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Effects of progestins on the estradiol-related accumulation of astrocytic granules in the hypothalamic arcuate nucleus

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Adult female rats treated with a single 2 mg injection of estradiol valerate (EV) develop anovulatory sterility (persistent estrus) and neuropathologic changes in the hypothalamic arcuate nucleus. A prominent feature of the latter is the accumulation of peroxidase-positive cytoplasmic inclusions in arcuate astrocytes which results from aberrant patterns of chronic, ovarian E_2 secretion in this model. In the present study, we tested an hypothesis that progesterone may antagonize the pathologic effects of E_2 within the medial basal hypothalamus analogous to effects in peripheral steroid target tissues. In intact EV-treated rats, we observed complete suppression of the astrocytic reaction by subsequent injections of medroxyprogesterone acetate (MPA). However, chronic progesterone exposure did not significantly antagonize this estrogenic affect in ovariectomized rats implanted subcutaneously with steroid-releasing Silastic capsules. Taken together, these results suggest that progestins may block the development of an E_2-related arcuate lesion by suppressing the pituitary-ovarian axis rather than by directly antagonizing the dystrophic effects of E_2 at the hypothalamic level.

Adult female rats treated with a single 2 mg injection of estradiol valerate (EV) develop a persistent estrus (PE) syndrome characterized by anovulation, polycystic ovaries, persistent vaginal cornification, and discrete neuropathologic changes in the hypothalamic arcuate nucleus[1,2]. The latter consist of axonal and dendritic degeneration with attendant microglial and astrocytic reactions[1]. In EV- and constant light-induced PE and in senile PE, the neuropathologic changes are progressive and dependent upon chronic exposure to ovarian estradiol (E_2)[2,14].

Identical pathologic changes occur in the arcuate nucleus of gonadectomized animals chronically estrogenized by means of E_2-containing subcutaneous implants, confirming that the lesion is generated by prolonged E_2 exposure. Interestingly, co-exposure to 5α-reduced androgen completely blocks the pathologic effects of the E_2[3]. Since progesterone antagonizes the action of E_2 in a variety of steroid target tissues[7], and given that progesterone (unlike 5α-reduced androgen) is present in relatively high serum concentrations in the female rat[5], the current study was undertaken to determine whether progesterone mitigates the pathogenic action of estradiol within the hypothalamic arcuate nucleus.

Treatment of animals. Six-week-old (130–200 g) sexually mature female Wistar rats were obtained from Canadian Breeding Farms and Laboratories. The animals were kept on regular 12-h light:12-h dark cycles. Food and water were given ad libitum. Vaginal smears were obtained from female rats for 2 weeks prior to treatment in order to verify normal vaginal cyclicity. Following the initiation of treatment, vaginal cytology was consistently recorded between 8:00 and 11:00 h. 5 days per week in all non-ovariectomized rats up until and including the day of perfusion.

Steroid injection protocol. Group (i): 4 normally-cycling control rats were treated weekly with steroid vehicle over a 4 month period up until the time of perfusion.

Group (ii): 5 normally-cycling rats were given a single intramuscular injection of 2 mg EV (Delestrogen, Squibb, Princeton, NJ) in 0.2 ml sesame oil. All 5 animals developed the PE condition and were perfused 4 months after treatment.

Group (iii): 3 animals were treated with EV as per group (ii). Following the EV injection, they received a loading dose of 50 mg MPA (Depo-Provera), half of which was given intramuscularly and half intraperitoneally. They subsequently received 11 weekly maintenance doses of 25 mg MPA i.m. The animals were perfused one week after the last injection (approx. 4 months after the administration of the loading dose. This progesterone

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regimen was employed to suppress luteinizing hormone (LH) and endogenous estrogen secretion[4].

Group (iv): 3 animals were treated with the MPA regimen only and perfused 4 months after the initiation of treatment.

Steroid implantation protocol. Normally-cycling female rats, were ovariectomized under ether anesthesia at 6 weeks of age. One and a half weeks after ovariectomy, all animals were again anesthetized with ether and implanted subcutaneously with Silastic capsules.

Group (v): 10 animals received a single capsule (Silastic medical grade tubing, 0.078 inches i.d. × 0.125 inches o.d.) filled with crystalline estradiol-17β (E_2). These capsules maintain plasma estradiol levels in the high physiologic range[3].

Group (vi): 10 animals received a single 3-cm long capsule (0.132 inches i.d. × 0.183 inches o.d.) containing crystalline progesterone (P). These implants maintain plasma progesterone at diestrus levels of about 30 ng/ml[5,9].

Group (vii): 10 animals received both the estradiol and progesterone implants (E_2P).

Group (viii): 10 animals received empty Silastic implants and served as controls (C). After 2.5 months, P implants were removed from all animals that contained them and E_2 capsules were inserted into those which did not initially receive E_2, including controls. Thus, each of the 40 animals now carried only one E_2 implant. The animals were kept for an additional two-week period up to the time of perfusion in order to allow plasma E_2 levels to equilibrate and P to clear. This standardization of circulating steroid levels among the groups ensured that any neurohistological changes observed reflected differences in *chronic* steroid hormone exposure and not the potential effects of varying gonadal hormone levels at the time of perfusion.

Tissue preparation. All animals were anesthetized with chloral hydrate and perfused transcardially with cold 1% paraformaldehyde-1% glutaraldehyde and 0.5 mg% calcium chloride buffered to pH 7.4 with 0.12 M phosphate buffer, as previously described[2]. Ovarian histology was assessed by dissection microscopy in all non-castrated animals at the time of perfusion. After overnight immersion of heads in cold fixative, brains were removed and small blocks containing the medial basal hypothalamus were excized, dehydrated, and embedded in Epon resin as previously described[2]. One-μm-thick coronal sections were cut through mid-regions of the arcuate nucleus (identified on the basis of previously published anatomical criteria[2]) with an ultramicrotome and stained with Toluidine blue for light microscopy. Astrocytes in the arcuate nucleus were identified as small, pale cells with euchromatic, ellipsoidal nuclei[1]. Astrocyte granules

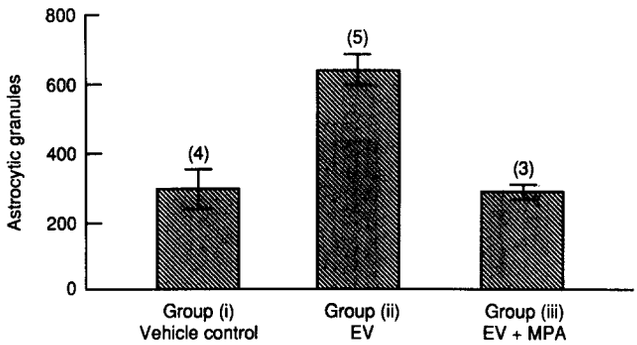

Fig. 1. Numbers of Toluidine blue-positive astrocyte granules in the arcuate nuclei of control and steroid-treated female rats. Group (ii) received a single injection of 2 mg estradiol valerate. Group (iii) received multiple injections of medroxyprogesterone acetate (see text for protocol) following a single injection of 2 mg estradiol valerate. Group (i) receive steroid vehicle only. Injections of MPA completely inhibit the EV-related accumulation of astrocytic inclusions. (n = number of animals per group).

which are chrome alum hematoxylin-positive and diaminobenzidine (peroxidase)-positive exhibit characteristic yellow-green metachromasia in Toluidine blue-stained sections and can be readily quantitated under light microscopy[2,3,13].

Morphology. Total numbers of Toluidine blue-stained astrocytic granules were counted and summed over 4 hemi-fields of arcuate nucleus per animal (see Fig. 1 in ref. 15). For each arcuate hemi-field, an area of 0.137 mm^2 was surveyed at 1000× magnification using a Zeiss photomicroscope with an ocular grid. Thus, numbers of astrocytic granules was quantitated over a total area of 0.548 mm^2 of arcuate nucleus per animal. Mean numbers of astrocyte granules were determined for each group.

All data were analyzed by the Mann–Whitney test for non-parametric statistics.

Reproductive profiles. Animals treated with EV only (group (ii)) developed the PE state within 4–5 weeks as defined by the presence of continuous vaginal cornification. Dissection microscopy confirmed the presence of aluteal, polycystic ovaries in all the PE rats. Polycystic ovaries were not observed in groups (i) and (iii) which exhibited normal estrous cyclicity and persistent diestrus (leukocytic) vaginal smears respectively.

Steroid injection experiments (Fig. 1). Toluidine blue-positive astrocyte granules were observed in the medial basal hypothalamus of all groups examined. The detailed morphology and topography of this specialized periventricular glial system have been previously reported[1,10].

EV-induced PE (group (ii)) was associated with a significant increase in numbers of astrocytic inclusions in the arcuate nucleus in comparison with normally-cycling controls (group (i); $P < 0.05$). Rats in group (iii), however, exhibited significantly fewer astrocytic inclusions in comparison with group (ii) ($P < 0.05$). Mean

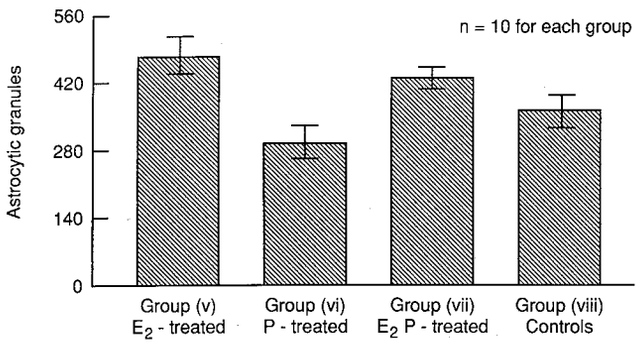

Fig. 2. Numbers of astrocytic granules in the arcuate nuclei of ovariectomized rats implanted with Silastic capsules releasing E_2 (group v), progesterone (group vi), E_2 and progesterone (group vii) or no steroid hormone (group viii). Progresterone does not significantly attenuate the E_2-related accumulation of glial granules in castrated animals.

astrocytic granule counts in group (iii) were within the range of vehicle-treated, normally-cycling control (group (i); $P > 0.05$) and animals receiving only MPA (group (iv), data not shown).

Steroid implantation experiments (Fig. 2). The astrocytic granule counts in the E_2 group were significantly greater than that observed in blank-implanted controls (C, $P < 0.05$) and in the P group ($P < 0.005$). The P group showed significantly less astrocytic activity than either the E_2 group ($P < 0.005$) or the E_2P group ($P < 0.01$), but did not differ significantly from controls ($P > 0.05$). As in the intact animals, progesterone inhibited the E_2-related increase in pituitary weights attesting to the biologic potencies of the steroid preparations (data not shown).

Rats rendered anovulatory with a single injection of 2 mg EV exhibit axonal and dendritic damage, a microglial response, and an accumulation of cytoplasmic granules in the hypothalamic arcuate nucleus[1]. We previously demonstrated that the neuroglial reactions characteristic of the EV-treated[2], constant light-exposed[2] and senile[14] PE rat are completely abrogated by prior ovariectomy. Moreover, Silastic implants producing tonic, physiological levels of unconjugated E_2, but not testosterone or 5α-dihydrotestosterone, elicit robust glial reactions in the arcuate nuclei of ovariectomized female rat[3]. These observations indicated that aberrant patterns of ovarian E_2 secretion characteristic of the PE state were responsible for the progressive development of arcuate neuropathology in this condition. The astrocytic inclusions which accumulate as a result of this unremitting estrogenic stimulus, stain intensely with diaminobenzidine, a marker for endogenous peroxidase activity[15]. We postulated that the latter may, in turn, catalyze the oxidation of E_2 to catecholestradiol and semiquinone radicals which may injure gonadotropin-regulating circuitry in this region of the brain[15].

In the present study, multiple injections of MPA completely prevented the estrogen-related accumulation of astrocytic granules in intact animals treated with EV. This finding raises the possibility that in normal female rats circulating progesterone may attenuate the neurodegenerative effects of E_2 within the medial basal hypothalamus. The attenuation of E_2-related acyclicity by progesterone[11] and the salutory effects of progesterone administration or pregnancy on the preservation of estrus cyclicity in aging female rodents[6,8,12] are consistent with this hypothesis. However, progesterone exposure did not block the E_2-induced accumulation of astrocytic granules in castrated animals implanted with E_2- and P-releasing Silastic capsules. It is possible that an inadequate progesterone dose was responsible for its failure to suppress the E_2-induced astrogliosis. This regimen did, however, prevent E_2-related pituitary enlargement attesting to the biological potency of P in this model. The possibility remains that in the ovariectomized animals, relatively rapid induction of astrocytic granules by E_2 administered to all animals for 2 weeks prior to perfusion (the standardization procedure) may have masked the inhibitory effects of progesterone in this system. The most plausible interpretation of the data is that unlike 5α-reduced androgens[3], P does not directly antagonize estradiol induction of astrocytic granules in the arcuate nucleus. Taken together, our findings suggest that in intact animals receiving both EV and MPA injections, astrocytic reactions in the arcuate nucleus fail to occur as an indirect effect of pituitary-ovarian (and hence endogenous E_2) suppression by the exogenous progestin. In this regard, MPA suppression mimics surgical ovariectomy which completely protects against hypothalamic damage resulting from EV or constant light exposure[2]. Ultrastructural evaluations of the arcuate neuropil will still be required to determine whether progestational steroids exert more subtle influences on E_2-related axonal and dendritic damage independent of astrocytic activity.

1 Brawer, J.R., Naftolin, F., Martin, J. and Sonnenschein, C., Effects of a single injection of estradiol valerate on the hypothalamic arcuate nucleus and on reproductive function in the female rat, *Endocrinology*, 103 (1978) 501–512.

2 Brawer, J., Schipper, H. and Naftolin, F., Ovary-dependent degeneration in the hypothalamic arcuate nucleus, *Endocrinology*, 107 (1980) 274–279.

3 Brawer, J., Schipper, H. and Robaire, B., Effects of long-term androgen and estradiol exposure on the hypothalamus, *Endocrinology*, 112 (1983) 194–199.

4 Brown-Grant, K., Corker, C. and Naftolin, F., Plasma and pituitary luteinizing hormone concentrations and peripheral plasma oestradiol concentration during early pregnancy and after the administration of progestational steroids in the rat, *J.*

Endocrinol., 53 (1972) 31–35.
5 Butcher, R., Collins, W. and Fugo, N., Plasma concentration of LH, FSH, prolactin, progesterone, and estradiol 17β, throughout the 4-day estrous cycle of the rat, *Endocrinology*, 94 (1974) 1704–1708.
6 Clemens, J., Amenonori, Y., Jenkins, T. and Meites, J., Effects of hypothalamic stimulation, hormones, and drugs on ovarian function in old female rat, *Proc. Soc. Exp. Biol. Med.*, 132 (1969) 561–563.
7 DiCarlo, F., Reboani, C., Conti, G., Portaleone, P., Viano, I. and Genazzani, E., Changes in estrogen receptor levels induced by pharmacological agents. In E. Genazzani, F. DiCarlo and W. Mainwaring (Eds.), *Pharmacological Modulation of Steroid Acion*, Raven Press, New York, 1980, pp. 61–74.
8 Flurkey, K., Randall, P., Sinha, Y., Erminu, M. and Finch, C., Transient shortening of estrous cycles in aging C57BL/6J mice: effects of spontaneous pseudopregnancy progesterone, L-dihydroxyphenylalanine and hydergine, *Biol. Reprod.*, 36 (1987) 949–959.
9 Goodman, R., A quantitative analysis of the physiologic role of estradiol and progesterone in control of tonic and surge secretion of LH in the rat, *Endocrinology*, 102 (1978) 142–150.
10 Keefer, D.A. and Christ, J.F., Distribution of endogenous diaminobenzidine staining cells in the normal rat brain, *Brain Research*, 116 (1976) 312–316.
11 Kohama, S., Anderson, C. and Finch, C., Progesterone implants extend the capacity for 4-day estrous cycles in aging C57BL/6J mice and protect against acyclicity induced by estradiol, *Biol. Reprod.*, 41 (1989) 233–240.
12 Matt, D., Jarver, P. and Lu, J., Relation of parity and estrous cyclicity to the biology of pregnancy in aging female rats, *Biol. Reprod.*, 37 (1987) 421–430.
13 Schipper, H., *Pathologic Effects of Estradiol on the Hypothalamic Arcuate Nucleus*, Ph.D. Thesis, McGill University, Montreal, 1982.
14 Schipper, H., Brawer, J.R., Nelson, J.F., Felicio, L.S. and Finch, C.E., Role of the gonads in the histologic aging of the hypothalamic arcuate nucleus, *Biol. Reprod.*, 25 (1981) 413–419.
15 Schipper, H.M., Lechan, R.M. and Reichlin, S., Glial peroxidase activity in the hypothalamic arcuate nucleus: effects of estradiol valerate-induced persistent estrus, *Brain Research*, 507 (1990) 200–207.