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Estrogen Reduces Aldosterone, Upregulates Adrenal Angiotensin II AT_2 Receptors and Normalizes Adrenomedullary Fra-2 in Ovariectomized Rats

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Abstract

We studied the effect of ovariectomy and estrogen replacement on expression of adrenal angiotensin II AT_1 and AT_2 receptors, aldosterone content, catecholamine synthesis, and the transcription factor Fos-related antigen 2 (Fra-2). Ovariectomy increased AT_1 receptor expression in the adrenal zona glomerulosa and medulla, and decreased adrenomedullary catecholamine content and Fra-2 expression when compared to intact female rats. In the zona glomerulosa, estrogen replacement normalized AT_1 receptor expression, decreased AT_{1B} receptor mRNA, and increased AT_2 receptor expression and mRNA. Estrogen treatment decreased adrenal aldosterone content. In the adrenal medulla, the effects of estrogen replacement were: normalized AT_1 receptor expression, increased AT_2 receptor expression, AT_2 receptor mRNA, and tyrosine hydroxylase mRNA, and normalized Fra-2 expression and catecholamine content. We demonstrate that the constitutive adrenal expression of AT_1 receptors, catecholamine synthesis and Fra-2 expression are partially under the control of reproductive hormones. Our results suggest that estrogen treatment decreases aldosterone production through AT_1 receptor downregulation and AT_2 receptor upregulation. AT_2 receptor upregulation and modulation of Fra-2 expression may participate in the estrogen- dependent normalization of adrenomedullary catecholamine synthesis in ovariectomized rats. The AT_2 receptor upregulation and the decrease in AT_1 receptor function and in the production of the fluid-retentive, pro-inflammatory hormone aldosterone partially explain the protective effects of estrogen therapy.

Keywords

Renin-angiotensin system; Angiotensin II AT_1 receptors; Adrenal medulla; Adrenal cortex; Reproductive hormones; Transcription factors; Fra-2; Tyrosine hydroxylase; Aldosterone

Introduction

It is well-established that hyperactivity of circulating and local renin-angiotensin-systems (RAS) is important for the development of cardiovascular and renal disorders and diabetes [1]. Increased RAS function as a result of estrogen deficiency has been considered a major contributor to the increase in cardiovascular and renal disease in postmenopausal women [2]. Estrogen has complex and sometimes contradictory effects on the RAS, but the overall effect

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appears to be a reduction in RAS activity [3,4]. This may partially explain the proposed, and controversial, protective effect of estrogen replacement in postmenopausal women [3,4].

The adrenal gland contributes to the regulation of cardiovascular function [5], with local RAS systems located in the zona glomerulosa and medulla [6,7]. Circulating and locally formed angiotensin II (Ang II), through AT_1 receptor stimulation, is established as a fundamental mediator of synthesis and release of aldosterone in the adrenal zona glomerulosa and of catecholamines in the adrenal medulla [8–11]. In rodents, there are two AT_1 receptor subtypes, AT_{1A} and AT_{1B}, which are pharmacologically identical but differently regulated [12,13]. Both AT_{1A} and AT_{1B} receptors are expressed in the adrenal zona glomerulosa, with a predominance of AT_{1B} receptors [12,13]. AT_{1B} receptors in the zona glomerulosa contribute to regulate aldosterone synthesis and release [10]. The adrenal medulla expresses only low levels of AT_{1A} receptors and no AT_{1B} receptors [7]. In this tissue, AT_1 receptors control Ang II- and stress-induced catecholamine formation and release [14–16].

Excess aldosterone production contributes to blood pressure increase, enhances peripheral and central sympathetic activity [17,18] and is an important inflammatory factor in the genesis of atherosclerosis [19]. Estrogen regulates adrenal gland function and the expression of adrenal RAS. In the adrenal gland, estrogen reduces AT_1 receptor expression, and as a consequence, Ang II-induced aldosterone secretion is decreased [20,21]. Conversely, most of the evidence indicates that estrogen enhances adrenomedullary catecholamine synthesis [22,23].

In addition to AT_1 receptors, the adrenal zona glomerulosa expresses substantial numbers of Ang II AT_2 receptors and in the adrenal medulla, AT_2 receptor concentration outnumbers that of AT_1 receptors tenfold [7,24]. The physiological functions of AT_2 receptors have not clarified, but their stimulation has been proposed to balance and oppose that of AT_1 receptor stimulation [25,26]. While adrenomedullary AT_2 receptors may participate in catecholamine formation and release in normal conditions and during stress [7,27], their role in aldosterone formation and secretion remains controversial [8,9].

We have previously reported that estrogen markedly upregulates AT_2 receptor expression in kidneys of female rats and mice, a factor likely to contribute to the protective effect of estrogens in kidney disease [28,29]. We asked whether estrogens influence the expression of AT_2 receptors in the adrenal gland, an effect that might contribute to their reported beneficial effects on cardiovascular function. In the present study we administered estrogen to ovariectomized (OVX) rats to determine the effect of estrogens on adrenal gland Ang II receptors, aldosterone and catecholamine synthesis.

Materials and Methods

Animals

These studies used 8-week-old female Wistar Hanover rats (Taconic, Germantown, N.Y., USA). Groups of 6 or 7 control intact rats, and 12–16 OVX rats were kept under controlled conditions with free access to water and food, according to protocols approved by the NIMH Animal Care and Use Committee. Fourteen days after surgery, the OVX rats were randomly divided into two groups. One group was implanted subcutaneously with 17β-estradiol (1.7 mg/pellet – 60 days release; Innovative Research of America, Sarasota, Fla., USA), under pentobarbital anesthesia (30 mg/kg, i.p.). This treatment provided about 28 μg 17β-estradiol per rat per day, resulting in blood levels of 180 ± 25.5 pg/ml, as previously reported [29]. The other group was implanted subcutaneously with cholesterol placebo pellets (Innovative Research of America). Ten days after the pellets were implanted, all animals were killed by decapitation between 10:00 and 11:00 h and the adrenal glands were immediately removed. For measurement of aldosterone, catecholamines and Fos-related antigen 2 (Fra-2), adrenals

were quickly dissected into cortex and medulla, and the tissues were frozen at −30°C by immersion in isopentane and stored at −80°C. For quantification of Ang II receptor binding by autoradiography and in situ hybridization determination of mRNA for Ang II receptors and tyrosine hydroxylase (TH) mRNA, whole adrenals were frozen and stored as above.

Quantitative Autoradiography of Angiotensin II Receptor Types

To determine Ang II receptor binding, we cut 16-μm-thick sections from adrenal gland in a cryostat at −20°C, thaw-mounted the sections on poly-L-lysine-coated slides (LabScientific, Inc., Livingston, N.J., USA), dried them overnight in a desiccator at 4°C, and stored them at −80°C until use. To determine binding to selective Ang II receptor types, we incubated adjacent sections as described [30]. We determined the total binding to Ang II receptors by incubating the sections with 0.5 nM of [^{125}I]Sar^{1}-Ang II (Bachem California, Inc., Torrance, Calif., USA) iodinated by the Peptide Radioiodination Service Center (University of Mississippi School of Pharmacy, University, Miss., USA), to a specific activity of 2,176 Ci/mmol). We determined the non-specific binding as the binding remaining after incubation of consecutive sections as above in the presence of 1 μM unlabelled Ang II (Bachem, Torrance, Calif., USA). Specific binding to Ang II receptors was the difference between total binding and non-specific binding. For selective binding to AT_1 receptors, we incubated consecutive sections with 0.5 nM of [^{125}I]Sar^{1}-Ang II in the presence of the selective AT_1 receptor antagonist losartan (10 μM, from DuPont-Merck, Wilmington, Del., USA) or the selective AT_2 receptor antagonist PD 123319 (1 μM, from Sigma, St. Louis, Mo., USA), chosen to give maximum specific displacement [30]. The number of AT_1 and AT_2 receptors was the binding displaced by the respective receptor antagonists. To quantify the autoradiographic images obtained after exposure to BioMax MR films (Eastman Kodak, Rochester, N.Y., USA), and development with GBX developer (Eastman Kodak) for 4 min followed by fixation, we compared the optical densities of the images with those generated by [^{14}C] microscales (American Radiolabeled Chemicals, Inc., St. Louis, Mo., USA) as described [31]. This was followed by computerized microdensitometry using the NIH Image 1.61 program (NIMH, Bethesda, Md., USA). At least two sections at similar planes were studied per animal by investigators unaware of each animal's treatment.

In situ Hybridization of TH mRNA

For in situ hybridization experiments, 16 μm-thick brain sections consecutive to those used for receptor binding were collected as above and stored at −80°C until assayed. We synthesized an antisense oligonucleotide of 48 bases as a probe for the rat TH cDNA sequence (Lofstrand Labs Ltd, Gaithersburg, Md., USA), localized in nt 1562–1609 [32]. We labeled the oligonucleotide using terminal deoxynucleotidyl transferase (GE Healthcare, Little Chalfont, Bucks., UK) to a specific activity of 3–4 × 10^{8} dpm/μg. Each reaction was performed with 70 pmol of oligonucleotide in the presence of 70 μCi of [α-^{35}S] ATP (GE Healthcare). The labeled oligonucleotide was separated from unincorporated nucleotides using MicroSpin G-25 columns (GE Healthcare). In situ hybridization of rat adrenal sections and post-hybridization washings were performed as described [33] in consecutive brain sections, one incubated with labeled antisense oligonucleotide (157 pmol/ml) and another with labeled oligonucleotide in the presence of a tenfold excess of unlabelled probe. After exposure to BioMax MR films, the films were then developed and quantified by comparison with ^{14}C standards (American Radiolabeled Chemicals, Inc.) [31]. At least two sections at similar planes were studied per animal by investigators unaware of each animal's treatment.

In situ Hybridization of Ang II Receptor Subtype mRNAs

To obtain rat AT_{1B} and AT_2 receptor-specific riboprobes, partial fragments of full-length cDNA were subcloned into the pBluescript KS^{+} vector (Stratagene, La Jolla, Calif., USA).

The rAT_{1B} cDNA was restricted with *Hin*dIII and *Eco*RI [34]. The restriction fragment of 398 bp (from nt 1832–2229) was prepared and ligated into the *Hin*dIII-*Eco*RI site of the vector. The fragment corresponded to the 3′ UTR of the gene. The rAT_2 cDNA was restricted with *Xba*I and *Bgl*II [34] and the fragment of 375 bp (from nt 1478–1852) was prepared and ligated into the *Xba*I-*Bgl*II site of the vector. The fragment corresponded to the 3′ UTR of the gene. The subclones rAT_{1B} and rAT_2 were confirmed by DNA sequencing.

For in vitro transcription of the ^{35}S-labeled antisense and sense (as a control) riboprobes, the subclones were linearized with *Hin*dIII or *Eco*RI for the rAT_{1B} or with *Xba*I or *Eco*RI for the rAT_2, and then with T3 or T7 RNA polymerase, respectively, were used in in vitro transcription reactions using an RNA labeling kit (GE Healthcare) as previously described [34]. The labeling of the ribo-probes was monitored with liquid scintillation counting. In a preliminary experiment, adrenal glands were used as positive controls.

To perform in situ hybridization, sections were fixed in 4% paraformaldehyde for 10 min, and acetylated for 10 min in 0.1 M triethanolamine HCl, pH 8.0, containing 0.25% acetic anhydride. Sections were then dehydrated in alcohols, and air-dried. Each section was covered with 150 µl hybridization buffer containing 50% formamide, 0.3 M NaCl, 2 mM EDTA, 20 mM Tris (pH 8.0), 1 × Denhardt's solution, 10% dextran sulfate, 100 µg/ml salmon sperm DNA, 250 µg/ml yeast tRNA, 150 mM dithiothreitol, 0.1% SDS, and 40,000 cpm/µl sense or antisense probe. Sections were hybridized overnight at 54°C, treated with 40 µg/ml ribonuclease A (Sigma) for 30 min, and washed in sodium chloride/sodium citrate with increasing stringency. After a final wash in 0.1 × standard saline citrate at 65°C for 60 min, sections were dehydrated through alcohols and exposed to Hyperfilm-3H (GE Healthcare) along with ^{14}C-labeled microscales (American Radiolabeled Chemicals, Inc.) for 1–2 days. Films were developed as described above. The intensities of the hybridization signals were quantified as nanocuries per milligram tissue equivalent by measuring optical film densities using the NIH Image 1.61 program after calibration with the ^{14}C-labeled microscales [31]. Non-specific hybridization was analyzed using sense (control) probes.

Determination of Fra-2 by Western Blot Analysis

After dissection of the adrenal cortex and medulla, tissues were homogenized on ice in buffer containing 10 mM Tris, pH 7.4, 1% SDS and a protease inhibitor cocktail (Roche Applied Science, Mannheim, Germany). The homogenate was centrifuged at 18,000 *g* for 5 min at 4° C. The protein concentration was determined by the Bradford procedure (Bio-Rad, Hercules, Calif., USA) in sample aliquots. Proteins were then fractionated by 10% SDS-polyacrylamide gel electrophoresis and transferred to a PVDF membrane (Millipore Corp., Bedford, Mass., USA). The membranes were blocked with 5% non-fat dried milk (Bio-Rad) in Tris-buffered saline (100 mM Tris-Cl, pH 7.5, and 100 mM NaCl) containing 0.1% Tween 20 (TBS-T) for 1 h. They were then incubated with anti-Fra-2 rabbit polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif., USA) at a dilution of 1:200 in TBS-T containing 5% dried milk at 4°C overnight. After washing the membranes with TBS-T, the membranes were incubated with horseradish peroxidase-conjugated donkey anti-rabbit IgG (GE Healthcare) for 1.5 h at a dilution of 1:2,000 in the blocking buffer. The proteins were visualized with the chemiluminescence system (GE Healthcare) and exposure to Kodak X-OMAT film. The amount of each protein was quantified using a Microsoft-based image processing system with Scion Image software (Scion Corp., Frederick, Md., USA) using actin to correct for differences in protein loading.

Determination of Adrenal Catecholamines by HPLC

We measured norepinephrine (NE) and epinephrine (E) content in homogenates of adrenal medulla. The adrenal medullas were homogenized in 0.3 N perchloric acid and centrifuged, and

the supernatant was stored at −80°C until measured by high-performance liquid chromatography with electrochemical detection (HPLC) after partial purification by adsorption on alumina [35].

Aldosterone Determinations

Whole adrenal glands were homogenized in 0.3 N perchloric acid and centrifuged 15 min at 4° C at 1,800 *g*. Clear supernatants from the homogenates were stored at −80°C until assayed. A commercial RIA kit (MP Biomedicals, Solon, Ohio, USA) was used to determine aldosterone concentrations following the manufacturer's recommended protocols as described earlier [35]. The intra-assay coefficient of variation was 4.5%

Statistics

We used one-way ANOVA followed by post-hoc analysis using the Tukey's multiple comparisons test to assess the significance of differences among groups. $p < 0.05$ was considered statistically significant. All values were expressed as the mean ± SEM; n = 5–7 in each group. All statistics were performed using GraphPad Prism 3.0 (GraphPad Software, Inc., San Diego, Calif., USA).

Results

Effects of Ovariectomy and Estrogen Treatment on Angiotensin II AT_1 and AT_2 Receptor Expression and mRNA in the Adrenal Zona Glomerulosa

In the zona glomerulosa, AT_1 receptor binding increased significantly in OVX rats when compared to non-operated control female rats (fig. 1, fig 2). There was a tendency for the expression of AT_{1B} mRNA to increase in OVX rats when compared to non-operated females, but the change did not reach statistical significance (fig. 1). Estrogen treatment of OVX rats reduced AT_1 receptor binding, normalizing AT_1 receptor expression to values similar to those found in control rats, and significantly reduced AT_{1B} mRNA expression below the levels found in OVX or control rats (fig. 1, 2).

There was no difference in AT_2 receptor expression or mRNA in OVX rats when compared to controls (fig. 3, 4). However, estrogen treatment produced a significant enhancement of both AT_2 receptor protein and mRNA expression (fig. 3, 4).

Effects of Ovariectomy and Estrogen Treatment on Adrenal Aldosterone

No significant differences in total adrenal aldosterone content were detected in OVX rats when compared to control rats (fig. 5). Estrogen treatment of OVX rats significantly decreased the adrenal aldosterone content (fig. 5).

Effects of Ovariectomy and Estrogen Treatment on Angiotensin II AT_1 and AT_2 Receptor Expression and mRNA in the Adrenal Medulla

In the adrenal medulla, OVX produced a significant increase in AT_1 receptor binding but no changes in AT_2 receptor expression or mRNA when compared to controls (fig. 2, 4, 6, 7). Estrogen treatment of OVX rats decreased AT_1 binding to levels similar to those of controls, and significantly increased expression of AT_2 receptor binding and mRNA (fig. 2, 4, 6, 7).

Effects of Ovariectomy and Estrogen Treatment on Adrenal Catecholamines, TH mRNA and Fra-2 Expression

In the adrenal medulla, OVX significantly decreased E and NE content when compared to control rats, but did not affect the expression of TH mRNA (fig. 8, 9). Estrogen treatment of OVX rats increased E and NE levels, partially restoring these levels to those of controls (fig.

8). Additionally, TH mRNA expression was significantly increased by estrogen replacement to OVX rats (fig. 9).

The expression of Fra-2 was significantly decreased in OVX rats when compared to control rats (fig. 10). Estrogen treatment of OVX rats increased Fra-2 expression to levels similar to those of controls (fig. 10).

Discussion

We report a profound estrogen influence on the adrenal gland of female rats, including control of aldosterone and catecholamine synthesis and regulation of the transcription factor Fra-2 in the adrenal medulla. These effects may be the result of modulation of local RAS systems [6–8,11,36] because estrogen exerts a powerful regulatory effect on adrenal AT_1 and AT_2 receptors, as reported here.

We administered estrogen at a dose that increases its blood levels about twofold over those found during the ovulatory peak, levels similar to those found during pregnancy [37]. Our use of ovariectomy as well as estradiol treatment models addressed both endogenous and exogenous estrogen effects.

Ovariectomy increased AT_1 receptor expression in the zona glomerulosa, in agreement with previous observations [20,21,38,39] and in the adrenal medulla, effects reversed by estrogen treatment. In addition, estrogen treatment downregulated AT_{1B} receptor mRNA in the zona glomerulosa. Our observations indicate that reproductive hormones control adrenal AT_1 receptor transcription and expression.

Ovariectomy did not alter AT_2 receptor binding or mRNA, suggesting that reproductive hormones may not be essential to maintain constitutive expression of adrenal AT_2 receptors. On the other hand, estrogen treatment produced major increases in AT_2 receptor expression and mRNA, in both the zona glomerulosa and the medulla. Receptor upregulation may be the consequence of altered estrogen/progesterone balance. Progesterone effects on adrenal AT_2 receptors have not been studied. However, in the pituitary gland, progesterone does not alter the AT_2 receptor expression induced after estrogen-induced hyperplasia [40]. AT_2 receptor upregulation may also be a response to the high estrogen levels during our experiments, above those occurring during the estrous cycle but similar to those found during pregnancy. Significant AT_2 receptor upregulation by estrogen has also been previously demonstrated in the human myometrium [41], and in the kidney of OVX rats and mice [28,29], a finding recently confirmed in mice deficient in apolipoprotein E [42].

Estrogen treatment of OVX rats altered the ratio of expression of AT_2/AT_1 receptors in favor of AT_2 expression in the zona glomerulosa and medulla. This observation agrees with the higher AT_2/AT_1 ratio seen in female rats [29,43] and mice [28] compared to male rats or mice, respectively. The estrogen-dependent downregulation of AT_{1B} receptor mRNA expression in the zona glomerulosa is similar to the downregulation of AT_{1B} receptors in the pituitary gland [12,44]. Possible mechanisms include modulation of receptor transcription through estrogen response elements [45] and/or the result of estrogen-induced decreased plasma K^+ concentration [46,47]. The AT_2 receptor promoter does not contain estrogen response elements [48]. Estrogen may activate AT_2 receptor transcription from alternative response elements such as the activator protein site (AP-1) [49,50], by increased intracellular sodium concentration through estrogen inhibition of Na^+,K^+-ATPase [51,52], a ouabain-sensitive mechanism [53] or by receptor activation potentiating its own expression, because AT_2 receptors stimulate endogenous adrenal ouabain, and this inhibits Na^+,K^+-ATPase [54].

The present results support the hypothesis of a mutual influence between AT_1 and AT_2 receptor function. AT_2 receptor gene disruption upregulates the number of AT_1 receptors in the adrenal gland, the kidney and the brain [55–57]. Conversely, cardiac-specific AT_2 receptor overexpression decreases AT_1-mediated effects [58], and AT_2 receptor activation negatively regulates AT_1 signal transduction in bovine chromaffin cells [26]. In turn, AT_2 receptor upregulation may be the consequence of the decrease in AT_1 receptor activity, because AT_1 receptor blockade enhances AT_2 receptor expression in rat brain, adrenal zona glomerulosa and cerebral vessels [35,36,59,60]. AT_2 receptor upregulation may compensate for AT_1 receptor downregulation, to maintain Na^+,K^+-ATPase and cellular sodium concentrations, since Ang II inhibits Na^+,K^+-ATPase through AT_1 receptor stimulation [61].

Modulation of Ang II receptor types has significant effects on adrenal zona glomerulosa function. Ovariectomy does not change the adrenal aldosterone content, but estrogen treatment of OVX rats very significantly decreases aldosterone concentration, a finding consistent with the report of decreased Ang II-induced aldosterone release by estrogen replacement in OVX rats [21,38]. That ovariectomy does not alter adrenal aldosterone levels in spite of enhanced AT_1 receptor expression may be explained by the concomitant decrease in circulating Ang II [62]. The estrogen-induced reduction in adrenal aldosterone is associated with a reversal of the ovariectomyinduced increase in AT_1 receptor expression. It also parallels the increase in AT_2 receptor transcription and expression. Since K^+ concentration in plasma is a major regulator of aldosterone release, estrogen-induced decrease in plasma K^+, directly and/or through a decrease in AT_1 receptor expression [46], may contribute to decrease aldosterone adrenal content. Our results support the hypothesis that AT_{1B} receptor stimulation enhances [10,63] and that AT_2 stimulation decreases aldosterone formation and release. The role of AT_2 receptors may be part of feedback mechanisms, because aldosterone infusion reduces the number of adrenal AT_2 receptors [64]. There are additional reports of opposing actions of adrenal AT_1 and AT_2 receptors in the adrenal zona glomerulosa including AT_2-mediated antagonism of the proliferative effect of AT_1 receptor stimulation [65] and regulation of local blood flow through dilation of cortical arteries, antagonizing AT_1-mediated vasoconstriction [66].

Estrogen modulation of adrenomedullary Ang II receptors parallels significant changes in catecholamine formation. Medullary catecholamine content decreases after ovariectomy, in agreement with previous observations [22,67]. Ovariectomy did not reduce TH mRNA expression, but lowered the content of the essential TH cofactor tetrahydrobiopterin [68], decreasing in vivo TH activity. We showed that estrogen treatment restored adrenomedullary catecholamine content in association with enhanced TH mRNA expression in the OVX rats. Other studies demonstrated that estrogen treatment replenishes the vascular content of tetrahydrobiopterin [68] and enhances the tetrahydrobiopterin content and gene expression of GTP cyclohydrolase, the rate-limiting enzyme for tetrahydrobiopterin synthesis [69]. Our results and those of the literature [22,23] can best be interpreted as decreased adrenomedullary catecholamine synthesis by ovariectomy, an effect reversed by estrogen treatment.

Estrogen may regulate TH transcription through activation of estrogen response elements in the tyrosine promoter [70]. In the adrenal medulla, basal and stress-induced TH transcription [7,71] are regulated by the Fra-2, a member of the Fos family contributing to formation of the inducible activator protein-1 (AP-1) transcription factor complex. We report here that ovariectomy decreased Fra-2 expression in parallel with the decrease in catecholamine content. The decrease in Fra-2 expression was reversed by estrogen treatment, coinciding with the increase in expression of TH mRNA and the restoration of catecholamine levels. This indicates that modulation of Fra-2 expression is a likely mechanism explaining the stimulant effect of estrogen on catecholamine synthesis. Ours is the first report of a direct in vivo modulation of Fra-2 by estrogen suggesting that estrogen upregulates Fra-2 transcription. It is of interest that

Fra-2 participates in the mechanisms of carcinogenesis and tumor recurrence [72], and that there is an inverse correlation between estrogen α receptor expression and Fra-2 levels in human breast cancer cells, indicating that estrogen α receptor signaling represses Fra-2 expression in these cells [73].

The regulation of adrenomedullary catecholamine synthesis by estrogen probably involves modulation of AT_1 and AT_2 receptor function. AT_1 receptor activation controls Ang II- and stress-induced enhanced TH transcription and catecholamine release in the adrenal medulla [14–16,35]. AT_1 receptor activation maintains constitutive adrenomedullary AT_2 expression, and both AT_1 and AT_2 receptors help to maintain TH transcription, adrenomedullary catecholamine levels, and Fra-2 mRNA expression [7].

Our results may be best interpreted as follows. In the adrenal medulla, AT_1, AT_2 and Fra-2 expression, TH transcription and catecholamine stores are partially dependent on estrogen effects. Ovarian hormones are necessary for the full constitutive Fra-2 expression. After ovariectomy, catecholamine formation decreases probably because of reduced tetrahydrobiopterin availability [68,69]. TH transcription is maintained by AT_1 receptor activation even in the presence of reduced Fra-2 expression. Estrogen treatment enhances TH transcription, an effect associated with normalization of Fra-2 expression, restoring catecholamine synthesis. Estrogen treatment enhances AT_2 receptor expression and transcription, demonstrating that AT_2 receptor activity is estrogen-dependent. We hypothesize that AT_2 receptor activation regulates catecholamine synthesis by modulating TH transcription through a Fra-2-dependent mechanism.

Our results may have clinical implications. We found that estrogen replacement reduced the content of aldosterone, a fluid-retentive, pro-inflammatory hormone [38], and in the adrenal medulla, reversed the AT_1 receptor upregulation that results from the loss of ovarian function. Estrogen-dependent AT_1 receptor downregulation, decreasing vasoconstriction, has been reported in the peripheral vasculature [74] and in the pituitary gland [75]. Downregulation of vascular and adrenal AT_1 receptors, and decreased aldosterone synthesis, may be considered important mechanisms contributing to the cardiovascular protective effects associated with estrogen.

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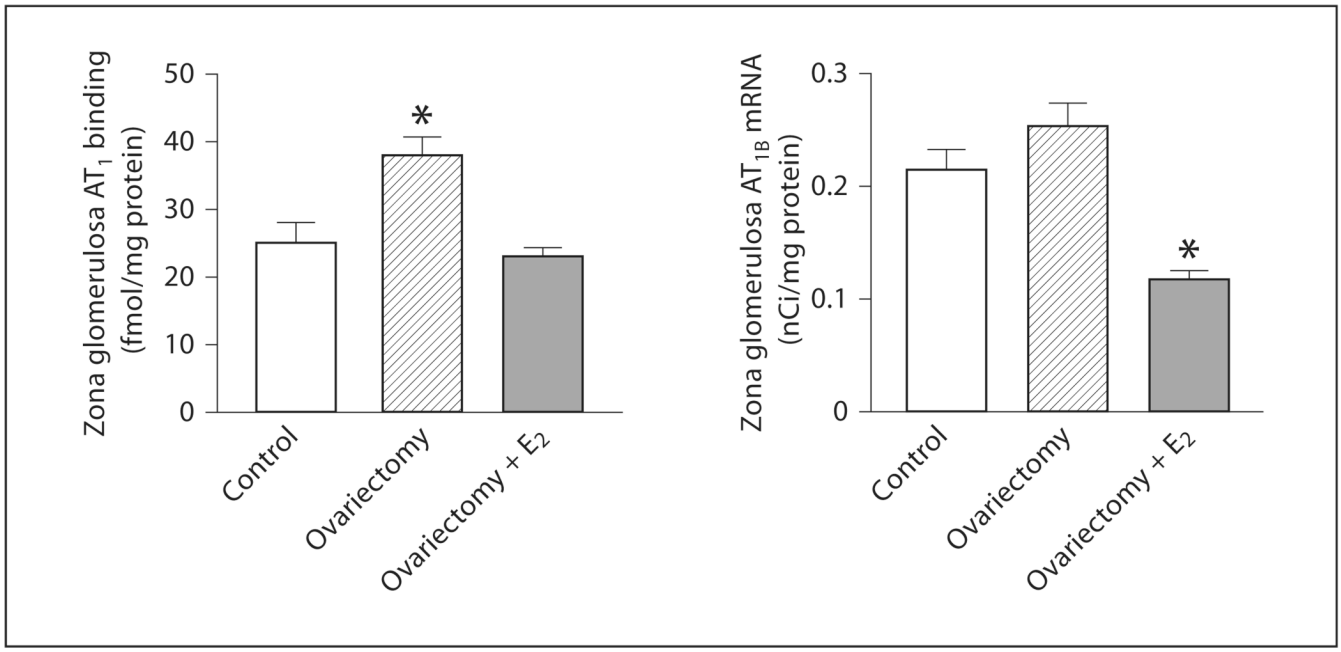

Fig. 1.

Effects of ovariectomy and estrogen treatment on AT_1 binding and AT_{1B} mRNA in the rat zona glomerulosa. AT_1 binding was measured by quantitative autoradiography. Consecutive sections were incubated with [^{125}I]Sar^1-Ang II in the presence or absence of losartan to determine binding to AT_1 receptors, and AT_{1B} mRNA was determined in additional consecutive sections by in situ hybridization, as described in Materials and Methods. E_2 indicates treatment with 17β-estradiol. Data are means ± SEM, n = 5–7 for each group. * $p < 0.05$ compared to all other groups.

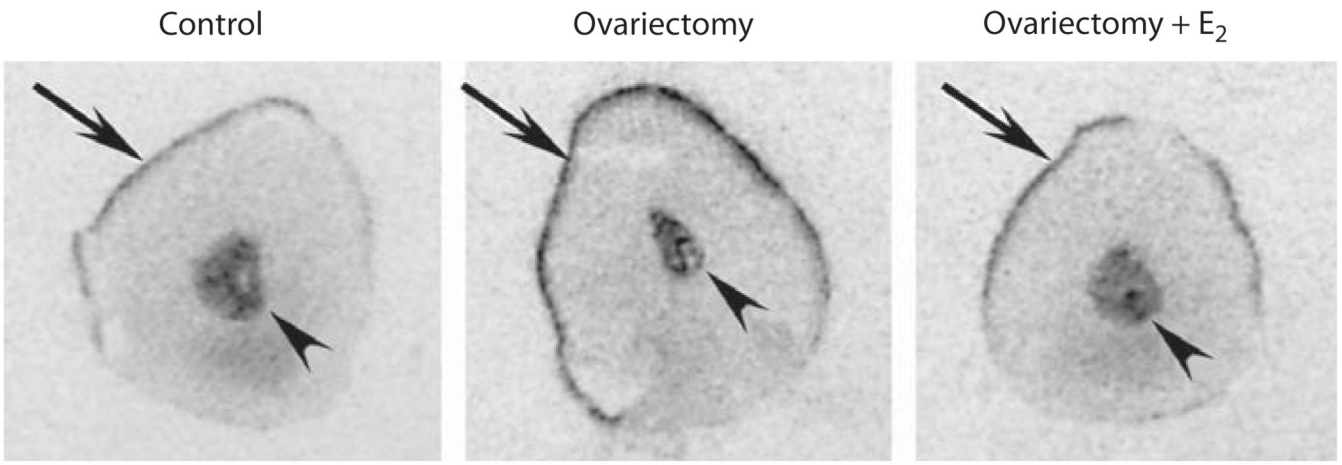

Fig. 2.

Effects of ovariectomy and estrogen treatment on AT_1 binding in the rat adrenal gland. Figures represent typical autoradiograms to show specific binding to AT_1 receptors. E_2 indicates treatment with 17β-estradiol. Sections were incubated with [^{125}I]-Sar^1-Ang II in the presence of PD 123319 to determine binding to AT_2 receptors, as described in Materials and Methods. Arrows point to the adrenal zona glomerulosa, arrowheads to the adrenal medulla.

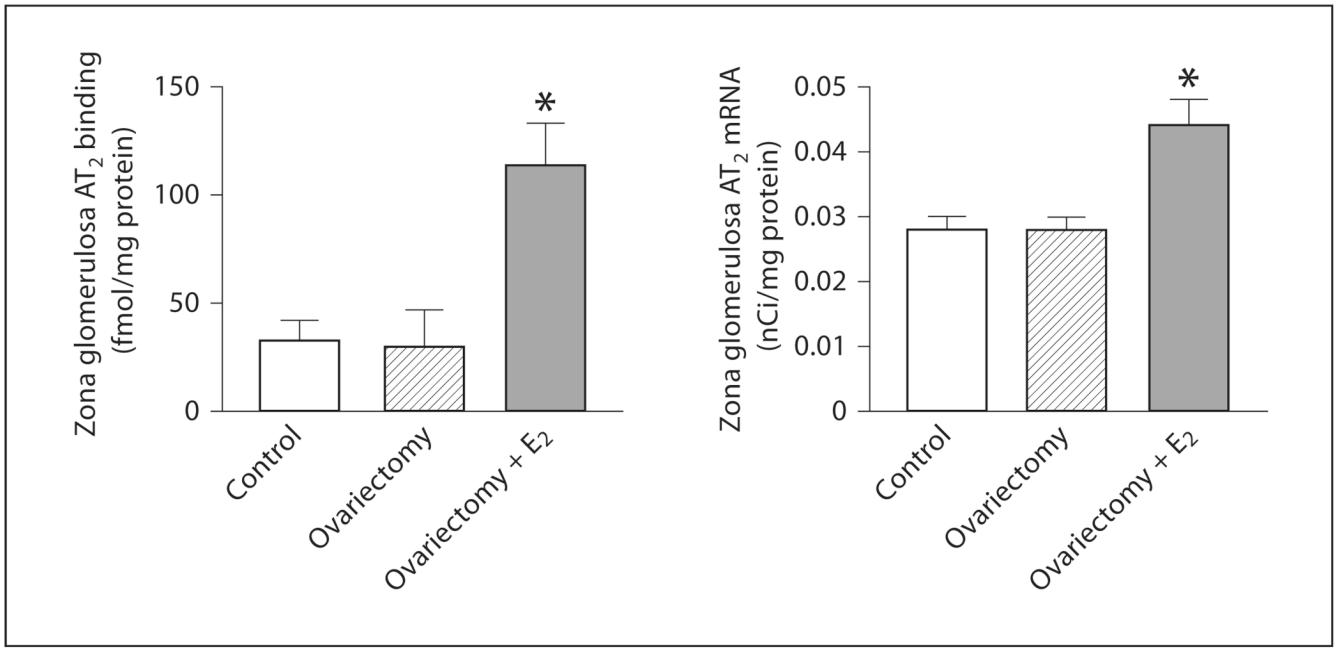

Fig. 3.

Effects of ovariectomy and estrogen treatment on AT_2 binding and mRNA in the rat zona glomerulosa. AT_2 binding was measured by quantitative autoradiography in consecutive sections, as described in Materials and Methods. AT_2 mRNA was determined by in situ hybridization as described in Materials and Methods. E_2 indicates treatment with 17β-estradiol. Data are means ± SEM, n = 5–7 for each group. * $p < 0.05$ compared to all other groups.

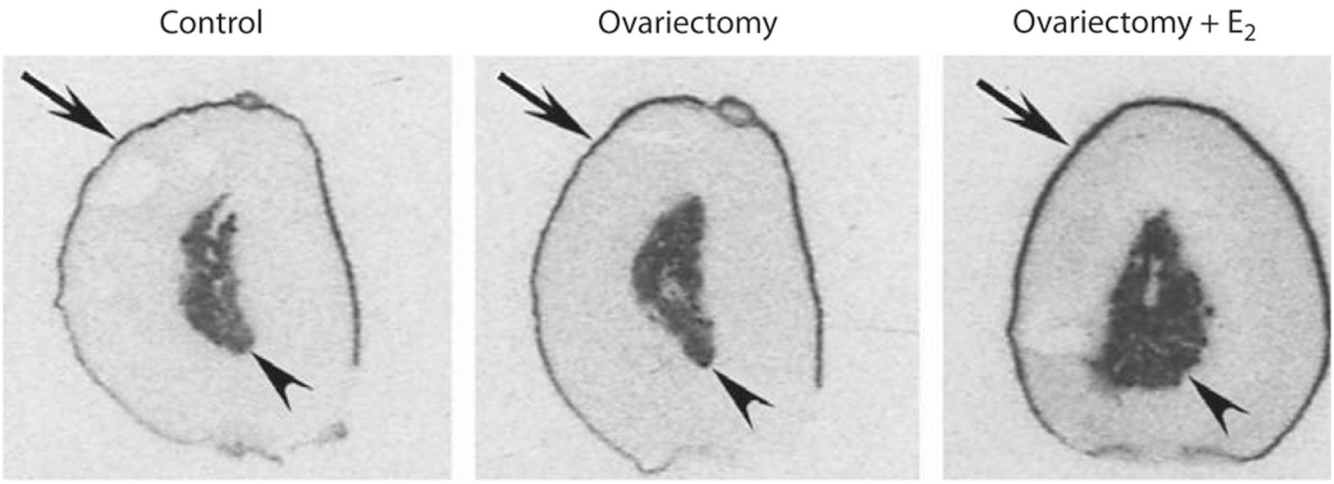

Fig. 4.

Effects of ovariectomy and estrogen treatment on AT_2 binding in the rat adrenal gland. Figures represent typical auto-radiograms to show specific binding to AT_2 receptors, determined by quantitative autoradiography as described in Materials and Methods. E_2 indicates treatment with 17β-estradiol. Arrows point to the adrenal zona glomerulosa, arrowheads to the adrenal medulla.

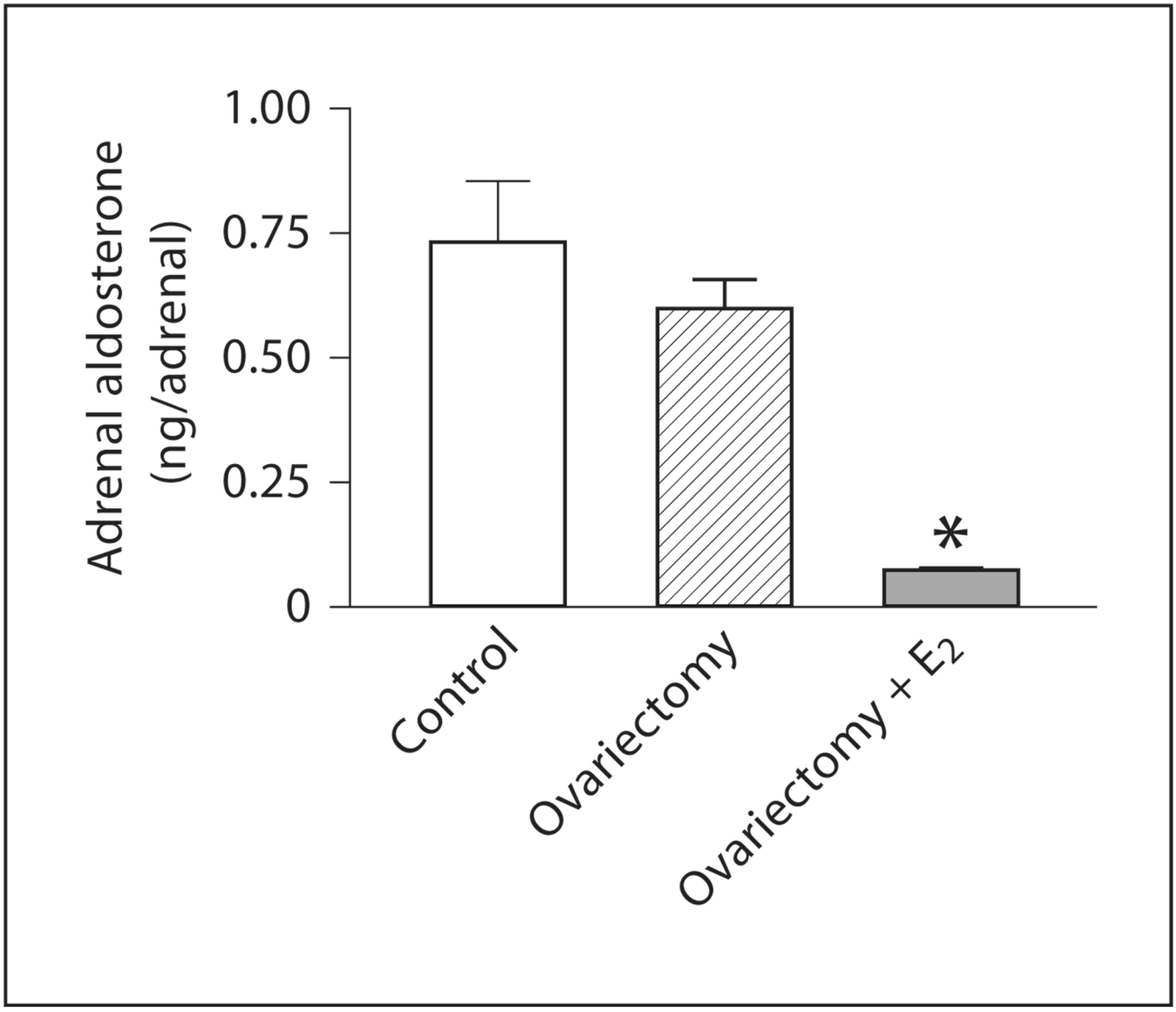

Fig. 5.

Effects of ovariectomy and estrogen treatment on aldosterone content in adrenal zona glomerulosa. Aldosterone was measured by RIA. E_2 indicates treatment with 17β-estradiol. Data are means ± SEM, n = 5–7 for each group. * $p < 0.05$ compared to all other groups.

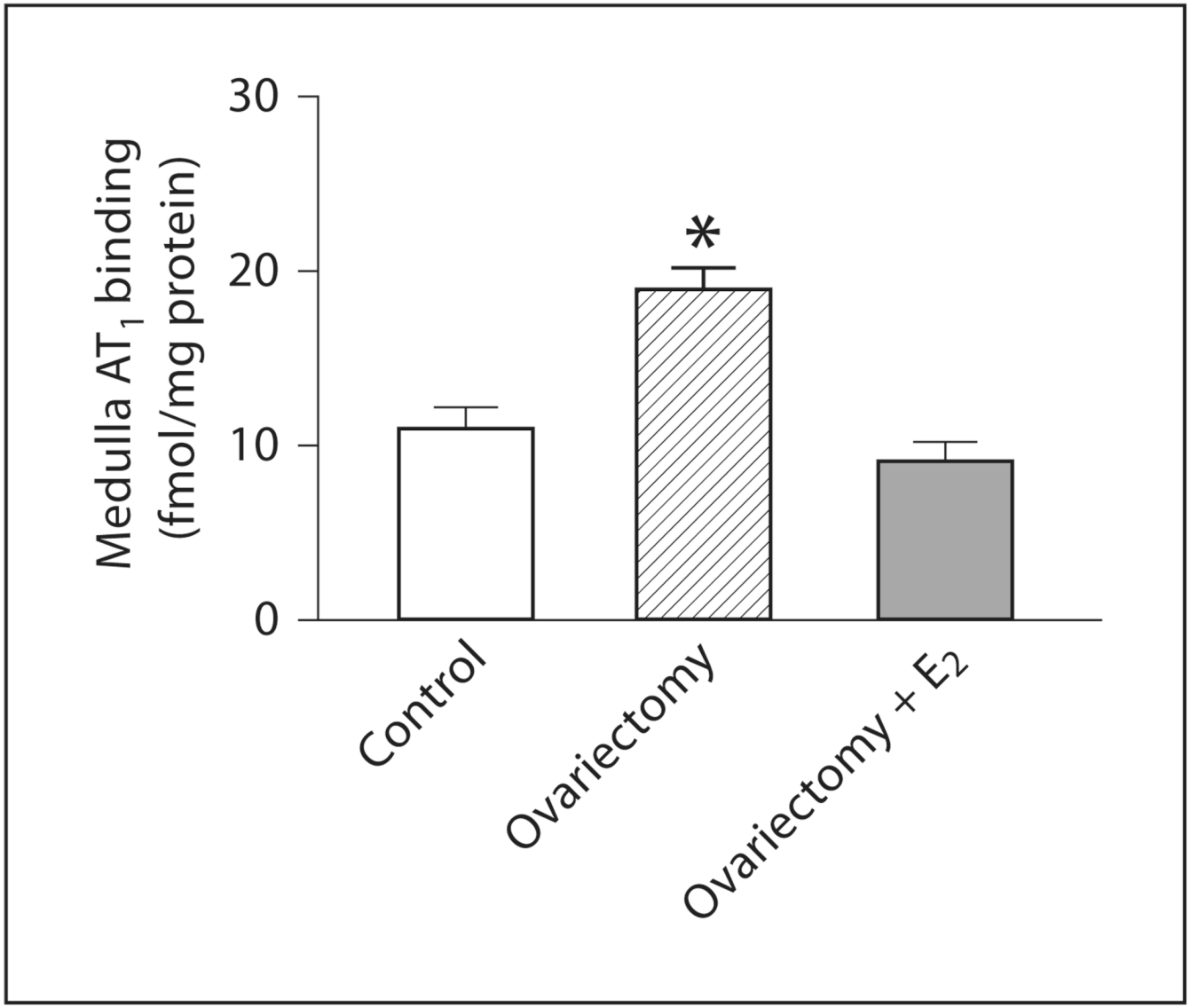

Fig. 6.

Effects of ovariectomy and estrogen treatment on AT_1 binding in the rat medulla. AT_1 binding was measured by quantitative autoradiography in consecutive sections, as described in Materials and Methods. E_2 indicates treatment with 17β-estradiol. Data are means ± SEM, n = 5–7 for each group. * $p < 0.05$ compared to all other groups.

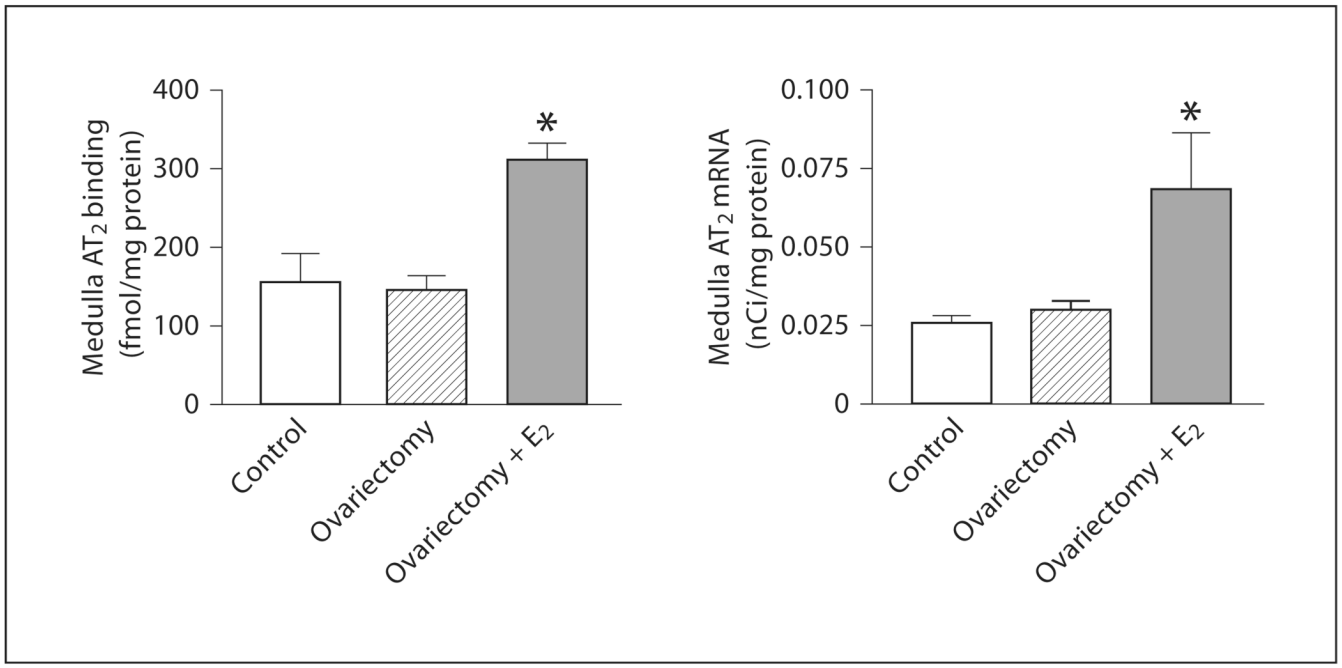

Fig. 7.

Effects of ovariectomy and estrogen treatment on AT_2 binding and mRNA in the rat adrenal medulla. AT_2 binding was measured by quantitative autoradiography as described in Materials and Methods. AT_2 mRNA was determined by in situ hybridization as described in Materials and Methods. E_2 indicates treatment with 17β-estradiol. Data are means ± SEM, n = 5–7 for each group. * $p < 0.05$ compared to all other groups.

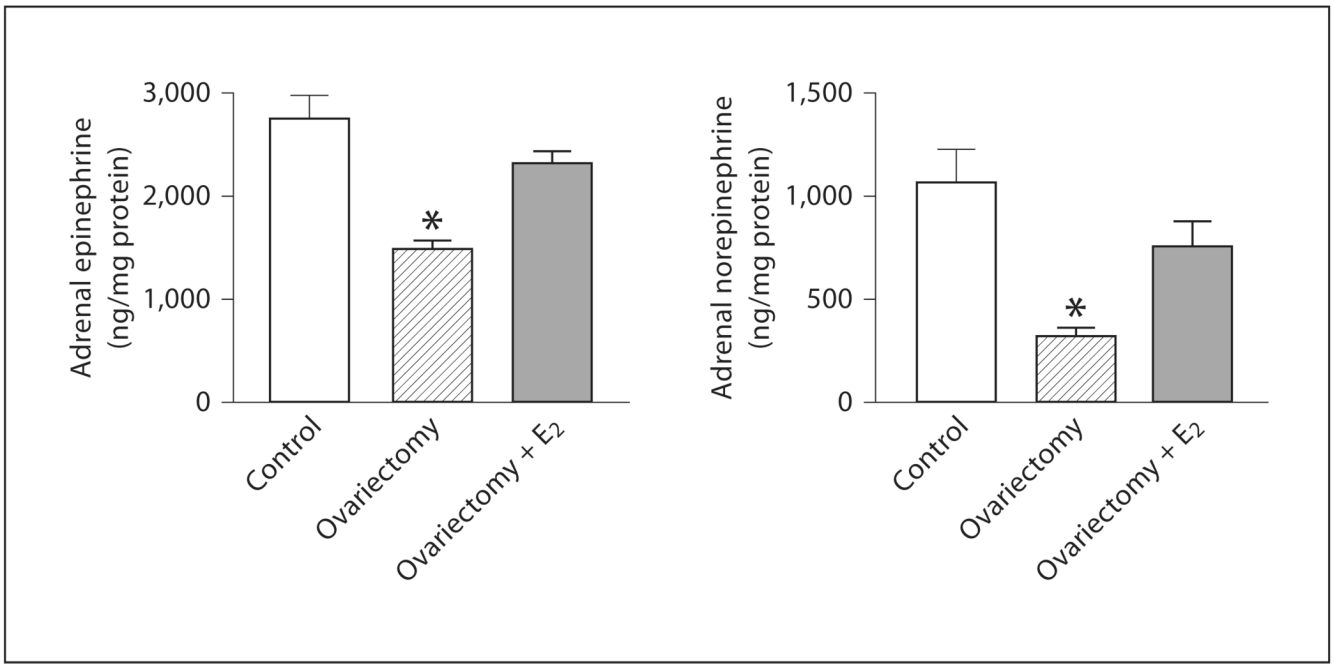

Fig. 8.

Effects of ovariectomy and estrogen treatment on catecholamine content in the rat adrenal medulla. E and NE were measured by HPLC. E_2 indicates treatment with 17β-estradiol. Data are means ± SEM, n = 5–7 for each group. * $p < 0.05$ compared to all other groups.

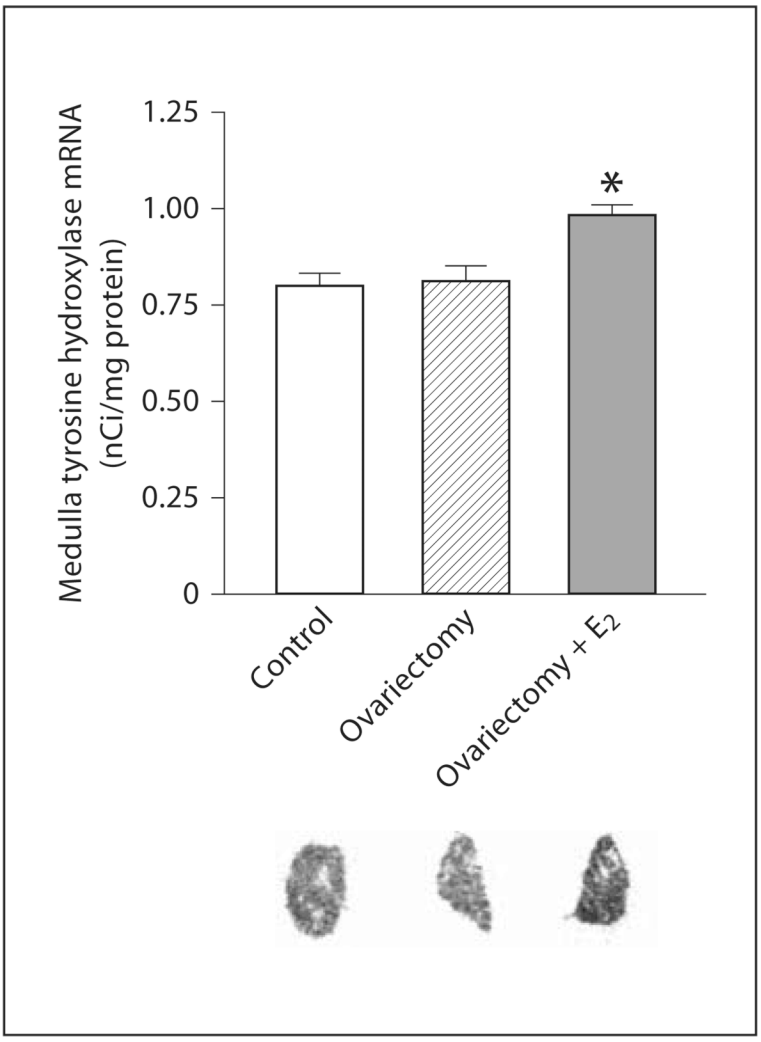

Fig. 9.

Effects of ovariectomy and estrogen treatment on TH mRNA in the rat adrenal medulla. TH mRNA was determined by in situ hybridization as described in Materials and Methods. E_2 indicates treatment with 17β-estradiol. Bars represent means ± SEM, n = 5–7 for each group. * $p < 0.05$ compared to all other groups. The photographs are typical autoradiograms of in situ hybridization of TH in adrenal medulla.

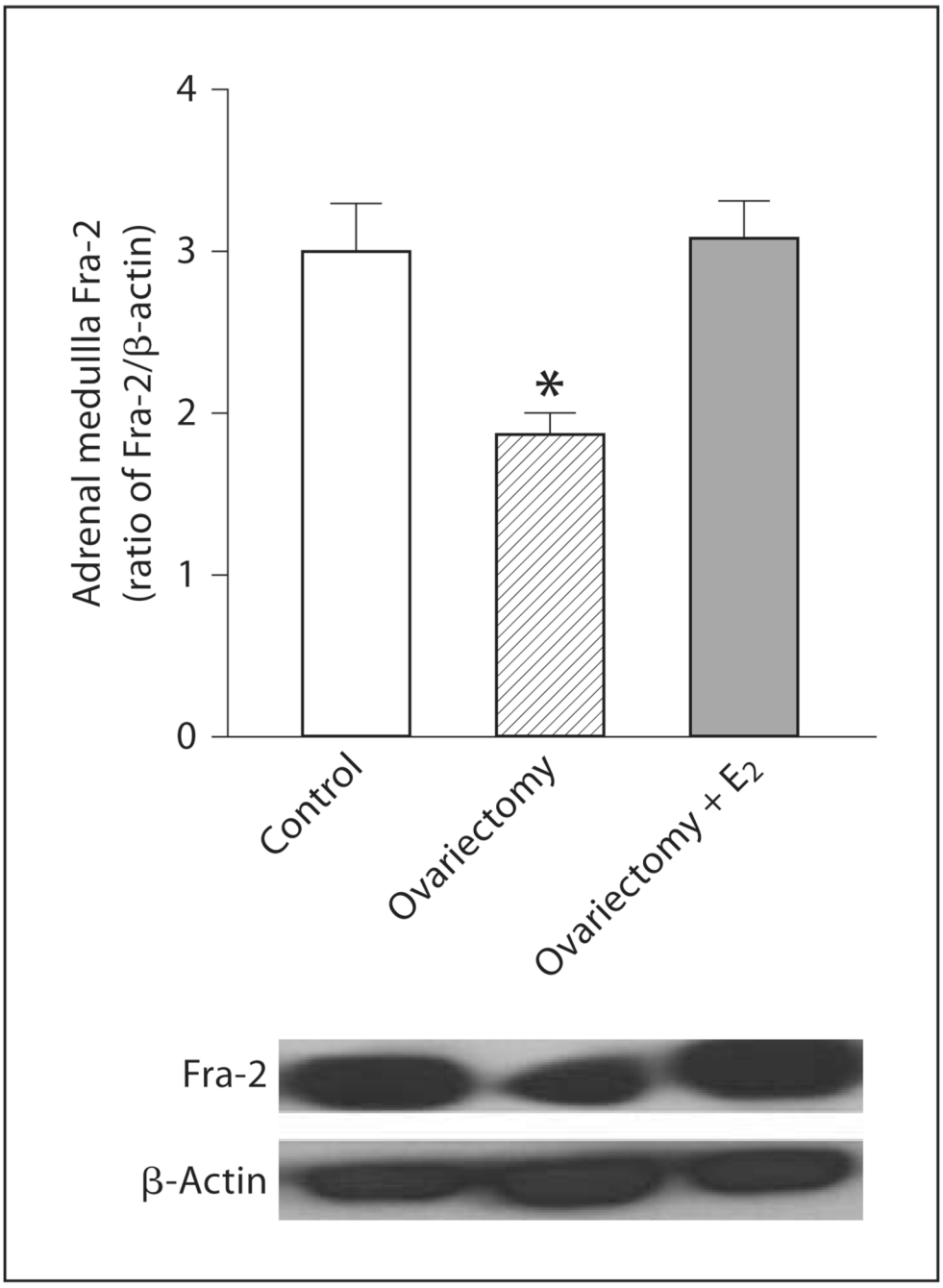

Fig. 10.

Effects of ovariectomy and estrogen treatment on Fra-2 expression in rat adrenal medulla. Fra-2 was determined by Western blot using β-actin as a control, as described in Materials and Methods. Bars represent means ± SEM, n = 5–7 for each group. The photographs are representative X-ray film images of Fra-2 and β-actin detected after treatment of membranes with a chemiluminescent substrate. * $p < 0.05$ compared to all other groups.