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Modulation of the response to estradiol-17β of rat vascular tissues by a non calcemic vitamin D analog☆

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Abstract

Estradiol-17β (E_2) increases creatine kinase (CK) specific activity in aorta (Ao) and left ventricle of the heart (Lv) from rat females. In the present study, we analyzed the effects of pretreatment with the non calcemic analog of vitamin D, JK 1624 F2-2 (JKF) on the response to E_2 (either 0.5 or 5 μg/rat) of Ao and Lv from prepubertal female rats. JKF did not affect CK in either organ. However, pretreatment with JKF (0.1 ng/g body weight for 1 or 2 weeks) increased the CK response to E_2 (0.5 μg/rat) by 50 ± 10% in Ao and by 150 ± 12% in Lv. The CK response to 5 μg/rat of E_2 in intact female rats, was increased by 118 ± 15% and 99 ± 11% in the Ao and by 89 ± 6% and 112 ± 13% in the Lv, in animals treated daily with JKF for 1 or 2 weeks, respectively, before administration of E_2. JKF also increased the response to 500 μg/rat raloxifene (Ral) by 47 ± 8% in Ao and by 56 ± 12% in Lv. Preliminary experiments showed that JKF treatment induced a ∼50% increase in estradiol receptor ERα in both organs. The results indicate that the vitamin D analog JKF upregulates the response and sensitivity of vascular tissues to E_2, in association with increased expression of their ERα. These results should prompt examination of the possibility that the effects estrogen therapy in postmenopausal women can be augmented by vitamin D or its analogs.

Keywords: Estrogen receptors α and β; "Non hypercalcemic" vitamin D analogs; Estrogen; Creatine kinase

1. Introduction

Despite the recent Women's' Health Initiative study report that continuous treatment with conjugated equine estrogens and medroxyprogesterone acetate may adversely affect cardiovascular disease in older women [1], a large body of evidence suggests that estrogen has multiple direct beneficial effects in the vasculature [2–4]. The lower incidence of heart diseases among pre-menopausal women compared with males or with postmenopausal women is likely related to protracted effects of estrogen during reproductive years. This protective effect of estrogen appears related to multiple effects such as favorable decrease in the ratio between LDL and HDL [5] and on lipid peroxidation [6] and vascular reactivity [7]. Overall, most studies in experimentally controlled conditions in the laboratory support the concept that estrogens are protective against coronary atherosclerosis [2–4,7–9].

The specific activity of creatine kinase (CK) is known to be induced by estrogens in vivo and in vitro [10,11], and, therefore, can be used as an estrogen receptor (ER) response marker. In previous reports, we showed that the non hypercalcemic analog of vitamin D, JK 1624 F2-2 (JKF) [12] increased the effects of E_2 in terms of both the absolute response and sensitivity to E_2 in skeletal cells and skeletal tissues [13] and in vascular cells in culture [14].

We now address the possibility that the effects of E_2 in the cardiovascular system [Aorta (Ao) and left ventricle of the heart (Lv)] can be modulated in vivo by JKF.

2. Materials and methods

2.1. Animals

Prepubertal female Wistar rats were used at the age of 25 days. Rats were housed in air-conditioned quarters with light from 05.00 to 19.00 h and received food and water ad libidum. Experiments were carried out according to the regulations of the Committee on Experimental Animals of the Tel-Aviv Sourasky Medical Center.

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2.2. Hormonal treatments

Rats were injected i.p. with vehicle (0.05% ethanol in saline) or JKF (0.1 ng/g body weight) daily for 4 days. On day 4, each of the rats above received an additional i.p. injection of either vehicle or E_2 (0.5 or 5 μg/rat), or raloxifene (Ral 500 μg/rat) or the combination of Ral and E_2. In some experiment the treatment was extended for another week. Animals were sacrificed 24 h after the vehicle, E_2 or Ral injection.

2.3. Creatine kinase extraction and assay

Rat organs: aorta (Ao) and left ventricle of the heart (Lv), were collected in isotonic buffer, homogenized and tissue extracts were obtained as described before [15–17]. Enzyme activity was assayed as described previously [15–17].

2.4. Statistical analysis

Differences between the mean values obtained from the experimental and the control groups were evaluated by analysis of variance (ANOVA).

3. Results

Treatment with JKF for 1 or 2 weeks did not affect significantly the specific activity of CK in Ao and Lv of prepubertal female rats. However, pretreatment with JKF (0.1 ng/g body weight) increased E_2-dependent CK stimulation: after 1 or 2 weeks of pretreatment with JKF. The induction by 0.5 μg E_2 of CK in Ao was increased by 50 ± 10%, and in Lv by 150 ± 12%. After 1 week of daily treatment with JKF, the induction by E_2 (5 μg) of CK in Ao was increased by 118 ± 15%. Two weeks of daily treatment with JKF increased the response to E_2 by 99 ± 11%. Similarly, 1 week of daily JKF treatment, increased the CK response to 5 μg of E_2 in Lv by 89 ± 6% and 2 weeks of pretreatment with JKF increased the CK response to 5 μg of E_2 by 112 ± 13% (Fig. 1). Using an identical pretreatment protocol we found that JKF administered for 1 or 2 weeks also increased the CK response to 500 μg raloxifene in both Ao and Lv. However, JKF abolished the blockade exerted by the estrogen receptor antagonist raloxifene on E_2 effects: such that in Ao the net increase was by 47 ± 8% and in Lv by 56 ± 12%, significantly more than with raloxifene/E_2 without JKF ($P < 0.05$). Treatment with JKF for 2 weeks also stimulated the level of estrogen receptor ERα in both Ao and Lv by about 50%, measured by Western blot analysis.

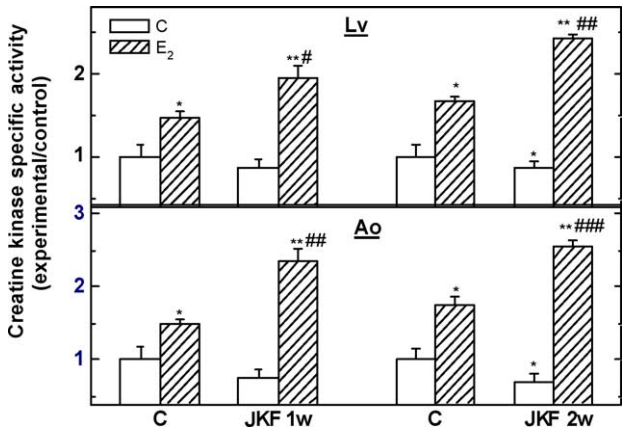

Fig. 1. Modulation by JKF, of CK specific activity in Ao and Lv from prepubertal female rats. Rats were injected as described in Section 2. Results are expressed as percentage increase of CK specific activity after JKF compared to E_2 alone. Results are the mean + S.E.M. of three experiments each performed in triplicates. $*P < 0.05$, $**P < 0.01$ for the comparison treatment with E_2 after vehicle alone. $\#P < 0.05$, $\#\#$ $P < 0.01$, $\#\#\#$ $P < 0.005$ for the comparison treatment with JKF alone. Data were assessed by ANOVA.

4. Discussion

The present in vivo study in intact prepubertal female rats, demonstrate that E_2 stimulates CK (an early "estrogen induced protein" in vivo and in vitro) which is a known estrogen response marker [11] in Ao and in Lv. Pretreatment with the vitamin D analog JKF alone, had only slight effects on CK specific activity in both Ao and Lv. More importantly, however, JKF upregulated CK response in Ao and Lv to E_2 and to raloxifene. Further, JKF blocked the inhibition of E_2 activity by raloxifene in vivo. The changes in the aortic and myocardial responses to E_2 and to raloxifene after vitamin D analog, probably reflects changes in ERα and ERβ as suggested by previous observations that JKF induces such effects on ER in the skeletal and vascular cells [13,14]. Indeed JKF treatment increased the level of ERα in Ao and in Lv as measured by Western blot analysis.

Although the recently published WHI report [1] seriously challenges beliefs that estrogens exert protective cardiovascular effects, multiple vascular influences of estrogen do appear to offer potential advantages [2,3,5,10]. Hence, there is a growing challenge to better understand all vascular effects of estrogenic compounds, such that selective activation of beneficial effects, with avoidance of potentially deleterious effects can be sought in the future. In this context our results not only define new metabolic targets for E_2 and raloxifene in the cardiovascular system, but also identify a novel pathway, that is, activation of vitamin D receptors, by which these effects can be modulated.

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