ICI 182,780 antagonizes the effects of estradiol on estrous behavior and energy balance in Syrian hamsters

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Wade, George N., J. Bradley Powers, Jeffrey D. Blaustein, and Deborah E. Green. ICI 182,780 antagonizes the effects of estradiol on estrous behavior and energy balance in Syrian hamsters. *Am. J. Physiol.* 265 (*Regulatory Integrative Comp. Physiol.* 34): R1399–R1403, 1993.—Three experiments examined the effects of ICI 182,780, a steroidal "pure" antiestrogen that is thought to be active peripherally but not in the brain when given systemically, on energy balance, estrous behavior, and in vivo cell nuclear binding of [^{3}H]estradiol in Syrian hamsters. Pretreatment with ICI 182,780 reduced in vivo uptake of [^{3}H]estradiol in uterus but not in pooled hypothalamus-preoptic area. Ovariectomized Syrian hamsters were treated with estradiol benzoate (EB, 5 μg/day), ICI 182,780 (250 μg/day), or both EB and ICI 182,780 for 4 wk. Estradiol treatment caused significant decreases in food intake, body weight and fat content, and linear growth. Given alone, ICI 182,780 had no effect on these measures. When they were given concurrently, ICI 182,780 attenuated the effects of estradiol on body weight, growth, and fat content but not on food intake. Treatment with ICI 182,780 significantly diminished estrous behavior induced with either EB plus progesterone or with EB alone. These findings support the hypothesis that, in addition to its actions in the brain, estradiol acts peripherally to modulate estrous behavior and energy balance.

body weight; body composition; uterus; food intake; estrogen receptors

IT IS CLEAR THAT OVARIAN steroids can act directly in the brain to affect a wide variety of behaviors and physiological functions, including social behaviors, regulatory behaviors, and energy balance (2, 4, 21, 23, 26). For example, appropriately placed intracerebral implants of estradiol facilitate estrous behavior, decrease food intake, or stimulate voluntary exercise in ovariectomized rats (6, 18, 27); lesions of these neural loci prevent the respective behavioral changes in response to systemic estradiol treatment (7, 9, 12).

In addition to these central actions, several lines of evidence support the hypothesis that estradiol can act on nonneural peripheral tissues to affect behaviors and energy balance in rats (22, 23, 26). The fact that the antiestrogen ICI 182,780 attenuates the effects of estradiol on energy balance and estrous behavior in ovariectomized rats is consistent with the existence of peripheral sites of action (22). ICI 182,780 differs from other antiestrogens in that it is highly potent peripherally but it does not appear to be active in the brain when it is administered systemically (22, 28, 29).

The idea that estradiol acts both centrally and peripherally to affect behaviors and energy balance has not been explored in species other than rats. The present experiments use ICI 182,780 to investigate this possibility in Syrian hamsters. Hamsters are of interest for several reasons. First, it is known that estradiol can act directly in the brain to facilitate sexual receptivity and affect other social behaviors (21), but the possibility of peripheral sites of action has not been tested. Second, it has been suggested that estradiol can act peripherally to alter lipid metabolism and energy balance in hamsters (3), but the evidence for this notion is rather indirect. Third, hamsters and rats differ in some of their responses to antiestrogens. The older nonsteroidal antiestrogens such as MER-25, CI-628, and tamoxifen inhibit steroid-induced estrous behavior in both species (13, 14, 17, 25). However, for regulation of energy balance these compounds are full estrogen agonists in rats, whereas they act as antagonists in hamsters (24, 25). Thus the newer, steroidal antiestrogens such as ICI 182,780 may have different effects in rats and hamsters.

METHODS

Animals and Housing

Adult, female Syrian hamsters (*Mesocricetus auratus*; initial body wt 90–110 g) of the Lak:LVG strain were obtained from Charles River Breeding Laboratories (Wilmington, MA). Hamsters were housed in wire-bottom stainless steel cages (17.5 × 17.5 × 25 cm) and given tap water and Purina Laboratory Rodent Chow (no. 5001) ad libitum. Food pellets were placed on the cage floor. A 14:10-h light-dark cycle was maintained (lights on at 0700 h), and room temperature was kept at 22 ± 2°C. After 1 wk of acclimation to the laboratory, animals were ovariectomized via bilateral flank incisions under pentobarbital sodium anesthesia (80 mg/kg; Sigma Chemical, St. Louis, MO).

Procedures

In vivo binding of [^{3}H]estradiol. Three weeks after ovariectomy, hamsters were given three daily injections of sesame oil ($n = 6$) or 250 μg ICI 182,780 ($n = 6$). One hour after the third injection, animals were injected intraperitoneally with 60 μCi [^{3}H]estradiol (sp act 103 Ci/mmol, New England Nuclear, Boston, MA). One hour after injection of [^{3}H]estradiol, hamsters were anesthetized with pentobarbital sodium (50 mg); a blood sample was taken via cardiac puncture with a heparinized syringe and then centrifuged. Hamsters were then perfused with cold saline (0.15 M). The hypothalamus-preoptic area and uterus were rapidly dissected. Tissues were homogenized, and a cell nuclear fraction was purified by a modification (8) of the method of Zigmond and McEwen (30). Radioactivity was extracted from the purified cell nuclei with 3 × 4 ml toluene-based scintillation fluid. A 100-μl aliquot of plasma was transferred to a scintillation vial containing 12 ml of scintillation fluid and shaken vigorously. Radioactivity was counted at an efficiency of ~45%, and counts were corrected for quenching by automatic external standardization. Protein in cell nuclear samples was precipitated with ethanol, dissolved in 0.3 N KOH, and estimated by the method of Bradford (5). Tissue cell nuclear concentrations of radioactivity are expressed as tissue to plasma ratios (i.e., disintegrations per minute per milligram tissue protein divided by disintegrations per minute per microliter blood plasma).

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Energy balance. Three weeks after ovariectomy, baseline food intake (pouching and spillage accounted for) and body weight were measured to the nearest 0.1 g. After 1 wk of data collection, animals were divided into four groups matched for baseline food intake and body weight. The groups were given daily subcutaneous injections of 0.1 ml sesame oil vehicle containing 2.5% ethanol ($n = 11$), 5 μg estradiol benzoate (EB, $n = 10$), 250 μg ICI 182,780 ($n = 11$), or 5 μg EB plus 250 μg ICI 182,780 ($n = 10$) for 4 wk. Body weight and food intake were measured twice a week. On the first and last days of injections, hamsters were anesthetized with pentobarbital sodium (80 mg/kg body wt), and naso-anal length was measured to the nearest millimeter with calipers while the animals were stretched with a constant 100-g weight (24). The difference between the two lengths is an index of linear growth during hormone treatment.

At the end of the experiment all animals were killed with an overdose of pentobarbital sodium (50 mg). Parametrial and retroperitoneal fat pads and uteri were removed and weighed. Eviscerated carcasses were dried to a constant weight at 70°C, and carcass lipid was estimated from carcass water content. In hamsters, percent carcass water and percent carcass lipid are highly correlated ($r = 0.98$; %lipid $= -1.32 \times$ %water $+ 96.54$; $n = 289$; Wade, unpublished data). Fat-free dry weight was calculated as the total eviscerated carcass weight less water and estimated lipid content.

Estrous behavior. The animals that were treated with EB, ICI 182,780, or EB plus ICI 182,780 (above) were tested for estrous behavior twice, once with and once without progesterone treatment. On the 3rd day of estrogen and/or antiestrogen treatment, all animals were given a subcutaneous injection of 200 μg progesterone in 0.1 ml sesame oil at 0900 h and tested for estrous behavior 5 to 6 h later. The second test, without progesterone, took place on the 23rd day of estrogen and/or antiestrogen treatment. For both tests, females were adapted to a small Plexiglas arena (30 × 36 × 30 cm) for 5 min. Sexual receptivity tests were begun by introducing a sexually active male to the arena. In addition, the female's flanks and perigenital region were continuously stimulated using an eyelid brush. Tests lasted for 180 s; the latency to display lordosis and the total time that the lordosis posture was maintained were recorded. Tests were conducted 6-8 h after lights-on.

Data Analyses

Data were analyzed by *t* tests and by one- or two-way analyses of variance followed by Newman-Keuls post hoc tests where appropriate. Differences were considered statistically significant when $P < 0.05$ for two tails. Data are expressed as means ± SE.

RESULTS

In Vivo Binding of [^{3}H]Estradiol

Pretreatment with ICI 182,780 significantly reduced in vivo cell nuclear binding of [^{3}H]estradiol in uterus but not in pooled hypothalamus-preoptic area (Fig. 1), similar to findings with rats (22). However, uterine [^{3}H]-estradiol binding was reduced by only ~50% in hamsters compared with ~90% in rats (22).

Energy Balance

EB treatment significantly reduced food intake and body weight gain (Fig. 2). ICI 182,780, given alone, had no effect on body weight, but it significantly attenuated the weight-reducing actions of EB. Given alone or in combination with EB, ICI 182,780 had no effect on food intake (Fig. 2).

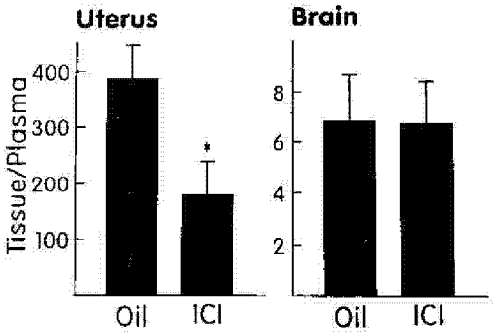

Fig. 1. Effects of ICI 182,780 (ICI) on in vivo uptake of [^{3}H]estradiol by cell nuclei in uterus and hypothalamus-preoptic area in ovariectomized hamsters. Animals were treated with sesame oil vehicle (0.1 ml) or ICI (250 μg) 48, 24, and 1 h before injection of [^{3}H]estradiol. Data are expressed as tissue to plasma ratios, that is, disintegrations per minute per milligram cell nuclear protein divided by disintegrations per minute per microliter plasma. * $P < 0.05$ vs oil-treated group.

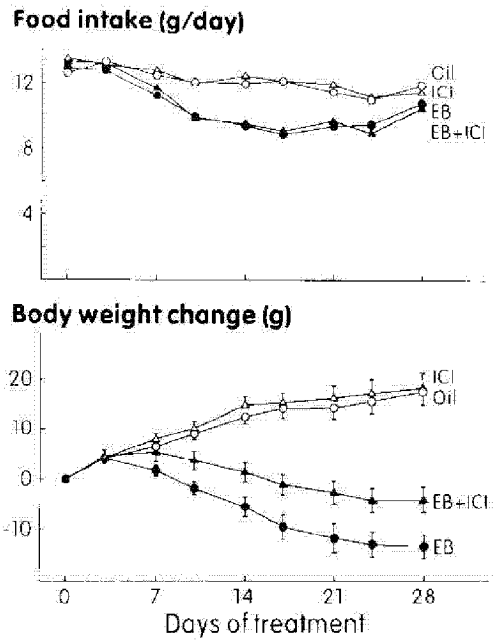

Fig. 2. Body weight gain and food intake of ovariectomized hamsters treated with sesame oil vehicle (0.1 ml), estradiol benzoate (EB, 5 μg/day), ICI (250 μg/day), or EB plus ICI for 4 wk. (Initial mean group body weights 134.2–135.9 g.)

Treatment with EB caused significant reductions in carcass water and lipid but not in fat-free dry weight (Fig. 3). Once again, ICI 182,780 alone had no effect, but it antagonized the effects of EB on carcass lipid content. The changes in body fat content were reflected in the weights of individual fat pads (Fig. 4). EB also caused a significant decrease in linear growth (change in nasoanal length) that was prevented by concurrent treatment with ICI 182,780 (Fig. 4). Administration of ICI 182,780 alone did not affect growth.

EB treatment caused a significant increase in uterine weight. As in other species (22, 28, 29), ICI 182,780 had no uterotrophic effect, and at this dose, the antiestrogen partially antagonized the effect of EB on uterine weight (Fig. 4).

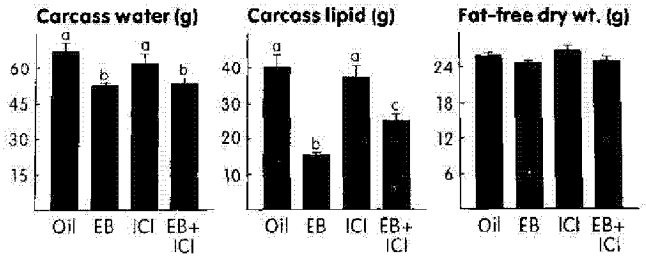

Fig. 3. Terminal carcass composition of ovariectomized hamsters treated with sesame oil vehicle (0.1 ml), EB (5 μg/day), ICI (250 μg/day), or EB plus ICI for 4 wk. Bars with different letters are significantly different ($P < 0.05$).

Estrous Behavior

Treatment with ICI 182,780, given alone or in combination with progesterone, did not induce any signs of sexual receptivity in ovariectomized hamsters (data not shown). However, ICI 182,780 did inhibit estrous behavior induced by treatment with either EB plus progesterone or EB alone. ICI 182,780 significantly increased lordosis latency and decreased lordosis duration in hamsters treated with EB for 2 days followed by progesterone (Fig. 5, *top*). In hamsters given EB for 23 days, concurrent treatment with ICI 182,780 significantly decreased lordosis duration but did not affect latency (Fig. 5, *bottom*).

DISCUSSION

These findings confirm a number of the unusual properties of ICI 182,780. As in other species (22, 28, 29), ICI 182,780 appears to be a pure antiestrogen in Syrian hamsters. It at least partially antagonized the actions of estradiol on body weight, body composition, linear growth, estrous behavior, and uterine weight without having any agonistic (estrogenic) effects when given by itself to ovariectomized animals. The present results also support the conjecture that ICI 182,780 does not act directly in the brain. As in rats (22), pretreatment with radioinert ICI 182,780, significantly reduced in vivo cell nuclear binding of [^{3}H]estradiol in uterus but not in pooled hypothalamus-preoptic area. Taken together, these two findings suggest that ICI 182,780 can be used as an experimental tool to dissociate central and peripheral actions of estradiol.

The fact that ICI 182,780 attenuated the effects of estradiol treatment on body weight and fat content is consistent with our assertion (23, 26) that ovarian steroids act both centrally and peripherally to affect energy balance. Several lines of work support the idea of distinct central and peripheral sites of estrogen action on energy balance in rats (22, 23, 26), but this appears to be the first evidence for this possibility in hamsters.

The fact that treatment with ICI 182,780 completely blocked the effects of estradiol on linear growth (change in naso-anal length) may indicate that estrogen effects on growth are predominantly due to nonneural actions of the steroid in hamsters. On the other hand, treatment with ICI 182,780 did not attenuate the suppressive effects of estradiol on food intake in hamsters. Thus it is likely that estradiol action in the brain is sufficient to decrease food intake and that no peripheral actions are required. However, we cannot exclude the possibility that a higher dose of ICI 182,780 than that used in this study would be sufficient to antagonize the effects of estradiol on food intake in hamsters.

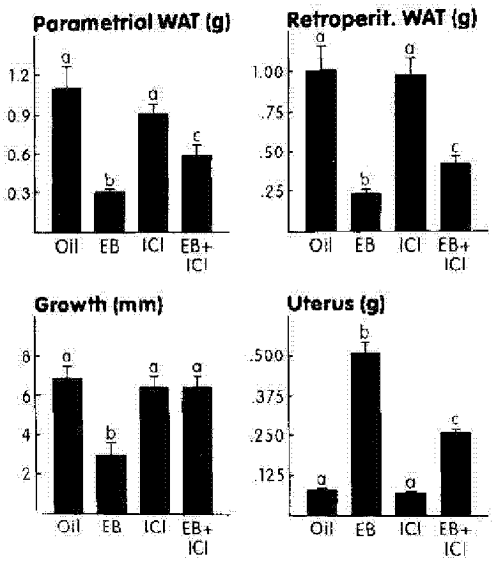

Fig. 4. Terminal weights of parametrial and retroperitoneal white adipose tissue (WAT) and uterus and change in naso-anal length (growth) of ovariectomized hamsters treated with sesame oil vehicle (0.1 ml), EB (5 μg/day), ICI (250 μg/day), or EB plus ICI for 4 wk. Bars with different letters are significantly different ($P < 0.05$).

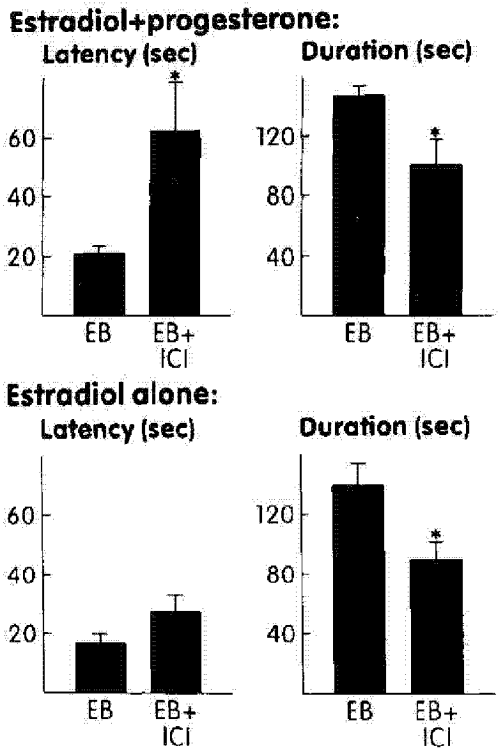

Fig. 5. Effects of ICI on estrous behavior in ovariectomized hamsters. *Top*: animals were injected with EB (5 μg) or EB plus ICI (250 μg) for 2 days followed by 200 μg progesterone on 3rd day, 4–5 h before testing. *Bottom*: animals were injected with EB or EB plus ICI for 23 days before testing. * $P < 0.05$ vs. EB-treated group.

These findings in hamsters contrast with those in rats where ICI 182,780 treatment actually potentiated the estradiol-induced decreases in food intake (22). We suggested that in rats this action of ICI 182,780 could be due to the induction of a "pharmacological hysterectomy," because surgical hysterectomy enhances behavioral responsiveness to estradiol in this species (1, 20). In contrast, surgical hysterectomy does not potentiate estradiol-induced sexual receptivity in hamsters (19), just as ICI 182,780 treatment does not potentiate the estradiol-induced decrease in food intake. Therefore, the difference between rats and hamsters in the effects of ICI 182,780 on food intake may be a reflection of the species difference in the actions of the uterus on behavioral responsiveness to estradiol.

ICI 182,780 attenuated the induction of estrous behavior, either by sequential treatments with estradiol and progesterone or by prolonged (23 days) treatment with estradiol alone. Thus, in hamsters, as in rats (22), estradiol appears to act peripherally, as well as centrally (2, 4, 21), to facilitate estrous behavior. We have suggested that in rats, ICI 182,780 could attenuate sexual receptivity at least in part by blocking the effects of estradiol on peripheral sensory fields that are important for estrous responsiveness (10, 11). Similar work examining the effects of ovarian steroids on peripheral sensory fields has not yet been done in hamsters, but it is known that somatosensory cues from the perineal region play a significant role in hamster copulatory behavior (15, 16).

To the extent that comparisons are possible, ICI 182,780 may be more effective at inhibiting estrous behavior in hamsters than in rats (22). If this is in fact the case, the uterus might play a role in this species difference, because surgical hysterectomy potentiates the effects of estradiol on sexual receptivity in rats but not in hamsters (1, 19). Thus in rats ICI 182,780 could concurrently inhibit estrous behavior (perhaps by inhibiting an action of estradiol on peripheral sensory fields) and facilitate estrous behavior (via a pharmacological hysterectomy facilitating estrogen action in the brain). According to this hypothesis, only the inhibitory actions would be evident in hamsters.

It appears as though the dose of ICI 182,780 we have used (250 μg/day) is more effective in reducing uterine weight and in vivo cell nuclear uptake of [^{3}H]estradiol in rats than in hamsters (22). At this time it is not clear whether this effect is due to *1*) species differences in ICI 182,780 absorption, delivery, or clearance; *2*) differences in affinity for estrogen receptors; or *3*) some other factor(s). Differences in the EB doses that were used (2 μg in rats, 5 μg in hamsters) could explain the differences in the inhibition of uterine weight but cannot account for the differences in inhibition of [^{3}H]estradiol binding. Whatever the basis for this species difference in ICI 182,780 potency in the uterus, the important point remains that in both rats and hamsters treatment with ICI 182,780 attenuates the effects of estradiol on energy balance and on estrous behavior without interfering with neural estrogen binding. Therefore, these results support the hypothesis that estradiol affects energy balance and estrous behavior via both neural and nonneural sites of action.

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