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VITAMIN E PROTECTS HYPOTHALAMIC BETA-ENDORPHIN NEURONS FROM ESTRADIOL NEUROTOXICITY

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Abstract: Estradiol valerate (EV) treatment has been shown to result in the destruction of 60% of beta-endorphin neurons in the hypothalamic arcuate nucleus. Evidence suggests that the mechanism of EV-induced neurotoxicity involves the conversion of estradiol to catechol estrogen and subsequent oxidation to free radicals in local peroxidase-positive astrocytes. In this study, we examined whether treatment with the antioxidant, vitamin E, protects beta-endorphin neurons from the neurotoxic action of estradiol. Our results demonstrate that chronic vitamin E treatment prevents the decrement in hypothalamic beta-endorphin concentrations resulting from arcuate beta-endorphin cell loss, suggesting that the latter is mediated by free radicals. Vitamin E treatment also prevented the onset of persistent vaginal cornification and polycystic ovarian condition which have been shown to result from the EV-induced hypothalamic pathology.

INTRODUCTION

A single injection of estradiol valerate (EV), which induces tonic ovarian secretion of physiological levels of estradiol, initiates the development of a progressive lesion in the hypothalamic arcuate nucleus characterized by degenerating axons and dendrites and the accumulation of peroxidase-positive astrocytic granules (1,2,3). Using quantitative immunocytochemistry, radioimmunoassay and unbiased stereological methods, we have recently shown that this lesion selectively affects beta-endorphin neurons (4). Following EV treatment, the total number of beta-endorphin-immunoreactive neurons in the arcuate nucleus was reduced by more than 60% as compared to controls. Furthermore, the absolute number of beta-endorphin immunoreactive cells lost corresponded precisely with the total number of neurons lost as determined by stereology in cresyl violet stained sections (4). In contrast, neighbouring neuronal populations including met-enkephalin-, neuropeptide-Y-, somatostatin-, neurotensin- and tyrosine hydroxylase-containing neurons were unaffected by EV treatment (4).

The mechanism whereby estradiol induces such selective degeneration of hypothalamic beta-endorphin neurons is not understood. However, evidence suggests that the production of lipid-damaging free radicals by local peroxidase-positive astrocytes may be involved (3). These astrocytes, present in normal rodent and human brain (5), are particularly prominent in the vicinity of degenerating neuronal elements in the arcuate nucleus of EV-treated rats (1,2). In vitro, these astrocytes have been shown to readily transform catechol estrogen (2- or 4-hydroxyestradiol), generated spontaneously and enzymatically from estradiol in hypothalamic tissue (6,7), to reactive O-semiquinone free radicals (8). Cytochemistry (9) and spectroscopy (10) has indicated that the peroxidase activity of the astrocytic granules may be due to the presence of heme (9). Estradiol has been shown to stimulate the production of delta-aminolevulinic acid synthetase, the rate limiting enzyme in heme biosynthesis (11,12). Heme and other peroxidases are catalysts in the formation of free radicals (6). Thus, estradiol may serve as both substrate and stimulator of the production of lipid damaging free radicals.

In order to test the hypothesis that EV-induced beta-endorphin cell loss is mediated by free radical-induced lipid peroxidation, animals injected with EV were concomitantly treated with vitamin E (alpha-tocopherol), a potent antioxidant. Vitamin E, which has been shown to accumulate in tissues in a concentration dependent manner, inhibits both iron-induced and spontaneous lipid peroxidation in brain and peripheral tissues (13, 14,15).

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MATERIALS AND METHODS

Nine week old female Wistar rats (Charles River, Canada) were housed under conditions of controlled light (12L:12D) and temperature (22°C) and divided into 4 separate groups (n=9). The first group was subjected to a single 2mg IM injection of estradiol-valerate (EV; Delestrogen, Squibb), a regimen previously shown to result in the destruction of beta-endorphin neurons (4). The second group was injected with EV and immediately treated with vitamin E, d-alpha-tocopherol (EV+VitE; Sigma Chemical, St-Louis, MO) via daily ingestion through the diet (150 IU alpha-tocopherol per kg powdered purina rat chow). The third group was treated with alpha-tocopherol only (VitE), and fourth group consisted of normally cycling controls (Cont). Eight weeks following the initiation of treatment, animals were sacrificed (between 0900 and 1100) and their brains rapidly removed and frozen in isopentane - 50°C for 15 sec. To control for possible effects of vitamin E treatment on the expression of beta-endorphin by arcuate neurons, additional animals from group 3 (alpha-tocopherol treated; n=5) and group 4 (controls; n=3) were sacrificed as above three days after initiation of treatment. Hypothalami from each group were evaluated for beta-endorphin, met-enkephalin (met-ENK) and neuropeptide-Y (NPY) concentrations. In the long-term treated animals, vaginal cytology was also monitored for two weeks prior to sacrifice. Ovaries were removed, fixed in Bouin's solution, and embedded in paraffin. Sections were stained with hematoxylin and eosin.

Radioimmunoassays

Hypothalami were homogenized in 1ml HCL 1N for 30s using a Polytron. Samples were centrifuged for 30s at 10 000g and the supernatant was utilized for the determination of beta-endorphin concentration. The beta-endorphin antibody (RIK 8626; Peninsula) was directed against human beta-endorphin and displayed 80% cross-reactivity with rat beta-endorphin. It did not crossreact with met-endorphin, gamma endorphin or beta-lipotropin. The NPY antibody (RIK-7172; Peninsula) showed cross reactivities with peptide YY, vasoactive intestinal polypeptide and avian pancreatic polypeptide of .003%, .001%, and .007%, respectively. The met-enkephalin antibody (#18100 Incstar) displayed 2.8% cross reactivity with leu-enkephalin and <.003% cross reactivities with alpha- and beta-endorphin.

Statistics

The means for each group of long-term treated animals were compared by one way analysis of variance followed by Student's t-test. The unpaired student's t-test was used for comparing controls to short-term vitamin E treated rats.

RESULTS

In conformity with previous results, EV treatment resulted in significant reductions in hypothalamic beta-endorphin concentrations, consistent with cell loss (Fig. 1). Met-enkephalin concentrations were unaltered and those of neuropeptide-Y were significantly increased (Table 1), also in agreement with earlier findings (4). In contrast, in animals treated concomitantly with EV and vitamin E, beta-endorphin, met-ENK and NPY levels were all comparable to those of controls (Fig. 1; Table 1). Animals chronically treated with vitamin E alone, displayed concentrations of beta-endorphin that were significantly higher than in controls (Fig. 1). However, both met-ENK and NPY levels were unaltered (Table 1). Vitamin E did not engender an increase in beta-endorphin concentrations in animals sacrificed after 3 days of treatment (87.68+13 pg/mg wet weight vs. 88.33+18; t-value 0.292).

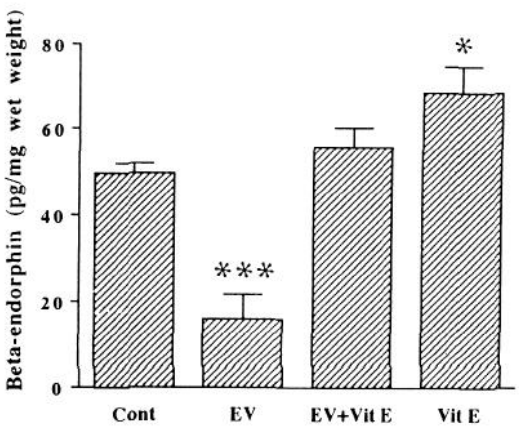

Figure 1. Effect of long-term Vitamin E treatment on EV-induced reductions in hypothalamic beta-endorphin concentrations. Values are expressed as mean ± S.E.M. for n=9; * and *** indicates significantly different ($p<0.05$ and $p<0.001$, respectively ANOVA) from age-matched controls. Abbreviations as described in materials and methods.

Ovaries from control animals exhibited the full range of healthy primary and secondary ovarian follicles as well as numerous corpora lutea (Fig. 2a). As previously described (16), animals treated with EV exhibited persistent vaginal cornification and developed typical polycystic ovaries devoid of corpora lutea (Fig. 2b). In contrast, 6 out of 9 of the animals chronically exposed to both estradiol and vitamin E displayed ovaries with abundant corpora lutea, moderate stromal development and a few healthy secondary follicles (Fig. 2c). These same animals displayed normal patterns of estrous cyclicity. The ovaries and cycling patterns from animals treated with vitamin E alone (long-term) were indistinguishable from those of controls (Fig. 2a,d).

Table 1. Effects of long-term Vitamin E treatment on hypothalamic met-enkephalin and neuropeptide-Y concentrations. Values are expressed as the mean ± S.E.M. for n=9; *indicates significantly different from age-matched controls ($p<0.05$).

	Met-enkephalin (pg/mg)	Neuropeptide-Y (pg/mg)
control	4494±411	212±10
EV-treated	5284±496	269±19*
EV+Vitamin E	4089±302	221±13
Vitamin E	4579±385	227±14

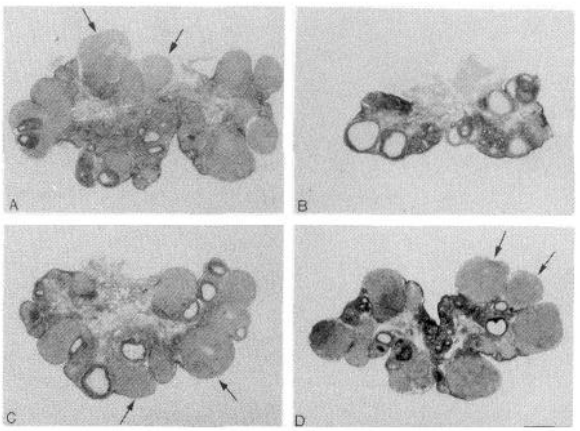

Figure 2. Ovaries from control a), EV-treated b), EV and Vitamin E treated c) and Vitamin E treated d) groups. Note the absence of corpora lutea (arrows) in the EV-treated group.

DISCUSSION

The pronounced reductions in hypothalamic beta-endorphin concentrations observed here in the EV-treated group are consistent with previous results (4, 17) and have been shown to result from the loss of an average 60% of beta-endorphin neurons in the arcuate nucleus (4). The maintenance of normal beta-endorphin concentrations in EV-injected animals co-treated with vitamin E suggests that the antioxidant prevented the estradiol-induced beta-endorphin cell loss. Furthermore, the fact that animals treated with alpha-tocopherol alone exhibited significantly higher levels of beta-endorphin than did untreated controls, implies that the antioxidant also protected against the well documented age-related decline in hypothalamic beta-endorphin content (18, 19, 20). It may be argued that the increase in beta-endorphin levels by vitamin E in both normal and EV-treated rats reflects increased expression of beta-endorphin by intact arcuate neurons. However, had this been the case relatively short-term treatment (3 days) with alpha-tocopherol should have been equally effective, contrary to what was observed.

The prevention of estradiol's degenerative effects by alpha-tocopherol treatment supports the hypothesis that estradiol neurotoxicity is mediated through free-radical induced lipid peroxidation. O-semiquinone free radicals are likely to be involved since these have been shown to be generated by peroxidase-positive cytoplasmic granules in nearby astrocytes (8). The high sulphydryl content previously demonstrated in peroxidase-positive astrocytes (21) may serve as buffer for estradiol-induced free radicals and thus could confer protection to the glia (8). The reasons for the selective vulnerability of beta-endorphin neurons to estradiol-mediated cell death are unknown but may be due to a relative paucity of protective antioxidant enzymes within these neurons or to the close proximity of these cells with peroxidase positive astrocytes.

Evidence from our laboratory indicates that the EV-induced degeneration of the beta-endorphin system, with consequent reduction in beta-endorphin input to the medial preoptic area (MPOA), evokes a compensatory upregulation of mu opioid binding sites in the MPOA, rendering GnRH neurons supersensitive to the inhibitory action of residual endogenous opioids (22, 23). The resultant persistent inhibition of endogenous GnRH release would thus account for the unique, chronically suppressed episodic plasma LH pattern to which the ovaries respond by becoming polycystic (24). The acyclic polycystic ovaries are thus the end-product of a cascade of defects originating with the EV-induced arcuate lesion. Since polycystic ovaries are the ultimate consequence of the EV-induced hypothalamic lesion, the appearance of cyclic, relatively normal ovaries in the EV-treated animals that had received alpha-tocopherol supports the interpretation that the EV-induced pathology represents free radical mediated destruction of beta-endorphin hypothalamic neurons.

In summary, this study supports the hypothesis that free radical induced lipid peroxidation is involved in the degeneration of beta-endorphin neurons following EV treatment and possibly also in reported age-associated reductions in beta-endorphin concentrations.

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