase cathepsin B and the aspartic proteinase cathepsin D, were reduced by long-term administration of E_2B (10 days) to 39 and 51% below normal, respectively (Fig. 1). Activity of cathepsin D, unlike that of cathepsin B, was never significantly elevated above normal.

The activities per microgram DNA of AcPase and of the two lysosomal glycosidases measured were less responsive to changes in estrogen levels. Activity of β-NAG was not affected by ovariectomy, but was increased significantly after administration of E_2B for 4 days (Fig. 1). The activity of AcPase was increased significantly postovariectomy (Fig. 1). Both β-NAG and AcPase could be restored to normal levels by administration of E_2B for 10 days. Activity of β-glucuronidase was not significantly changed from the normal level either after ovariectomy or upon estrogen replacement therapy (data not shown).

Males. Cathepsin B activity per microgram DNA in control male rat hearts was slightly (12%), yet significantly ($P = 0.05$), greater than in normal female rat hearts. Administration of E_2B for 4 days resulted in a dramatic increase of 62% in cathepsin B activity

TABLE I. EFFECT OF ESTROGEN ON HEART WEIGHT AND PROTEIN TO DNA RATIO IN RAT HEARTS

Condition	*N*	Heart wet wt (mg/g body wt)	Protein/DNA (mg/mg)
Females			
Normally cycling	8	4.25 ± 0.12	96 ± 8
3-week postovariectomy	4	3.83 ± 0.08*	107 ± 14
E_2B—4 days	10	3.86 ± 0.10*	124 ± 6*
E_2B—10 days	6	4.06 ± 0.10	87 ± 4
Males			
Control	6	4.57 ± 0.10	92 ± 4
E_2B—4 days	6	4.08 ± 0.11*	97 ± 3
E_2B—10 days	6	4.16 ± 0.08*	76 ± 3*

Note. DNA and protein were determined as described under Materials and Methods. Values are means ± SE.
* $P \leq 0.05$ compared to normal or control rats.

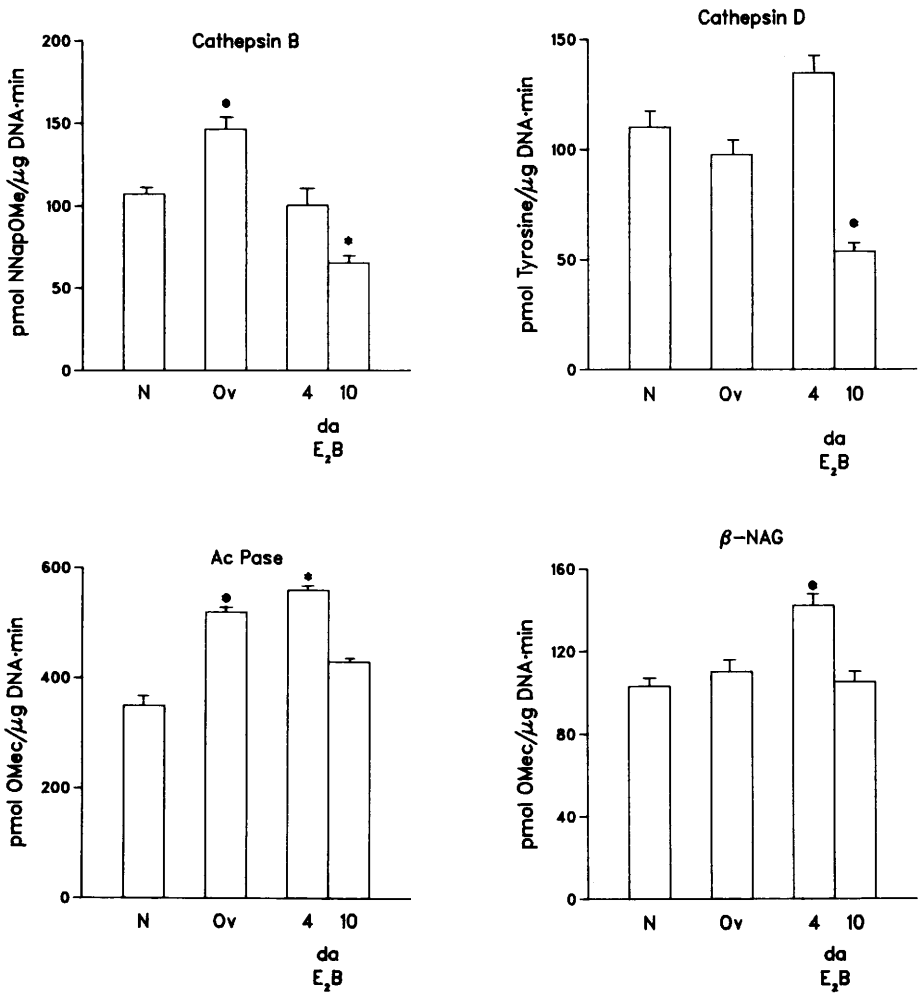

FIG. 1. Lysosomal enzyme activity in homogenates of female rat hearts as affected by estrogen withdrawal or replacement. Activity is expressed as pmole reaction product formed/μg DNA · min; $\bar{x}$ ± SEM (N = 4–11). *$P \leq 0.05$ compared to N. AcPase = acid phosphatase; β-NAG = β-*N*-acetylglucosaminidase; N = normally cycling females; Ov = 3-week postovariectomy; E_2B = 17β-estradiol-3-benzoate.

(Fig. 2). However, extended administration of E_2B for 10 days decreased cathepsin B activity to 48% below the control male value. Cathepsin D activity per microgram DNA in control male rat hearts was 26% less than in female hearts and was not affected by short-term estrogen administration, but was reduced by extended estrogen administration to 52% less than control (Fig. 2). Thus, in the male rat heart as in the female rat heart administration of E_2B for 10 days resulted in a 40–50% reduction in lysosomal proteinase activities. However, in the male rat heart this reduction was accompanied by catabolism whereas in the female rat heart the anabolic rate, characterized by the protein/DNA ratio, did not appear to be altered.

In male rat hearts, the activities per microgram DNA of the lysosomal glycosidases, β-NAG and β-glucuronidase, seemed to be more sensitive to estrogen administration than in female rat hearts. β-NAG activity per microgram DNA in male rat hearts was dramatically increased (~200%) above the control value by administration of E_2B for 4 days (Fig. 2). β-Glucuronidase activity was

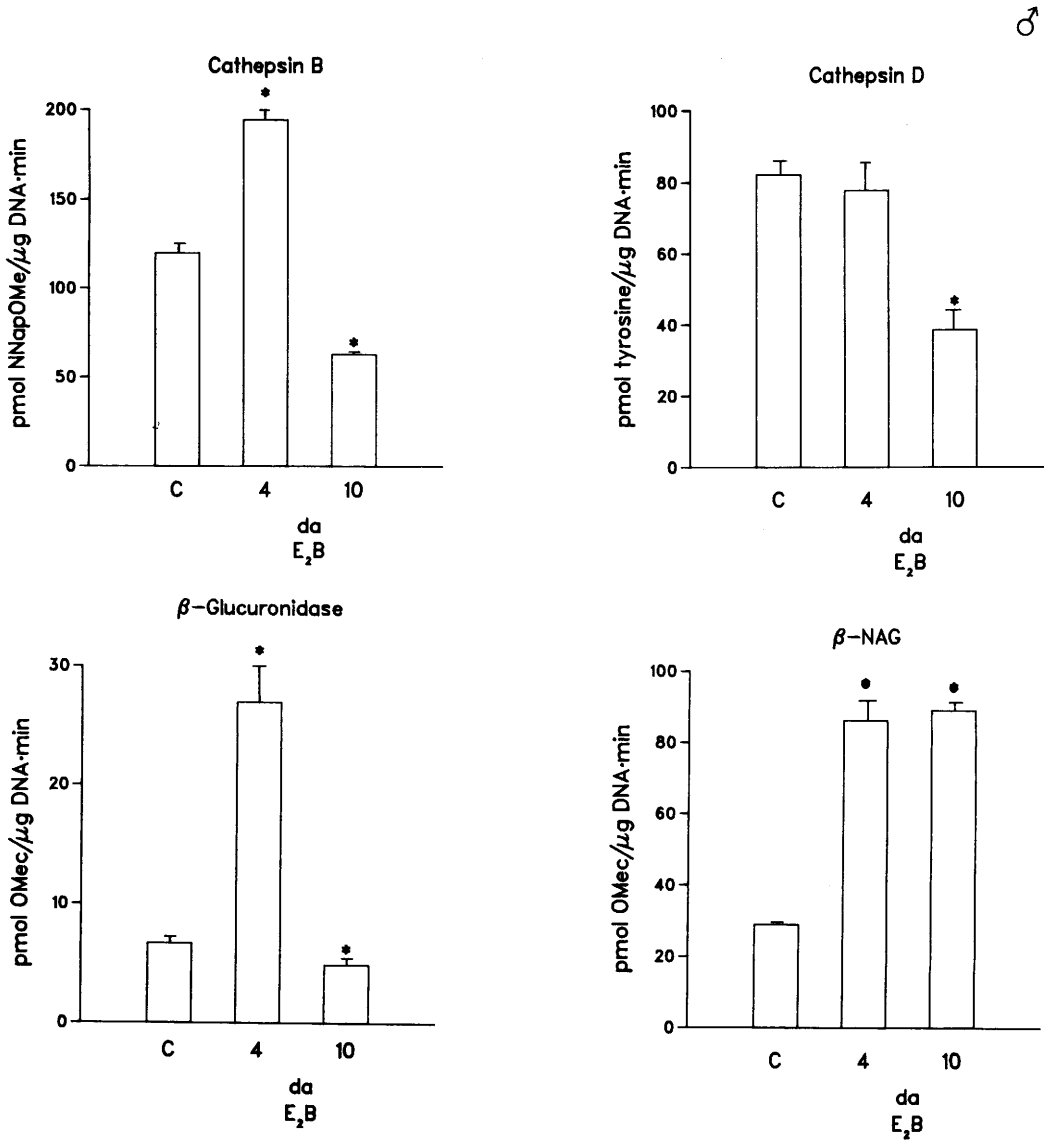

FIG. 2. Lysosomal enzyme activity in homogenates of male rat hearts as affected by estrogen. Activity is expressed as pmole reaction product formed/μg DNA · min; $\bar{x} \pm$ SEM ($N = 6$). *$P \leq 0.05$ compared to C. β-NAG = β-*N*-acetylglucosaminidase; C = control untreated males; E_2B = 17β-estradiol-3-benzoate.

also dramatically increased (~300%) by E_2B (4 days), but was then subsequently reduced below the control level upon longer administration of E_2B (Fig. 2). In male rat hearts, in contrast to female hearts, AcPase activity was not affected by estrogen administration (data not shown).

Discussion. The differential responses of lysosomal enzyme activities in the female and male rat heart to E_2B might reflect differences in estrogen receptors in the male and female cardiovascular systems. Stumpf *et al.* (1) have reported that there are estrogen receptors in the atrial and auricular muscle cells, but not in the ventricular muscle cells of both male and female rat hearts. In a more extensive study of the cardiovascular system of the baboon, McGill and Sheridan (5) reported that male hearts and arteries possess more estrogen receptors than do fe-

male hearts and arteries. Such a sex difference in estrogen receptors might account in the present study for the greater response of lysosomal enzyme activities to E_2B in the male rat heart.

The differential responses to E_2B among the five lysosomal enzymes assayed in this study may be due to heterogeneity of lysosomal populations. Biochemically, investigators have established that as many as five lysosomal populations can be isolated from the heart although three of the lysosomal populations are apparently of nonmyocyte origin (13). By assaying a spectrum of five lysosomal enzymes we were attempting to assess changes in enzyme activities in more than one cell type within the heart. Welman and Peters (13) had suggested that β-NAG is a marker enzyme for lysosomes in the myocytes and β-glucuronidase for lysosomes in the vascular smooth muscle cells. AcPase, although frequently used as a lysosomal marker enzyme, is not entirely lysosomal in the heart (13). In addition to these three hydrolases, we assayed two lysosomal proteinases, cathepsins B and D, which have been demonstrated to respond differentially to interventions that alter protein metabolism in the heart (14). Welman and Peters (13) had reported that cathepsin B activity sediments with that of β-NAG, their presumptive marker for lysosomes in myocytes. Cathepsin D, on the other hand, sediments with two separate lysosomal populations, one apparently of phagocytic (macrophage) origin (13).

Our studies cannot localize the changes in lysosomal enzyme activities to specific cell types, however, it is apparent that estrogen can regulate lysosomal enzyme activities in the heart. Our previous work (7) indicated that estrogen decreases specific activities of both cathepsin D and β-glucuronidase in female rat hearts. In the present study we have confirmed that cathepsin D and β-glucuronidase activities per microgram DNA can be reduced by estrogen.

Sex steroids have been shown to modify activities of lysosomal enzymes in several tissues including the heart (6), skeletal muscle (15), the aorta (16), the uterus (17), and the myometrium (7). The mechanism(s) by which sex steroids can modify activities of lysosomal enzymes is not yet known. Koenig *et al.* (6) found that androgen-induced increases in lysosomal enzyme activities are associated with a general anabolic response and with a decreased stability of the lysosomal membrane. Elangovan and Moulton (17) found that estrogen increases the activity of cathepsin D in the uterus in association with the general anabolic effect. Progesterone, on the other hand, only increases the rate of cathepsin D synthesis. In the present study, the heart weight/body weight ratio and the protein/DNA ratio were decreased in response to E_2B in male rat hearts, suggesting a generalized catabolic response, yet in female rat hearts E_2B induced a generalized anabolic response. Changes in enzyme activities did not necessarily parallel changes in the heart weight/body weight ratio or the heart protein/DNA ratio (compare Table I with Figs. 1 and 2).

In this study we measured the catalytic activities of the enzymes and not their total concentration. The changes in enzyme activities could be due to changes in the rates of synthesis, to changes in the proteolytic activation of the enzyme from high-molecular-weight precursors, to changes in the activation of the enzyme due to altered levels of endogenous inhibitors or activators or to changes in the rates of degradation of the enzymes. Samarel *et al.* (18) have shown that cathepsin D activity in rabbit heart is regulated by changing the proteolytic activation of the high-molecular-weight precursor of cathepsin D.

In the present study we demonstrated that estrogen can decrease the activities of two lysosomal proteinases, cathepsin B and cathepsin D, in both male and female rat hearts. Koenig *et al.* (6) have demonstrated that endogenous or exogenous androgens decrease lysosomal membrane stability and increase the activities of lysosomal acid hydrolases (β-glucuronidase, hexosaminidase, β-galactosidase, and aryl sulfatase) in male and female rodent hearts. These two studies suggest that sex steroids could alter the properties of heart lysosomes and thereby the presumptive role of lysosomes in myocardial ischemic damage [for review see (19)].

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