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Differential Regulation of Aortic Growth in Male and Female Rodents Is Associated With AAA Development

Brenda S. Cho, Ph.D.^{†,*}, Derek T. Woodrum, M.D.[†], Karen J. Roelofs, D.V.M.[†], James C. Stanley, M.D.[†], Peter K. Henke, M.D.[†], and Gilbert R. Upchurch Jr, M.D.^{†,1}

^{*}Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan

[†]Department of Surgery, Jobst Vascular Research Laboratories, Section of Vascular Surgery, University of Michigan, Ann Arbor, Michigan

Abstract

Background—The objective was to examine effects of gonadal hormone manipulation on aortic diameter and macrophage infiltration in rodents during abdominal aortic aneurysm (AAA) formation.

Methods—Experiment 1: 17- β estradiol and testosterone pellets were implanted in male (ME) and female (FT) rats. No pellet was implanted in shams (MES, FTS). Experiment 2: Testes and ovaries were removed from males (MO) and females (FO), respectively. No organs were removed from shams (MOS, FOS). Experiment 3: Male and female rats were orchiectomized and oophorectomized, respectively. Four weeks post-castration, testosterone (MOT) and 17- β estradiol (FOE) pellets were implanted. Shams underwent castration, but no pellet was implanted (MOTS, FOES). All rats underwent infrarenal aortic infusion with elastase postimplantation/postcastration. Diameters were measured on postoperative d 14. Tissue was stained for macrophages by immunohistochemistry.

Results—Diameter ($P = 0.046$) and macrophage counts ($P = 0.014$) decreased in ME compared with shams, but not in females treated with testosterone (FT). Diameter ($P = 0.019$) and macrophage infiltration ($P = 0.024$) decreased in MO compared with shams, but not in FO. Diameter increased in MOT compared with MOTS ($P = 0.033$), but decreased in FOE compared with FOES ($P = 0.002$). Macrophages decreased in FOE compared with FOES ($P = 0.002$).

Conclusion—This study documents a decrease in AAA diameter in males treated with estrogen or undergoing orchiectomy, but no changes in females treated with testosterone or undergoing oophorectomy; and an increase in diameter in MOT and a decrease in FOE. These data suggest that gonadal hormones differentially regulate AAA growth in association with changes in macrophages.

Keywords

aorta; aneurysm; hormone; estrogen; testosterone; gender; rodent; elastase; cytokine

INTRODUCTION

Abdominal aortic aneurysms (AAAs) are the 14th leading cause of overall death in the United States [1]. Controllable risk factors include smoking and hyper-tension, while

uncontrollable risk factors include age (risk increases with increasing age) and gender [2, 3]. Epidemiological studies have shown that males form AAAs four times as often as females [4]. Premenopausal women have a lower incidence of AAAs than do men of the same age, but after menopause, the incidence of AAAs in women begins to approach men. The molecular mechanisms behind this observation are unknown.

There have been a limited number of experimental studies examining the role of sex, in particular the role of estrogen, during AAA development. Most studies suggest estrogen may be protective during AAA formation [5]. Ailawadi *et al.* reported an estrogen-mediated reduction in matrix metalloproteinase (MMP)-9 production, an enzyme that degrades extracellular matrix (ECM) proteins, in male rats given exogenous estrogen [3]. Estrogen also influences remodeling of elastin and collagen in rats [6]. Pirila *et al.* reported that type I collagen levels were decreased in ovariectomized rats, but returned to baseline levels upon treatment with estrogen [7]. Other cardiovascular protective roles for estrogen include: (1) estrogen receptor-dependent changes that result in inhibition of smooth muscle cell (SMC) proliferation, and (2) stimulation of prostacyclin and nitric oxide synthesis that results in vasodilation [8–11].

In contrast to the protective role that estrogen plays in the cardiovascular system [12–14], testosterone appears to increase risks [15, 16]. In a mouse model of myocardial infarction, estrogen improves cardiac function, whereas testosterone has the opposite effect [17]. Androgens also enhance monocyte adhesion to the endothelium and impair vascular reactivity [18, 19]. In the angiotensin (Ang) II rodent model of AAAs, androgens, rather than estrogen, mediate a higher incidence and severity of aneurysms, as demonstrated by a reduction in AAA incidence in orchietomized males compared with females [20].

The purpose of this study was to examine changes in aortic diameter in males compared with females, using gonadal hormone manipulation, both surgical and pharmacological, to alter AAA phenotype.

MATERIALS AND METHODS

Adult male and female Sprague Dawley rats weighing 190 to 210 g were obtained from Charles River Laboratories (Wilmington, MA) and were used for all experiments in this study. Surgical procedures and experiments were approved by the University of Michigan Universal Committee on the Use and Care of Animals, per Protocol no. 8833.

Rodent Elastase Infusion AAA Model

Adult male and female Sprague Dawley rats ($n = 6$ per group) were anesthetized with 2% to 3% isoflurane with 100% oxygen. Using sterile techniques, a ventral midline incision was made, and the infrarenal abdominal aorta was isolated and infused with pancreatic porcine elastase as previously described (Fig. 1A, B) [21]. Briefly, the aorta was exposed from the iliac bifurcation (distal) to the level of the left renal vein (proximal), and all side branches within the exposed length of the aorta were tied off with 7-0 silk suture (Ethicon Inc., Somerville, NJ) to ensure pressurization of the aorta. Using 4-0 cotton suture (Genzyme Corp., Fall River, MA) and a Heifitz Clip, temporary distal and proximal aortic control was obtained. A 30 gauge needle was used to make an aortotomy just proximal of the iliac bifurcation. A polyethylene-10 (PE-10) tube was inserted through the aortotomy, and either saline (control, 1 mL) or pancreatic porcine elastase (1 mL of 6 units/mL, lot number 083K7655; Sigma, St. Louis, MO) was infused into the isolated infrarenal aorta for 30 min (infusion rate of 2 mL/h). Saline or elastase permeated out of the aorta during infusion, and residual fluid was flushed out of the terminal aorta. There was no distal effect of 30 min of ischemia in the elastase-infused animals and saline-infused controls. Following infusion, a

10-0 nylon monofilament suture (Surgical Specialties Corp., Reading, PA) was used to repair the aortotomy in the aorta.

Aortic diameters (proximal, mid, and distal) were measured preand postinfusion. Mean percent increase in diameter was calculated: $(\text{max postinfusion o.d.} - \text{max pre-infusion o.d.}) / (\text{max pre-infusion o.d.}) \times 100$. Images were taken using a Spot Insight Color Optical Camera (Diagnostic Instruments, Sterling Heights, MI) that was attached to the operating microscope (Nikon, Melville, NY), and aortic diameters were measured using Image Pro Express Software (Media Cybernetics Inc., Silver Spring, MD). 3-0 Vicryl Suture (Ethicon Inc., Somerville, NJ) was used to close the abdomen and the rats were recovered. Fourteen days postoperation, rats were anesthetized, infrarenal aortas were exposed, and aortic diameters were measured prior to aortic explantation. The aortas were then harvested for further analyses.

Male Estradiol Pellet Implantation

Male and female Sprague Dawley rats were anesthetized as described above and a small skin incision (approximately 0.5 cm in length) was made on the posterior neck (Table 1). A 21-d slow-release 17- β estradiol pellet (0.1 mg; Innovative Research of America, Sarasota, FL) was implanted subcutaneously, and the skin was closed with 3-0 Vicryl suture. Sham rats underwent the same procedure, but no pellet was implanted. Previous studies have shown that this dose of estradiol results in serum levels of 44.7 ± 6.1 pg/mL 2 to 3 wk postimplantation, similar to females, compared with 15.0 ± 2.2 pg/mL in male control rats ($P < 0.05$) [3, 22]. All rats were infused with elastase 5 d postimplantation and aortas harvested for further analyses 14 d postinfusion.

Male and Female Testosterone Pellet Implantation

Male and female Sprague Dawley rats were anesthetized as described above and a small skin incision (approximately 0.5 cm in length) was made on the posterior neck. A 21-d slow-release testosterone propionate pellet (0.25 mg; Innovative Research of America) was implanted subcutaneously, and the skin was closed with 3-0 Vicryl suture. The sham rats underwent the same procedure, but no pellet was implanted. Testosterone propionate is an ester of testosterone, which has a relatively short duration of effect, and has been given to rats at similar doses in previous studies [23]. All rats were infused with elastase 5 d postimplantation and aortas harvested for further analyses 14 d postinfusion.

Male Orchiectomy

Male Sprague Dawley rats were anesthetized and a small skin incision (approximately 1 cm in length) was made along the midline of the lower abdomen. Another small incision was made in the midline. The *vas deferens* were exteriorized and ligated anterior of the testes with 5-0 Prolene suture (Ethicon Inc., Somerville, NJ), and the testes exteriorized. The *vas deferens* were returned into the abdomen. The muscle layer and skin were then closed with 3-0 Vicryl suture. All male rats in the second set of experiments were infused with elastase 4 wk postoperation and aortas harvested for further analyses 14 d postinfusion. In the third set of experiments, 4 wk postorchiectomy, 21-d slow-release testosterone propionate pellets were implanted subcutaneously in the posterior neck of male rats. All of these rats were infused with elastase 5 d postimplantation and aortas harvested for further analyses 14 d postinfusion.

Female Oophorectomy

Female Sprague Dawley rats were anesthetized as described above and a small skin incision (approximately 1 cm in length) was made along the midline of the abdomen. Another small

incision was made in the muscle layer. The left fallopian tube and ovary were exteriorized, the fallopian tube was ligated with 5-0 Prolene suture, and the ovary removed. These procedures were repeated for the right ovary. The fallopian tubes were returned into the abdomen. The muscle layer and skin were closed with 3-0 Vicryl suture. All female rats in the second set of experiments were infused with elastase 4 wk postoperation and the aortas harvested for further analyses 14 d postinfusion. In the third set of experiments, 4 wk postoophorectomy, 21-d slow-release 17- β estradiol pellets were implanted subcutaneously in the posterior neck of female rats. All of these rats were infused with elastase 5 d postimplantation and aortas harvested for further analyses 14 d postinfusion.

Measurement of Hormone Levels

17- β Estradiol levels of female control rats, oophorectomized female rats, and oophorectomized female rats treated with exogenous estrogen replacement therapy were measured using an Estradiol ELISA Kit (Neogen Corporation, Lexington, KY) unpublished data, [24, 25], vaginal swabs (uterus) were performed to document loss of epithelium and to document a relative “post-menopausal” state. Briefly, samples or standards were added to the microplate, followed by the diluted enzyme conjugate. The mixture was incubated for 1 h at room temperature, during which competition for binding sites took place. Absorbance readings at 650 nm of the samples and standards were compared to obtain quantitative results. Testosterone levels were not measured since accurate and reliable testosterone enzyme-linked immunosorbent assay (ELISA) kits are not commercially available [26].

Histology

Aortic tissue was fixed in 4% paraformaldehyde for 20 h, transferred to and stored in 70% ethanol, and subsequently embedded in paraffin for immunohistochemistry. Sections were stained for elastin with Verhoeff's von Gieson Stain.

For macrophage staining, aortic sections were deparaffinized in xylene and rehydrated in graded alcohols. Heat induced epitope retrieval was performed using 10 mM sodium citrate, pH 6.0, in a microwave. Sections were then incubated with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity, followed by a blocking buffer to prevent nonspecific binding. A mouse anti-rat CD68 primary antibody 1:100 (Serotec, Raleigh, NC) was used for staining; followed by an anti-mouse IgG biotinylated secondary antibody and an avidin-biotin-HRP complex, available in the Mouse IgG Elite Vectastain ABC Kit (Vector Laboratories, Burlingame, CA). Sections were then visualized using a DAB Peroxidase Substrate Kit (Vector Laboratories) and counterstained with Hematoxylin QS (Vector Laboratories). Macrophages were quantified by a blinded reader and are presented as “average cells per 10 high powered fields (hpfs).”

Data Analysis

Statistical analyses were performed using Sigma Stat 2.03 (Systat Software Inc., Point Richmond, CA). Quantitative results were analyzed by unpaired two-tailed Student's *t*-test and two-way ANOVA, where a *P* value less than 0.05 was considered statistically significant.

RESULTS

17- β Estradiol Treatment of Males, Not Testosterone Supplementation in Females, Decreases AAA Diameters

Experimental AAA Formation—17- β Estradiol levels of female control rats were significantly higher than that of oophorectomized female rats (18.6 pg/mL *versus* 4.7 pg/mL,

$P < 0.001$). Oophorectomized female rats also had decreased 17- β estradiol levels compared with oophorectomized females with estrogen replacement (4.7 pg/mL *versus* 18.5 pg/mL, $P < 0.001$). There was no difference in 17- β estradiol levels between female control rats and oophorectomized females with estrogen replacement (18.6 pg/mL *versus* 18.5 pg/mL, $P = 0.954$).

Preliminary cytokine ELISA showed that there were no significant differences between elastase-infused males and elastase-infused females in monocyte chemoattractant protein-1 and macrophage inflammatory protein 2 levels for all time points (data not shown).

In the first set of experiments, the mean percent increase in aortic diameter of 17- β estradiol implanted male (ME) and sham male (MES) aortas 14 d following elastase infusion were $190\% \pm 31\%$ and $342\% \pm 55\%$, respectively ($P = 0.046$, Fig. 2). In contrast, 14 d following elastase infusion, there was no difference in aortic diameters for testosterone propionate implanted female (FT) and sham female (FTS) aortas (FTS: $159\% \pm 32\%$ *versus* FT: $165\% \pm 39\%$, $P = 0.93$). There was a corresponding decrease in total macrophage counts in estradiol implanted male compared with sham aortas (MES: 141 ± 14 average per 10 hpfs *versus* ME: 59 ± 23 average per 10 hpfs, $P = 0.014$, Table 1, Fig. 3), but there was no difference in macrophages in female aortas (FTS: 83 ± 14 average per 10 hpfs *versus* FT: 76 ± 12 average per ten hpfs, $P = 0.74$). Elastin degradation selectively paralleled total macrophage counts (Fig. 4).

Orchiectomized Males, Not Oophorectomized Females, Alters AAA Formation Experimental AAA Formation

In the second set of experiments, the mean percent increase in aortic diameter of orchiectomized male (MO) and orchiectomy sham male (MOS) aortas 14 d following elastase infusion were $177\% \pm 27\%$ and $373\% \pm 95\%$, respectively ($P = 0.019$, Fig. 5). Fourteen days following elastase infusion, there was no difference in aortic diameters for oophorectomized female and oophorectomy sham female (FOS) aortas (FOS: $215\% \pm 98\%$ *versus* FO: $227\% \pm 51\%$, $P = 0.88$). In a separate group of animals, oophorectomized females were infused with elastase 8 wk postoperation and the aortas harvested on post-infusion d 14. There was no difference in aortic diameters between this group and the sham group (FOS: $215\% \pm 98\%$ *versus* 8 wk FO: $124\% \pm 10\%$, $P = 0.087$). Macrophage infiltration decreased significantly in orchiectomized male compared with sham aortas (MOS: 141 ± 14 average per ten hpfs *versus* MO: 106 ± 10 average per ten hpfs, $P = 0.024$, Table 1, Fig. 3), but there was no difference in female aortas (FOS: 83 ± 14 average per ten hpfs *versus* FO: 94 ± 8 average per ten hpfs, $P = 0.54$), with elastin degradation paralleling macrophage infiltration (Fig. 4).

Hormone Replacement Restores AAA Phenotype in Orchiectomized Males and Oophorectomized Females

Experimental AAA Formation—In the third set of experiments, the mean percent increase in aortic diameter of orchiectomized males without testosterone replacement (MOTS), and orchiectomized males with testosterone replacement (MOT), 14 d following elastase infusion were $137\% \pm 23\%$ and $285\% \pm 33\%$, respectively ($P = 0.033$, Fig. 6). Fourteen days following elastase infusion, the mean percent increase in aortic diameter of oophorectomized females without estrogen replacement (FOES) was significantly higher than that of oophorectomized females with estrogen replacement (FOE) (FOES: $227\% \pm 51\%$ *versus* FOE: $99\% \pm 13\%$, $P = 0.002$). Although there was no difference in the mean percent increase in aortic diameter of testosterone propionate implanted male (MT) and sham males (MTS) (MTS: $392\% \pm 103\%$ *versus* MT: $343\% \pm 43\%$, $P = 0.52$), when testosterone pellets were implanted in males, there was a 30% incidence of aortic rupture at

early time points (rat deaths: 2 on d 1, 1 on d 6, 2 on d 7, 1 on d 11 postinfusion). Total macrophage counts did not increase in orchiectomized males with testosterone replacement compared with those without testosterone replacement (MOTS: 106 ± 10 average per 10 hpfs versus MOT: 54 ± 8 average per 10 hpfs, Table 2), but decreased in oophorectomized females with estrogen replacement compared with those without estrogen replacement (FOES: 94 ± 8 average per ten hpfs versus FOE: 29 ± 5 average per ten hpfs, $P = 0.002$).

DISCUSSION

The present study documents a decrease in experimental AAA diameter in male rats treated with exogenous estrogen, but not in females treated with exogenous testosterone. In addition, there was a reduction in aneurysm size in orchiectomized male rats, but no increase in oophorectomized females. This lack of difference seen in oophorectomized females compared with native females is perhaps the result of persistent circulating estrogen or estrogen receptors, following castration, providing continued protection against AAA formation. It may also be related to insufficiently altering the estrogen to progesterone balance, thus not altering AAA phenotype in females. When orchiectomized males were treated with exogenous testosterone replacement therapy, they were susceptible to developing AAAs as were native males; whereas orchiectomized males without testosterone replacement were phenotypically similar to native females and did not develop AAAs. Following this reasoning, one might deduce that testosterone is associated with an increase in AAA diameter, and expect females treated with testosterone to show an increase in AAA size. We speculate that this is not the case because the endogenous circulating estrogen in native female rats protects them from developing AAAs. In contrast, female rats treated with exogenous estrogen replacement therapy following surgical castration demonstrated a decrease in AAA diameter. These changes were all associated with altered macrophage recruitment, except in orchiectomized male rats treated with exogenous testosterone, suggesting that aortic macrophage infiltration is differentially regulated by gonadal hormones during AAA growth.

Although the aortic diameter increased in orchiectomized males with testosterone replacement, macrophage infiltration unexpectedly decreased, rather than returning to macrophage infiltration seen in native males. Rather than being macrophage-dependent, this change in aortic diameter may be associated with factors, such as blood pressure, which has been shown to be influenced by testosterone [27, 28]. Interestingly, when native males were treated with exogenous testosterone, they had larger body mass and developed AAAs similar to native males (data not shown). Following elastase infusion, these rats often lost use of their hind legs ($n = 5$ out of 8), and many subsequently thrombosed their aorta. Those who survived infusion had a high rate of early rupture.

The results in this study extend a previously published study in which Ailawadi *et al.* demonstrated that male rats formed larger AAAs and at a higher incidence than female rats. They also showed that there was increased macrophage infiltration and increased MMP-9 production and activity in males compared with females [3]. When female aortas were transplanted into male recipients, resistance to form aneurysms was lost, which supports the tenet that testosterone increases susceptibility of developing AAAs. Males treated with estrogen also formed smaller AAAs, demonstrated a decrease in macrophage infiltration, and showed a decrease in MMP-9 compared with native males. In a recent study, Yoshimura *et al.* showed that MMP-9 and phosphorylated JNK were localized in vascular SMCs and macrophages in male AAA tissue [29]. Similarly, Pan *et al.* showed colocalization of macrophage migration inhibitory factor in macrophages, endothelial cells, and SMCs in aneurysmal, but not normal tissue [30]. Others previously described the elevated gene expression of proinflammatory cytokines, such as interferon- γ , IL-1 β , and tumor necrosis

factor (TNF)- α in AAAs [29], and reported that JNK activation may lead to activation of MMP-9 and proinflammatory signaling, critical in AAA formation [31–33]. Collectively, these data provide evidence of gender-related differences in AAA development, which may reflect an estrogen-mediated reduction in macrophage-dependent MMP-9 production.

The present findings are also in agreement with a study by Henriques *et al.* in which they reported a significant decrease in AAA incidence in apolipoprotein E-deficient orchiectomized male mice compared with females, but no change in AngII-induced reductions in plasma renin concentration or spleen AngII receptor density [20]. AngII's effects depend on the hyperlipidemic state. AngII may promote development of aneurysms by contributing to the inflammatory response in the vessel wall, or by altering SMC migration, inducing ECM and MMP production, or stimulating reactive oxygen species (ROS) formation. Medial macrophage accumulation precedes aortic dilation in the suprarenal aorta. There is extensive aortic remodeling, and thrombus is resolved and replaced by fibrous tissue containing inflammatory cells.

Oophorectomy had no effect on the incidence or severity of AAAs in female mice. They concluded that orchiectomy, but not oophorectomy, regulates AngII-induced vascular diseases in apolipoprotein E-deficient mice. Additionally, the study suggested that androgens mediate a higher incidence of AngII-induced AAA formation, through mechanisms other than those involving plasma renin concentration or AngII receptor density, and that estrogen does not mediate gender differences in AngII-induced AAA.

Another possible mechanism for the differential effects of gonadal hormones on AAA development is the role of estrogen receptors. Estrogen receptor α mediates the beneficial effects of estrogen on injured vessels by inhibiting SMC proliferation and matrix deposition in mice [34, 35]. Since SMCs are a major source of chemokines that attract macrophages, a reduction in aortic wall SMCs may result in a decrease in macrophage infiltration observed in the current study. Production of nitric oxide (NO), a vasodilator and anti-inflammatory molecule by human aortic endothelial cells was up-regulated when treated with 17 β -estradiol but was unchanged with testosterone [36], and may also be involved.

Numerous studies in the trauma and sepsis literature highlight the importance of hormonal regulation of leukocyte trafficking. Angele *et al.* showed that macrophage immune responses are depressed in males after trauma and hemorrhage, whereas they are enhanced in females [37]. There is a significant decrease in the release of proinflammatory cytokines in castrated mice treated with testosterone. In a separate study, this group reported that female sex steroids exhibit immuno-protective properties after trauma because estrogen treatment in castrated male mice prevented immunodepression [38]. Knoferl *et al.* hypothesized that decreased estrogen levels following oophorectomy are responsible for the reduction in immune responses in proestrus females after trauma [39]. Release of the cytokines inter-leukin (IL)-6 and IL-12 were significantly decreased in oophorectomized female mice after trauma and hemorrhage, with levels restored following estrogen treatment.

In agreement with previously conducted studies, there seems to be an estrogen effect on the macrophages. Multiple groups have reported that males and females respond differently to injury because of differences in leukocyte infiltration. Studies in rodents with ischemic kidney injury show that tissue leukocyte infiltration is greater in males after ischemia than females, due to hormonal differences [40]. A separate study suggests that estradiol, *via* TNF- α , decreases leukocyte binding, thereby protecting against myocardial ischemia-reperfusion injury [13].

One study has shown that prostate specific antigen stimulates ROS generation [41]. Oxidative stress increases in and contributes to the pathophysiology of inflammation [42].

ROS regulates MMPs and may contribute to the pathogenesis of hypertension, a risk factor for AAAs. Since testosterone stimulates production of prostate specific antigen, one can speculate that the regulation of testosterone influences AAA development *via* ROS levels and/or activity.

To further investigate the role of gonadal hormones in AAA formation in association with leukocyte trafficking during AAA development, additional experiments should be conducted. Soluble mediators, such as chemokines and cytokines, can be quantified using gene array techniques and RT-PCR in order to study more of the mechanism. In particular, arrays and RT-PCR will be useful in examining the roles of cytokines, including IL-1 β and TNF- α , which have been shown to be elevated in aneurysmal tissue in male rats compared with females [43]. Potential mechanisms through which gonadal hormones may work include: (1) inhibition of chemokine-driven leukocyte infiltration and inflammation, (2) changes in MMP expression, levels, or activity, (3) alterations in ECM protein expression or levels, and (4) changes in estrogen receptors, leading to gender-based differences in AAA formation.

The present study documents: (1) a decrease in experimental AAA diameter in male rats treated with exogenous estrogen, but no changes in females treated with exogenous testosterone, (2) a decrease in AAA diameter in orchietomized male rats, but no effect in females undergoing oophorectomy, and (3) a decrease in AAA diameter in females and an increase in AAA diameter in males treated with exogenous hormone replacement therapy following surgical castration. These changes appear to be associated with altered macrophage recruitment.

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FIG. 1. (A) Schematic of elastase aortic infusion. (B) Image of elastase-infused aneurysmal aorta 14 d following elastase infusion. (Color version of figure is available online.)

FIG. 2.

Percent increase in aortic diameter post-exogenous hormone implantation 14 d following elastase infusion; 17- β estradiol implanted male (ME) aortas had significantly smaller AAAs compared with male sham (MES) aortas post-elastase infusion (* $P = 0.046$). There was no difference in aortic diameters in female rats who underwent testosterone propionate implantation (FT) compared with female sham (FTS) aortas ($P = 0.93$).

FIG. 3. Representative images of macrophage stained aortic sections, 14 d following elastase infusion. Macrophages are present in all three groups, but are more abundant in male shams (A), compared with males treated with 17- β estradiol (B) and orchiectomized males (C). Magnification, 80 $\times$. (Color version of figure is available online.)

FIG. 4.

Representative images of von Gieson stained aortic sections, 14 d following elastase infusion. Elastin is disrupted in male shams (A), but is intact in males treated with 17- β estradiol (B) and orchiectomized males (C). Magnification, $\times 20$. (Color version of figure is available online.)

FIG. 5.

Percent increase in aortic diameter post-surgical hormone manipulation 14 d following elastase infusion. Orchiectomized male (MO) aortas had significantly smaller AAAs compared with male sham (MOS) aortas on d 14 post-elastase infusion ($*P = 0.019$). There was no difference in aortic diameters for oophorectomized female (FO) compared to female sham (FOS) aortas ($P = 0.88$).

FIG. 6.

Percent increase in aortic diameter post-hormone replacement therapy 14 d following elastase infusion. Aortas of orchietomized males with testosterone propionate (MOT) had significantly larger AAAs compared with orchietomized male sham (MOTS) aortas ($*P = 0.033$). Aortas of oophorectomized females with $17\text{-}\beta$ estradiol implantation (FOE) had significantly smaller AAAs compared with oophorectomized female sham (FOES) aortas ($*P = 0.002$).

TABLE 1**Experimental Design**

Gender	Hormonal/surgical treatment	Acronym	Sample size
Male	Estradiol implantation sham	MES	6
Male	Estradiol implantation	ME	15
Female	Testosterone implantation sham	FTS	10
Female	Testosterone implantation	FT	9
Male	Orchiectomy sham	MOS	8
Male	Orchiectomy	MO	8
Female	Oophorectomy sham	FOS	7
Female	Oophorectomy	FO	14
Male	Orchiectomy, testosterone implantation sham	MOTS	9
Male	Orchiectomy, testosterone implantation	MOT	15
Female	Oophorectomy, estradiol implantation sham	FOES (same as FO)	14
Female	Oophorectomy, estradiol implantation	FOE	11

TABLE 2

Total Macrophage Counts, Day 14 Post-Elastase Perfusion

Group	Total macrophages (mean $\pm$ SEM)
Male control	141 $\pm$ 14
Male + estradiol (ME)	59 $\pm$ 23 *
Male + orchiectomy (MO = MOTS)	106 $\pm$ 10 *
Male + orchiectomy + testosterone (MOT)	54 $\pm$ 8

* *P* value < 0.05, compared with male control.