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Short communication

The 21-aminosteroid antioxidant, U74389F, prevents estradiol-induced depletion of hypothalamic β-endorphin in adult female rats

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Abstract

A single intramuscular injection of 2 mg estradiol valerate (EV) results in neuronal degeneration and β-endorphin depletion in the hypothalamic arcuate nucleus of adult female rats. We have hypothesized that peroxidase-positive astrocytes in this brain region oxidize estrogens and catecholestrogens to semiquinone radicals which mediate oxidative neuronal injury. In the present study, dietary administration of the potent antioxidant 21-aminosteroid, U-74389F, completely blocked EV-induced β-endorphin depletion in the hypothalami of adult female rats. Neither EV nor 21-aminosteroid treatment had any effect on hypothalamic concentrations of neuropeptide Y and Met-enkephalin, confirming that the estradiol lesion is fairly selective for the β-endorphin cell population. The present findings support the hypothesis that the toxic effect of estradiol on hypothalamic β-endorphin neurons is mediated by free radicals.

Key words: Estradiol; β-Endorphin; 21-Aminosteroid; Free radical; Hypothalamus

Adult female rats treated with a single intramuscular injection of 2 mg estradiol valerate (EV) develop anovulatory acyclicity, persistent vaginal cornification and discrete neuropathological changes in the hypothalamic arcuate nucleus [3,5]. The arcuate lesion is characterized by the presence of dendritic degeneration, increased numbers of reactive microglial cells and the accumulation of peroxidase-positive astrocytic inclusions [3,5,22]. The pathologic effect of estradiol appears to be selective for β-endorphin neurons [9]. The estradiol-induced compromise of the β-endorphin system results in a compensatory up-regulation of μ opioid binding sites in the medial preoptic area (MPOA; [8]). The subsequent hypersensitivity of the MPOA to opioids engenders a cascade of neuroendocrine aberrations ultimately resulting in anovulation and multicystic ovaries [2].

We recently demonstrated that the antioxidant, α-tocopherol (vitamin E) blocks estradiol-induced depletion of hypothalamic β-endorphin and prevents the development of the anovulatory condition [7]. This observation suggests that estrogen-derived free radical intermediates may play an important role in the pathogenesis of the arcuate lesion akin to the pro-oxidant effects of estradiol in endometrium and other peripheral sex steroid target tissues [13,14,18,20,22]. Peroxidase-positive astrocytes in primary culture readily convert catechol-estrogens and dopamine to orthosemiquinone radicals with proven neurotoxic activity [21]. Thus, the peroxidase activity in arcuate astroglia, augmented by estradiol treatment, may catalyze the oxidation of estrogens to free radical intermediates and thereby promote the development of hypothalamic pathology [20,22].

To exclude the possibility that vitamin E interferes with the neurotoxic effects of estradiol through some mechanism distinct from its role as an antioxidant, we tested the 21-aminosteroid, U74389F, a potent antioxidant chemically unrelated to α-tocopherol, for its ability to similarly protect against EV-induced depletion of hypothalamic β-endorphin in adult female rats.

Animals were divided into 4 groups ($n = 11$) and treated as follows: the first group received a single 2 mg i.m. injection of EV, a regimen previously shown to result in neuronal degeneration and β-endorphin de-

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pletion in the arcuate nucleus. The second group was injected with EV and immediately begun on continuous dietary treatment with U74389F, 25 mg per kg b.wt. per day, as recommended by the manufacturer (administered in powdered Purina rat chow; provided by Upjohn Inc., Kalamazoo, MI). The third group was treated with the 21-aminosteroid alone, and the fourth group consisted of normally cycling untreated controls.

Eight weeks following the initiation of treatment, animals were sacrificed (between 09.00 and 11.00 h) and their brains rapidly removed and frozen in isopentane/liquid nitrogen at −50°C for 15 s.

Hypothalami were dissected from the brains as previously described [9] using the optic chiasm and mammillary bodies as rostro-caudal borders, and the lateral recess of the hypothalamus as the lateral border. The tissues were homogenized in 1 ml of 1 N HCl for 30 s using a Polytron (Brinkmann Instruments, Westbury, NY). Samples were centrifuged for 30 s at 10,000 × g, and the supernatants were used for determination of the β-endorphin concentrations by radioimmunoassay (RIA). The β-endorphin antibody (RIK 8626; Peninsula, Belmont, CA) is a rabbit-derived anti-human antiserum that displays 80% cross-reactivity with rat β-endorphin. It does not cross-react with Met-enkephalin, γ-endorphin, or β-lipotropin. The neuropeptide-Y (NPY) antibody (RIK-7172; Peninsula) shows cross-reactivities with peptide-YY, vasoactive intestinal polypeptide, and avian pancreatic polypeptide of 0.003%, 0.001%, and 0.007%, respectively. The Met-enkephalin antibody (no. 18100; Incstar) displays 2.8% cross-reactivity with Leu-enkephalin and less than 0.003% cross-reactivities with α- and β-endorphin. Student's t-test (2-tailed) was used to determine significance between groups at the $P < 0.05$ level.

The effects of the various treatments on hypothalamic β-endorphin, neuropeptide and Met-enkephalin concentrations are depicted in Fig. 1. Animals treated with EV exhibited hypothalamic β-endorphin concentrations (116.9 ± 3.0 pg/mg wet wt.) which were significantly lower than those of untreated, normally cycling controls (152.3 ± 3.8 pg/mg; $P < 0.05$). Animals injected with EV and treated with U-74389F exhibited β-endorphin concentrations (161.0 ± 3.9 pg/mg) which were similar to the untreated controls ($P > 0.05$), and significantly greater than the levels measured in the group receiving only EV ($P < 0.05$). Animals receiving U-74389F alone exhibited a trend towards increased hypothalamic β-endorphin levels relative to controls which did not reach statistical significance ($P > 0.05$). No differences in the hypothalamic concentrations of neuropeptide-Y and Met-enkephalin were noted between any of the groups ($P > 0.05$ for each comparison).

As described previously [9], a single injection of EV resulted in the significant depletion of hypothalamic

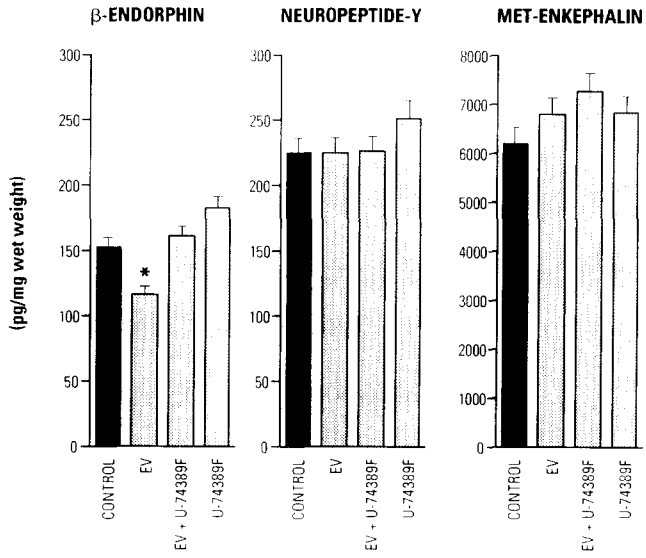

Fig. 1. Effects of estradiol valerate (EV) and U-74389F on hypothalamic concentrations of β-endorphin, neuropeptide Y and Met-enkephalin in adult female rats. Columns and vertical bars represent means + S.E.M. ($n = 11$ per group). * $P < 0.05$ relative to untreated controls.

β-endorphin in adult female rats. In contrast, EV treatment did not alter the hypothalamic concentrations of either Met-enkephalin or neuropeptide-Y, corroborating our earlier observations that the pathologic effects of estradiol on the β-endorphin neuronal system are fairly specific.

The present results demonstrate that U-74389F, a 21-aminosteroid with potent antioxidant activity, shares with α-tocopherol [7] the ability to prevent the estradiol-induced decrement in hypothalamic β-endorphin. This observation strongly suggests that the previously documented neuroprotective effect of α-tocopherol in the EV-treated rat model is due to the vitamin's antioxidant activity rather than being secondary to some alternative mechanism of action. 21-Aminosteroids have been shown to effectively scavenge free radicals and inhibit lipid peroxidation in brain tissues both in vitro and in whole animal models. For example, these compounds protect against free radical-related neural damage in models of vasogenic brain edema [11], post-ischemic reperfusion injury [12] and spinal cord trauma [10]. By scavenging estrogen-derived pro-oxidant intermediates within the medial basal hypothalamus, both U74389F and α-tocopherol may abrogate neural degeneration and β-endorphin depletion which result from unremitting estrogenic stress.

A unique feature of the estrogen-induced hypothalamic lesion is the accumulation of diaminobenzidine (DAB)-positive cytoplasmic granules in a sub-population of periventricular (Gomori) astrocytes. The DAB reaction within these glial inclusions is due to non-enzymatic peroxidase activity which is most likely medi-

ated by ferrous iron and/or other transition metals [4,22]. This glial peroxidase activity may play a pivotal role in the development of estrogen-related neural damage in the medial basal hypothalamus. In the course of estradiol metabolism, highly reactive semiquinones and oxyradicals are generated in a variety of estrogen target tissues [13,15,16]. In hypothalamus, 2-hydroxylases and peroxidases convert estradiol to 2-hydroxyestradiol or catecholestrogen [1,17,19]. Catecholestrogens may, in turn, be transformed to semiquinone radicals via a *peroxidase*/H_2O_2-catalyzed reaction or by spontaneous autoxidation. The latter pathway also generates potentially neurotoxic O_2-derived free radicals, including H_2O_2 and superoxide anion [15,16]. In peripheral estrogen target tissues, semiquinones and O_2-derived free radicals have been implicated in the carcinogenic and teratogenic effects of estradiol and related compounds [13,14,18]. Similar estrogen-derived free radicals, generated by glial peroxidase activity, may stimulate lipid peroxidation and thereby promote neuronal degeneration within the arcuate nucleus [22]. Why β-endorphin neurons should be selectively vulnerable to estradiol neurotoxicity is a question that remains to be answered. As recently reviewed elsewhere [2], estrogen-induced damage to central β-endorphin neurons may play an important role in aging of the neuroendocrine hypothalamus, in a host of mammalian anovulatory disorders, and in human anorexia nervosa. The results of the present study lend further support to the hypothesis that this estradiol neurotoxicity is mediated, at least in part, via oxidative mechanisms [20–22]. We previously demonstrated that hypothalamic degeneration in the EV-treated rat model is abrogated by prior ovariectomy [5], and that identical neuropathological changes are induced in the hypothalami of castrated female rats in which levels of plasma estradiol-17β are chronically maintained in the upper physiologic range by implantation of steroid-releasing Silastic capsules [6]. Taken together, our findings suggest that oxidative injury to β-endorphin neurons and resulting neuroendocrine disorders are mediated by sustained exposure to high physiological as opposed to pharmacological levels of circulating estradiol.

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