Heme oxygenase contributes to estradiol and raloxifene-induced vasorelaxation in estrogen deficiency

Anikó Pósa, Imre Pávó, Csaba Varga

Department of Physiology, Anatomy and Neuroscience, Faculty of Science and Informatics, University of Szeged, Kozep fasor 52, H-6726 Szeged, Hungary

Address for correspondence:

Anikó Pósa PhD

University of Szeged

Kozep fasor 52, H-6726 Szeged, Hungary

Tel: +36 62 544884, fax: +36 62 544291

E-mail: paniko@bio.u-szeged.hu

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Despite of disappointing lack of evidences for benefits in outcome studies, hormone replacement therapy or treatment with the selective estrogen receptor modulators like raloxifene (RAL) has a number of potentially beneficial cardiovascular effects in certain conditions of postmenopausal women. Similarly, estradiol (E_2) or RAL administration improves various cardiovascular indices in the ovariectomized (OVX) rat, a frequently used model of estrogen depletion [1]. A principal mediator of cardioprotection for both estrogens and RAL appears to be the endothelial nitric oxide (NO) [2, 3]. Recent data indicate that carbon monoxide (CO), another endothelium-derived smooth muscle relaxant also induced by estrogen and may contribute to cardioprotection [4]. No data exist, however, as concerns the role of estrogen replacement on heme oxygenase (HO) activity and its impact in estrogen-deficient state. Moreover, the contribution of HO to cardiovascular effects of RAL has not been evaluated. We hypothesized that besides NO, HO- generated CO may also contribute to the E_2 and RAL- induced vasodilatation observed under estrogen-deficient conditions.

Four-month-old female Wistar rats (230-250 g; at least 10 animals per group) underwent OVX surgery. After six weeks recovery period, oral E_2 (0.10 mg/kg/day) or RAL (RAL 0.33: 0.33 mg/kg/day, RAL 1: 1.0 mg/kg/day) treatment was introduced for 2 weeks. HO activity was inhibited with tin protoporphyrin (SnPP; 30.0 μg/kg, 24-h and 1-h prior experiments). HO activity was determined in homogenized aortic or cardiac tissues [5]. Arginine vasopressin (AVP) induced heart perfusion and contraction of abdominal aortic rings *ex vivo* was studied [5]. All manipulations were performed in full accordance with the EU guidelines on the care and use of laboratory animals and had been approved by the Institutional Ethics Committee. Data are reported as means ± S.E.M. of the findings from at least 3 independent experiments. Statistical comparisons were performed by using ANOVA; $p \leq 0.05$ taken as statistically significant.

OVX decreased HO activities in the cardiac left ventricle and in the aortic tissues (Fig. 1A,

2A). E_2 or RAL increased the HO activities similar to values of the intact rats. In the OVX animals, AVP caused a significantly larger decrease of heart perfusion than in the intact female group. E_2 and RAL treatment abolished the decreased heart perfusion response observed, while HO activity inhibition caused a significant augmentation in all groups (Fig. 1B.). Similarly, OVX resulted in a significantly increased AVP-induced aortic contraction (Fig. 2B.). E_2 or RAL decreased the tension to a level similar to that observed in the ovary-intact animals. When HO activity inhibition was combined with either E_2 or RAL administration, aortic contraction increased to a similar level as that seen in the non-treated OVX animals.

The estrogen depletion caused by OVX was accompanied by reduced activity of the HO system both in the myocardium and the aorta and increased sensitivity to vasoconstriction and myocardial hypoperfusion. These adverse effects could be partially reversed by the exogenous administration of E_2 or RAL. On the other hand, the beneficial changes of E_2 and RAL did not occur when the HO activity was inhibited, suggesting an important role of HO pathway in these findings.

Our results are the first demonstration that RAL increases HO activity in the cardiovascular system. Similarly to E_2, RAL was found to restore the HO activity of HO in the heart and aorta of OVX rats. We found that the AVP-induced aortic contraction and myocardial hypoperfusion was aggravated following OVX. These results may come from the interplay between the NOS and HO systems. Indeed, the overexpression of HO-1 restored endothelial nitric oxide synthase (eNOS) activity in the endothelial cells under oxidative stress [6]. A low concentration of CO induced NO release, while a high concentration inhibited eNOS activity and NO generation [7]. Our findings support the role of the basal, constitutive HO activity in the protection against vascular constriction: HO-1 knockout mice exhibited an impaired relaxation of the superior mesenteric arteries and an increased contractility to phenylephrine

as compared with the vessels from wild-type animals [8, 9]. Previous studies have also demonstrated that overexpression of HO-1 was associated with an increase in HO activity and a decrease in the blood pressure in spontaneously hypertensive rats [10].

In conclusion, we have demonstrated here that RAL, similarly to E_2, is a potent inducer of the HO system. The data presented provide the first evidence that HO may play an important role in the RAL-induced beneficial effects on the cardiovascular system and extend the evidence relating to a similar role of estrogens.

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Figure 1.

Heme-oxygenase activity in the cardiac left ventricle (LV) (**A**) and the effects of HO inhibition on the decrease in heart perfusion (**B**). Data are expressed as means ± S.E.M. of the results on a minimum of 9 rats per group. Statistical significance: $^{a}p<0.05$ as compared with the ovary-intact group. $^{b}p<0.05$ a significant difference between the groups with and without SnPP pretreatment.

Figure 2.

The effects of heme-oxygenase activity in the aorta (**A**) and the heme oxygenase inhibitor tin protoporphyrin (SnPP) on the aorta contraction by arginine-vasopressin (**B**).

Results are shown as means ± S.E.M. for 10 animals in each group. Statistical significance: $^{a}p<0.05$ as compared with the ovary-intact group. $^{b}p<0.05$ a significant difference between the groups with and without SnPP pretreatment.