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Artículo original

Hormonal status modifies renin-angiotensin system and vasopressin-degrading activity in the hypothalamic-pituitary-adrenal axis of the female mice

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EL ESTADO HORMONAL MODIFICA AL SISTEMA RENINA-ANGIOTENSINASA DEGRADANDO LA ACTIVIDAD HIPOTALÁMICAS PITUITARIA EN LA RATONA

RESUMEN

El eje hipotálamo pituitaria adrenal (HPA) ha sido relacionado con la regulación de la presión arterial. En el trabajo analizamos los efectos de la ovariectomía estradiol (E) el reemplazo con varios solubles(s) relacionados con la membrana a término y regulación sistema renina-angiotensina (aminopéptidasa M y amino-peptidasa B) y la actividad degradatoria en el eje HPA. Cincuenta ratones hembras se distribuyeron en cinco grupos: operados falsos (c), ovariectomizados (ov-c) y ovariectomizados tratados con (10-20 y 40 mgms/kg) E. En el hipotálamo los aminopétidos A y S aumentaron después de ovariectomía mientras que el reemplazo regreso la actividad a niveles control. Por el contrario, ni la ovariectomía ni el reemplazo E modificaron los aminopeptidasas solubles (s) o de membrana (b). En las suprarrenales la aminopeptidasa soluble no cambio después de la ovariectomia pero la dosis alta del estradiol aumento la actividad. Dado el papel de la aminopeptidasas en el sistema renina angiotensina los cambios hormonales en condiciones fisiológicas o patológicas pueden modificar la regulación de la presión arterial a través de estos sistemas.

Palabras clave: ratón, aminopeptidasas A, B, M, aminopeptidasa rasopresora degradante, ovariectomía.

ABSTRACT

The hypothalamic-pituitary-adrenal (HPA) axis has been implicated in the regulation of blood pressure. In the present work, we analyze the effects of ovariectomy and estradiol (E) replacement on several soluble (s) and membrane bound (b) aminopeptidases involved in the regulation of the renin-angiotensin system (RAS) (aminopeptidase A (APA), aminopeptidase M (APM) and aminopeptidase B (APB)) and vasopressin-degrading activity (AVP-DA) in HPA axis. Fifty female mice (Balb/C) were distributed in five groups: Sham-operated control (C), ovariectomized (OV-C) and ovariectomized treated with (10, 20 and 40 mg/Kg) E. In hypothalamus, APAs and APAb increased after ovariectomy while E replacement return the activitiy to the control levels. On the contrary, neither ovariectomy nor E replacement modified pituitary APAs and APAb. In adrenal, APAs did not change after ovariectomy, but the highest dose of E increased this activity. However, APAb increased in adrenal after ovariectomy, whereas E replacement return

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the activity to control levels. APMs and APBs did not change neither after ovariectomy nor E replacement in hypothalamus. In the same way, APMb did not change after ovariectomy, but the highest dose of E increased it. APBb increased after ovariectomy but return to control values after E administration. In pituitary, neither ovariectomy nor E replacement modifies APMb and APBb. However, ovariectomy increased APBs but not APMs. E replacement return APBs to control levels, and the lowest dose of E decreased APMs. In adrenal, ovariectomy did not change APMs and APBs, but E replacement decreased both activities. Ovariectomy increased APMb and APBb and E replacement only return APBb to control levels. AVP-DAs did not change neither after ovariectomy nor E replacement in the HPA axis. On the contrary, AVP-DAb increased after ovariectomy only in hypothalamus, and E replacement return the values to control levels. Only pituitary AVP-DAb increased with the highest dose of E used. Due to the role of these aminopeptidases on the systemic and local renin angiotensin systems, hormonal modifications during physiological and/or pathological conditions could modify blood pressure regulation through these systems.

Key words: aminopeptidase A, M, B, vasopressin-degrading aminopeptidase, ovariectomy, mouse.

It is well known that renin-angiotensin system (RAS) is involved in the control of blood pressure. All or part of the components concerning the RAS have been reported to be synthetized and secreted outside of classical organs and tissues at sites including the brain, pituitary and other periferic tissues[1]. In addition to the circulating RAS, a local system has been postulated in the pituitary by immunodetection of its components in various mammalian species[2]. This local RAS has been implicated in the central regulation of the cardiovascular system, body water balance and also exert some influence over the secretion of pituitary hormones[3]. Classically, angiotensin II (Ang II) has been considered the main effector peptide of the RAS, but Ang II is not the only active peptide. Several of its degradation products, including angiotensin III (Ang III) and angiotensin IV (Ang IV), also posses biological functions. These peptides are formed via the activity of several aminopeptidases[4]. Thus, Ang III is obtained by delection of the N-terminal aspartic residue by glutamyl-aminopeptidase (GluAP) (EC 3.4.11.7.) and aspartyl-aminopeptidase (AspAP) (EC 3.4.11.). AspAP and GluAP have been named together as amino-peptidase A (APA) or angiotensinase. Ang III is futher converted to Ang IV by arginyl-aminopeptidase (aminopeptidase B, APB) (EC 3.4.11.6) or alanyl-aminopeptidase (aminopeptidase M, APM) (EC 3.4.11.14.)[5,6]. Ang III possesses most of the properties of Ang II and shares the same receptors. This peptide is particularly important in brain and especially in pituitary physiology and plays a major role in the secretion of arginine-vasopressin[7] (AVP). AVP is also implicated in the regulation of blood pressure[8] and is metabolized by vasopressin-degrading cystyl-aminopeptidase[5] (AVP-DA) (EC 3.4.11.3). In previous reports[9,10], we studied the possible existence of physiological sex differences in serum AP activities in mice, by evaluating the effect of gonadectomy and the in vitro response to the presence in the medium of steroid hormones. APN and APB activities were measured in sera from male, female, orchiectomized and ovariectomized mice. Our results demonstrated highly significant sex differences, and an influence of steroid hormones on AP activity. Depending on the nature of the AP, these enzymes responded in different ways to the presence of these substances and also responded differently to gonadectomy.

Due to the hypothalamic-pituitary-adrenal (HPA) axis has been implicated in the regulation of blood pressure, in the present work we analyse the effects of ovariectomy and estradiol (E) replacement on soluble and membrane-bound APA, APB and APM activities, involved in RAS regulation, and AVP-DA, at different levels of the HPA axis.

MATERIAL AND METHODS

Fifty virgin female Balb/C mice were used (21.65 « 0.68 g body weight). The animals were randomly divided into 5 groups of 10 mice each one. All the animals had free access to food and water and were housed at a constant temperature of 25 1C with lights on from 7:00 am to 7:00 pm. Four groups of mice were ovariectomized and the fifth group was sham-operated and used as control (C). Fifteen days after gonadectomy, three of this ovariectomized groups were treated subcutaneously with 10, 20 and 40 mg/kg of E dissolved in sesame oil, during ten days. The fourth group ovariectomized (OV-C) and the sham-operated only were treated with 3 ml/Kg of sesame oil, used as vehicle (figure 1). After this time, the animals were anaesthetized by an intraperitoneal administration

of chloral hydrate and perfused with saline through the ventricle. Then, hypothalamus, pituitary and adrenal glands were obtained. The tissues were frozen to -801C until their use.

Tissue samples were homogenized in 10 volumes of 10 mM HCl-Tris buffer (pH 7.4) and ultracentrifuged at 100,000 g for 30 min (4 1C) to obtain the soluble (s) fraction. The resulting supernatants were used to measure soluble enzymatic activity and protein content, assayed in triplicate. To solubilize membrane-bound proteins (b), the pellets were rehomogenized in HCl-Tris buffer (pH 7.4) plus 1% Triton X-100. After centrifugation (100,000 g, 30 min, 4 1C) the supernatants were used to measure membrane bound activity and proteins, also in triplicate. To ensure complete recorvery of activity, the detergent was removed from the medium by adding adsorbent polymeric Biobeads SM-2 (100 mg/ml) and shaking the samples for 2 h at 4 1C.

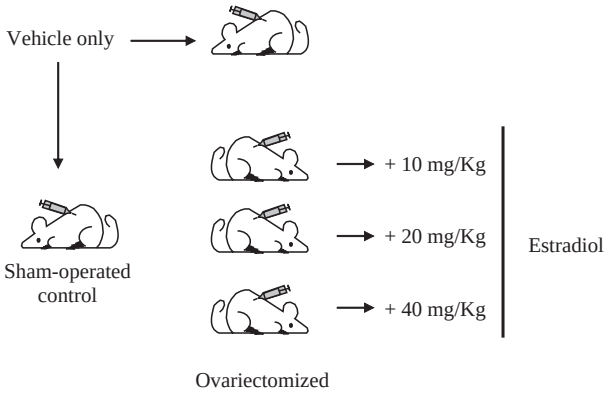

Figure 1. Experimental design. Fifty virgin female Balb/C mice were randomly divided into 5 groups of 10 mice each one. Four groups of mice were ovariectomized and the fifth group was sham-operated and used as control. Fifteen days after gonadectomy, three of this ovariectomized groups were treated subcutaneously with 10, 20 and 40 mg/Kg of estradiol dissolved in sesame oil, during ten days. The fourth group ovariectomized and the sham-operated control only were treated with the vehicle.

APA, APB, APM and AVP-DA activities were measured in a spectrophotometric assay using aspartyl- and glutamyl-ß-naphthylamide (AspNNap and GluNNap), arginyl-ß-naphthylamide (ArgNNap), alanyl-ß-naphthylamide (AlaNNap) and Cystyl-ß-naphthylamide (CysNNap) in accordance with the methods of Cheung and Cushman[11], Tobe *et al* [12] and Greenberg[13], with modifications. The amount of ß-naphthylamine released as a result of the enzymatic activity was measured spectrophotometrically at 550 nm[14]. Proteins were quantified in triplicate by the method of Bradford[15], using BSA as a standar. Specific soluble and membrane-bound APA, APB, APM and AVP-DA activities were expressed as pmol of their correspondign aminoacyl-NNap hydrolyzed per min per mg of protein by using a standard curve prepared with the latter compound under corresponding assay conditions. The spectrophotometric assays were linear with respect to time of hydrolysis and protein content. We used one-way analysis of variance (ANOVA) to analyze differences between groups. Post-hoc comparisons were made using the least significant difference test; P values below 0.05 were considered significant.

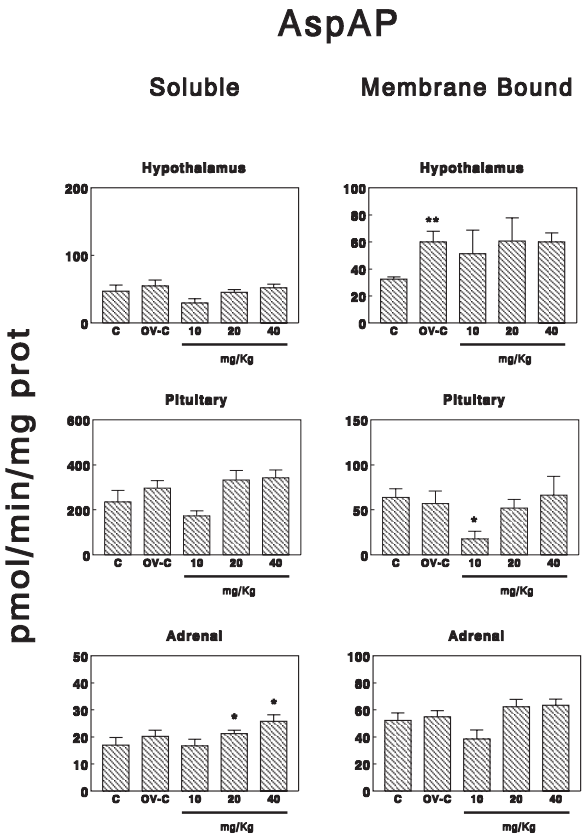

Figure 2. Specific soluble soluble and membrane bound AspAP activity in hypothalamus, pituitary and adrenal, of sham-operated control (C), ovariectomized (OV-C) and ovariectomized groups administrated with 10, 20 and 40 mg/Kg of estradiol. Results are expressed in picomoles of Aspartyl-â-naphthylamide hydrolyzed per min and per mg of protein (Mean»SEM; n=10; * p<0.05; ** p<0.01).

RESULTS

Specific soluble and membrane bound AspAP and GluAP (APA), APB, APM and AVP-DA activities in the hypothalamus, pituitary and adrenal are shown in figures 2 to 6.

Regarding AspAP activity, in hypothalamus,

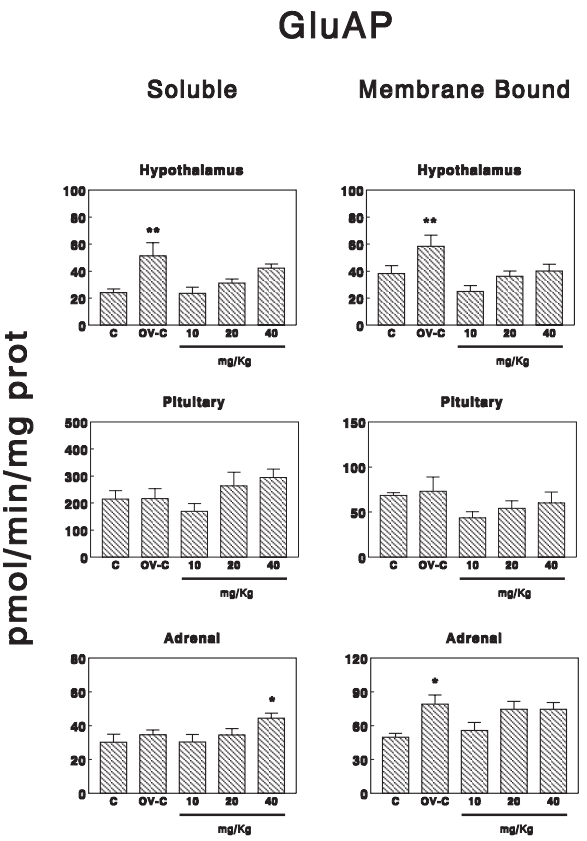

Figure 3. Specific soluble soluble and membrane bound GluAP activity in hypothalamus, pituitary and adrenal, of sham-operated control (C), ovariectomized (OV-C) and ovariectomized groups administrated with 10, 20 and 40 mg/Kg of estradiol. Results are expressed in picomoles of Glutamyl-â-naphthylamide hydrolyzed per min and per mg of protein (Mean»SEM; n=10; * p<0.05; ** p<0.01).

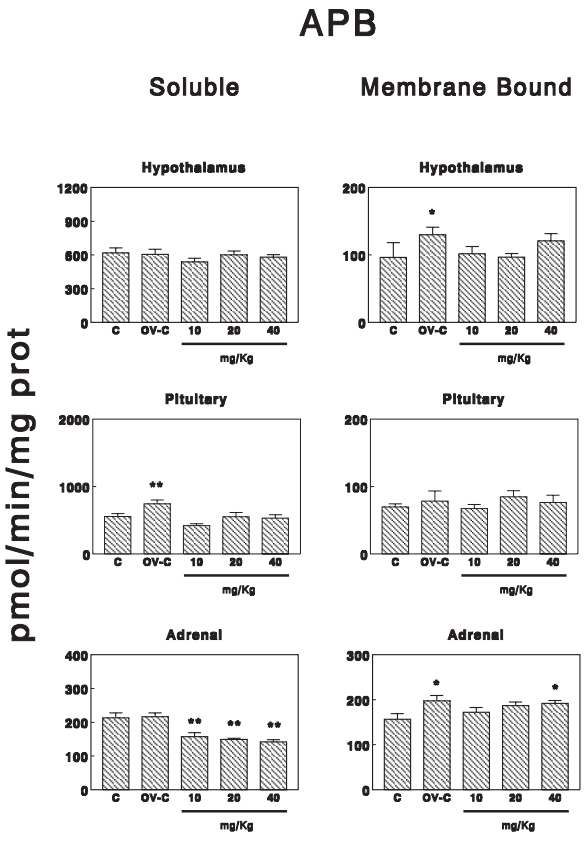

Figure 4. Specific soluble soluble and membrane bound APB activity in hypothalamus, pituitary and adrenal, of sham-operated control (C), ovariectomized (OV-C) and ovariectomized groups administrated with 10, 20 and 40 mg/Kg of estradiol. Results are expressed in picomoles of Arginyl-â-naphthylamide hydrolyzed per min and per mg of protein (Mean»SEM; n=10; * p<0.05; ** p<0.01).

neither ovariectomy nor E replacement modified AspAps. However, ovariectomy increased significantly ($p < 0.01$) AspAPb while E replacement at all the doses used, return the activity to control levels. In pituitary, AspAPs did not change neither with ovariectomy nor 20 or 40 mg/Kg E change AspAPb activity. However, administration of 10 mg/Kg E decreased significantly ($p < 0.05$) AspAPb activity. In adrenal, ovariectomy did not change AspAPs activity. However, administration of 20 and 40 mg/Kg E increased significantly ($p < 0.05$ in both cases) AspAPs activity. Neither ovariectomy nor E replacement modified AspAPb activity.

Regarding GluAP activity, in hypothalamus, ovariectomy increased significantly ($p < 0.01$) GluAPs and GluAPb activities, while E replacement return the activities to control levels. In pituitary, neither ovariectomy nor E replacement modified GluAPs nor GluAPb activities. In adrenal, ovariectomy did not change GluAPs activity, but 40 mg/Kg E increased significantly ($p < 0.05$) GluAPs activity. Ovariectomy increased GluAPb activity, while E replacement return the activity to control levels at all doses used.

Regarding APB activity, in hypothalamus, APBs did not change neither with ovariectomy nor with E replacement. However, ovariectomy increased significantly ($p < 0.05$) APBb activity while E replacement return the activity to control levels at all doses used. In pituitary, ovariectomy increased significantly ($p < 0.01$) APBs, whereas E replacement also return the activity to control levels at all doses used. However, neither ovariectomy nor E replacement modified APBb activity. In adrenal, ovariectomy did not change APBs. However, the administration of E decreased significantly ($p < 0.01$) APMs activity in a concentration-dependent manner. APBb activity increased with ovariectomy ($p < 0.05$), while 10 and 20 mg/kg E return the activity to control levels. However, 40 mg/kg E increased significantly ($p < 0.05$) APBb.

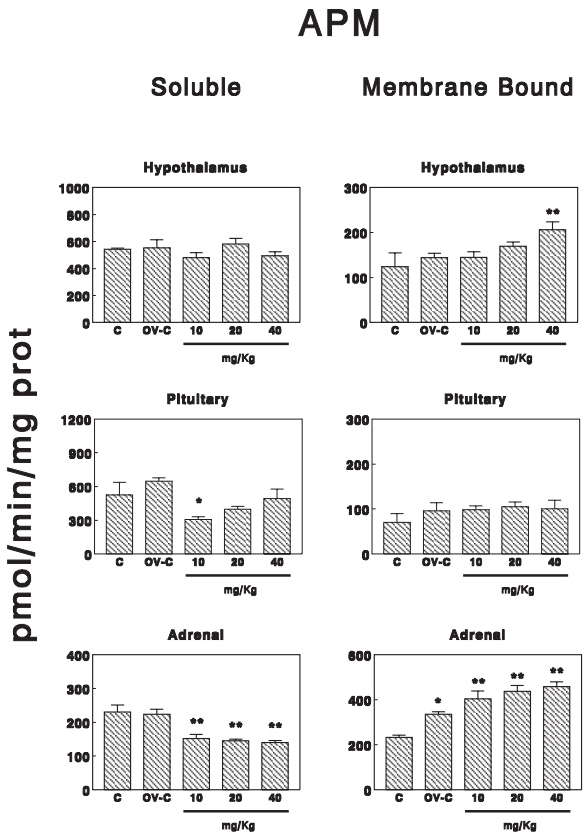

Figure 5. Specific soluble soluble and membrane bound APM activity in hypothalamus, pituitary and adrenal, of sham-operated control (C), ovariectomized (OV-C) and ovariectomized groups administrated with 10, 20 and 40 mg/Kg of estradiol. Results are expressed in picomoles of Alanyl-â-naphthylamide hydrolyzed per min and per mg of protein (Mean»SEM; n=10; * p<0.05; ** p<0.01).

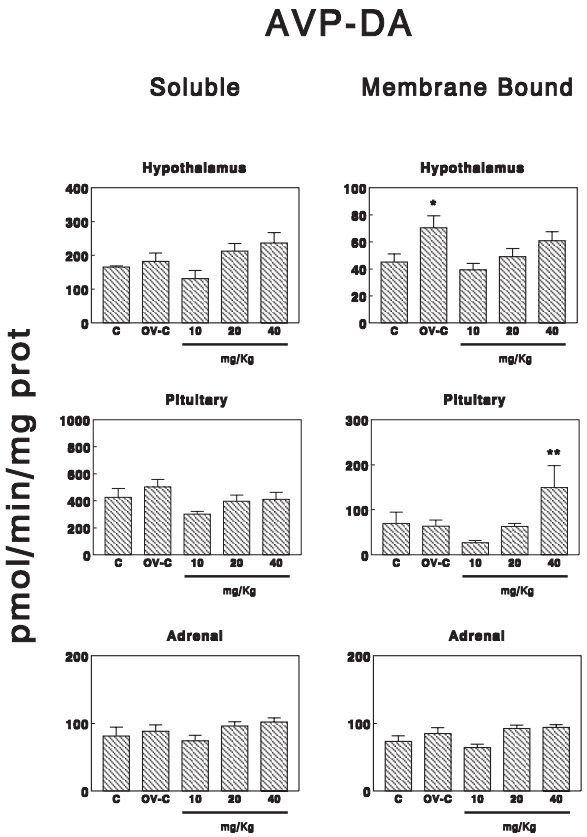

Figure 6. Specific soluble soluble and membrane bound AVP-DA in hypothalamus, pituitary and adrenal, of sham-operated control (C), ovariectomized (OV-C) and ovariectomized groups administrated with 10, 20 and 40 mg/Kg of estradiol. Results are expressed in picomoles of cystyl-â-naphthylamide hydrolyzed per min and per mg of protein (Mean»SEM; n=10; * p<0.05; ** p<0.01).

Regarding APM, in hypothalamus, APMs and APMb activities did not change neither with ovariectomy nor with E administration. Hovewer, APMb increased significantly ($p<0.01$) after the administration of 40 mg/kg E. In pituitary, ovariectomy did not change APMs, although this activity decreased after the administration of 10 mg/kg E ($p<0.05$). Neither ovariectomy nor E replacement modified significantly APMb. In adrenal, APMs activity did not change with ovariectomy, but E replacement decreased it significantly in a concentration-dependent manner ($p<0.01$). On the contrary, ovariectomy increased significantly ($p<0.05$) APMb activity, although the administration of E increased significantly ($p<0.01$) APMb activity in a concentration-dependent manner.

Regarding AVP-DA, in hypothalamus, neither ovariectomy nor E replacement modified AVP-DAs. However, ovariectomy increased significantly ($p<0.05$) AVP-DAb, while E replacement return the activity to control levels at all doses used. In pituitary, neither ovariectomy nor E replacement modified AVP-Das and AVP-DAb, although AVP-DAb increased significantly ($p<0.01$) after the administration of 40 mg/Kg E. In adrenal, neither ovariectomy nor E replacement modified AVP-Das and AVP-DAb.

DISCUSSION

Although in the study of the RAS much attention has been focused on their effector peptides, the regulatory mechanism of these peptides was rarely analyzed. In the present work, we have studied the effect of gonadectomy and estradiol replacement on several RAS-regulating and AVP-degrading amino-peptidase activities in the HPA axis. Previous in vitro studies performed in our laboratory have demonstrated the influence of steroid hormones on aminopeptidase activities, as previously cited[9,10].

Two of the most highly recognized factors

implicated in the pathogenesis of hypertension, artherosclerosis, congestive heart failure and associated cardiovascular diseases are the RAS and estrogen. A major effect of estrogen results from its influence on the RAS[16]. Estrogen has been shown to modulate Ang II-regulated behaviors, such as thirst, and may do so by influencing the central RAS[17]. In fact, estrogen replacement therapy decreases mean arterial pressure, increases cardiac output, and significantly reduces the risk of cardiovascular disease in postmenopausal women[18,19]. Administration of transdermal estradiol with or without progesterone has a hypotensive effect, particularly at night, in postmenopausal women. Furthermore, estradiol appears to have a dual effect on renin: it increases its production but inhibits its activation[20].

Ang II has been reported to stimulate HPA axis[21]. Furthermore, Ang II type-1 receptors are present in areas of the brain controlling autonomic nervous activity and the HPA axis[22].

APA is a membrane-bound metalloprotease, wich cleaves Ang II into Ang III. Pituitary is one of the richest source of APA[23]. Our results show that ovariectomy increased GluAPs and GluAPb in hypothalamus. However, only AspAPb activity increased after ovariectomy in hypothalamus. In adrenal, we found an increase of GluAPb activity after ovariectomy. These data may indicate that Ang II is converted into Ang III more rapidly without estradiol. Ang III possesses most of the properties of Ang II and may also activate AT_1 and AT_2 receptors[7,24]. Furthermore, Ang III is converted into Ang IV by their action of APB and APM[25]. Our results showed an increase on APBb activity after ovariectomy in hypothalamus and APBs in pituitary. These activities return to control levels after E replacement. In adrenal, we found some pharmacological effects and only APBb activity increased after ovariectomy and return to control levels with E replacement.

AVP-DA is the aminopeptidase which hydrolyses AVP. In the present work, we found an increase after ovariectomy in AVP-DAb activity and return to control levels with E replacement. To conclude, estradiol influence some, but not all, aminopeptidase activities involved in the regulation of the RAS and AVP at different levels of the HPA axis, and this can be useful to the knowledge of the effect of estrogen therapy in blood pressure regulation.

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