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Antidepressant effects of ERβ-selective estrogen receptor modulators in the forced swim test

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Abstract

Estradiol (E_2) may influence depressive symptomology of women and decrease depressive behavior among rodents. The mechanism(s) for E_2's antidepressant effects are not well understood. To determine whether antidepressant effects of E_2 may involve actions at intracellular estrogen receptor (ER) α or β isoforms, selective ER modulators (SERMs) were administered (10 μg sc) to ovariectomized rats 48 h before testing in the forced swim test, an animal model of depression, and the horizontal crossing task. Rats received sesame oil vehicle, 17β-E_2, which has a high affinity for ERα and ERβ, SERMs that vary in their activity at ERα and β, or a tricyclic antidepressant (desipramine; 30 mg/kg ip), as a positive control. ERα-selective SERMs were propyl pyrazole triol (PPT) and 17α-E_2. PPT has more selective effects at ERα than does 17α-E_2, which also binds ERβ. ERβ-selective SERMs were diarylpropionitrile (DPN) and 7,12-dihydrocoumestan (coumestrol). DPN is more selective at ERβ than coumestrol, which also binds ERα. 17β-E_2, ERβ-selective SERMs (DPN, coumestrol), and desipramine administration produced antidepressive behavior (decreased immobility, increased struggling and swimming). ERα-selective SERMs (PPT, 17α-E_2) were not different from vehicle. There were no differences among groups in the number of beam breaks made in the horizontal crossing task. These data suggest that E_2's antidepressive effects may involve actions at ERβ.

Keywords: Estradiol; Estrogen receptor; ERα; Affect; Sex differences

1. Introduction

The ovarian hormone, 17β-estradiol (E_2), may influence incidence of, or negative symptomology associated with, depression. Women, compared to men, are twice as likely to experience depression and have longer and more recurrent depressive episodes (Earls, 1987; Nolen-Hoeksema and Ahrens, 2002). Gender differences in depression emerge at puberty (Kessler and Walters, 1998; Lewinsohn et al., 1998; Weissman and Olfson, 1995). Perimenopausal women with depression that receive E_2 replacement have improved mood (Cohen et al., 2003; Schmidt et al., 2000; Soares et al., 2001). In addition, depression scores of postmenopausal women who are not depressed and are taking E_2-replacement therapy are lower than age-matched women that are not on E_2 (Miller et al., 2002). Although these data suggest that E_2 may influence depression, E_2's effects on, and mechanisms for, depression are not well understood.

Among rodents, there is evidence for antidepressive effects of E_2. Proestrous rats, which typically have higher E_2, progestin, and androgen levels than do diestrous or male rats, demonstrate decreased depressive behavior in the forced swim test (less immobility; Frye and Walf, 2002; Frye and Wawrzycki, 2003). Removal of the primary source of E_2, progestins, and androgens [ovariectomy (ovx)] increases depressive behavior of female rats and mice and E_2 replacement reverses this effect (Bernardi et al., 1989; Estrada-Camarena et al., 2003; Frye and Wawrzycki, 2003; Hilakivi-Clarke, 1996; Okada et al., 1997; Rachman et al., 1998; Walf and Frye, submitted for publication). Thus, endogenous increases in, and exogenous replacement of, E_2 may mediate antidepressive behavior of female rodents.

How E_2 has its antidepressant actions is an important question. E_2 may mediate depressive behavior via ligand-dependent actions at intracellular estrogen receptors (ERs).

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E_2's effects at ERs involve diffusion of an E_2 and sex hormone binding protein complex through the plasma membrane from the bloodstream. This complex binds to intracellular ERs, causing a conformational change. There is a dissociation of heat shock proteins, and a dimerized pair of E_2/ER then binds to the E_2 response element (ERE). Following this binding to the ERE and interaction with transcription factors, there is transcription of genes regulated by ERE that carry out cell's functional response (Etgen, 1984; Falkenstein et al., 2000; O'Malley and Means, 1974).

However, traditional actions at a single, ligand-activated transcription factor, ER, may not explain E_2's antidepressant effects. In 1996, a second form of ER, ERβ, was discovered (Kuiper et al., 1996; Tremblay et al., 1997). ERα and ERβ have distinct N-terminal regions, but share approximately 97% DNA- and approximately 60% ligand-binding domains (Tremblay et al., 1997). ERα and ERβ are encoded by different genes (Green et al., 1986; Kuiper et al., 1996), show distinct patterns of gene regulation, (Kuiper et al., 1997; Paech et al., 1997; Mitchner et al., 1998; Tena-Sempere et al., 2004), and different patterns of expression in the body and brain at different points in development (Shugrue et al., 1997). Notably, binding affinity, and ligands for, ERα and ERβ differ (Kuiper et al., 1997), which has led to the development of selective ER modulators (SERMs).

E_2 binds with a high affinity to ERα and ERβ (Kuiper et al., 1997); thus, actions at either ERα and/or ERβ may mediate its antidepressive effects. To begin to dissociate the extent to which actions at ERα and/or ERβ may underlie E_2's antidepressive effects, actions of E_2 and various SERMS and a positive control, a tricyclic antidepressant (desipramine), were compared for their effects on depressive behavior in the forced swim task and motor behavior in the horizontal crossing task. If actions at ERα or ERβ mitigate antidepressant effects, then SERMs selective for ERα or ERβ should influence behavior in the forced swim test, an animal model of depression.

2. Method

These methods were preapproved by the Institutional Animal Care and Use Committee at SUNY Albany.

2.1. Animals and housing

Female Long–Evans rats ($N=224$), approximately 55 days old, were either obtained from the breeding colony at SUNY-Albany (original stock from Taconic Farms, Germantown, NY) or were purchased from Taconic Farms. Rats were group housed (four to five per cage) in polycarbonate cages ($45 \times 24 \times 21$ cm) in a temperature-controlled room (21 ± 1 °C) in the Laboratory Animal Care Facility. The rats were maintained on a 12:12-h reversed light cycle (lights off 8:00 a.m.) with continuous access to Purina Rat Chow and tap water.

2.2. Surgery

All rats were ovx under Rompun (12 mg/kg; Bayer, Shawnee Mission, KS) and Ketaset (80 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) anesthesia 1 week prior to testing.

2.3. SERMs and desipramine

Rats were administered sesame oil vehicle, 17β-E_2 (Steraloids, Newport, RI), which has equal affinity for ERα and ERβ (Kuiper et al., 1997), or one of the following SERMs.

ERα-specific SERMs: propyl pyrazole triol (PPT; Tocris Cookson, Ellisville, MO) is a potent selective ER agonist that has 410-fold selectivity for ERα over ERβ (Stauffer et al., 2000). 17α-E_2 (Sigma Chemical, St. Louis, MO) is five times more active at ERα than ERβ (Kuiper et al., 1997).

ERβ-specific SERMs: diarylpropionitrile (DPN; Tocris Cookson) is a highly selective ERβ agonist, with 70 times greater activity at ERβ than ERα (Meyers et al., 2001). 7,12-dihydrocoumestan (coumestrol; Sigma) is a less selective ERβ agonist, with a sevenfold greater affinity for ERβ compared to ERα (Kuiper et al., 1997).

As a positive control, some rats were administered a single injection of desipramine hydrochloride, a tricyclic antidepressant (Sigma). Desipramine was freshly made on each day. Desipramine was dissolved in saline to a concentration of 30 mg/ml and was administered to rats via intraperitoneal injection.

2.4. Procedure

Ovx rats were randomly assigned to receive vehicle or 10 μg 17β-E_2, PPT, 17α-E_2, DPN, or coumestrol 48 h before behavioral testing in either the forced swim test or horizontal crossing task. This dosage and regimen for SERMs was chosen because 10 μg E_2 produces physiological levels of E_2 (Vonghер and Frye, 1999) and activation of sex behavior, 24–48 h after administration (Frye et al., 1998). Another group of ovx rats was assigned to receive an antidepressant drug regimen consisting of a single intraperitoneal injection of 30 mg/kg desipramine, as has been previously described (Kitamura et al., 2002). Effects of desipramine on depressive and motor behavior after acute administration (30 min before testing) and a regimen analogous to those used with SERMs regimen (48 h before testing) was assessed. This group of desipramine-administered rats served as a positive control in this experiment to validate the forced swim test procedure that was utilized as an animal model of depression.

2.5. Behavioral testing

Most rats were tested on two occasions approximately 1–3 weeks apart. Of this group, the majority of rats were first tested in the forced swim test and then in the horizontal crossing task on a second occasion; however, some rats in

each group were tested in the opposite order and there was no observed effect of testing order. Some rats were only tested in the forced swim test or the horizontal crossing task; there was no evidence of differences in these rats' performance compared to rats that were tested in both tasks. Forced swim test behavior was observed, and manually recorded, by one trained investigator (AAW). The number of beam breaks mechanically recorded in the horizontal crossing task was documented by one of four trained observers. All were blind to the hypothesized outcome of the experiments.

2.5.1. Forced swim task

A modified version of the forced swim test, behavior which is sensitive to antidepressant treatment, was utilized (Frye and Walf, 2002; Overstreet et al., 1995; Porsolt et al., 1977; Zangen et al., 1997). The duration rats spent struggling, swimming, and immobile in a cylindrical container filled with 30 cm of 30 °C water was recorded for 10 min. This temperature of water was utilized because it has been used by our laboratory and others in the past to assess steroids' or neurotransmitters' effects on depressive behavior (Chau et al., 2001; Frye and Walf, 2002; Frye and Wawrzycki, 2003; Rada et al., 2003) and lower temperature water, such as 25 °C water, reduces body and brain temperature and/or produces hypothermia simultaneously with the expression of immobility behavior of rodents (Abel, 1993; Arai et al., 2000; Taltavull et al., 2003). Struggling is defined as the movement of the forelimbs in rapid fashion that break the surface of the water, climbing, and/or clinging to the walls of the chamber. Swimming is defined as movement of the forelimbs and/or hindlimbs that do not break the surface of the water and/or the movement of rats while they are underwater. Immobility, an index of depressive behavior, consisted of the lack of any movements typical of struggling or swimming and only the slight, if any, movements necessary for the rat to keep its head above water (i.e., floating).

2.5.2. Horizontal crossing task

To assess effects of SERMs and desipramine on motor behavior of rats, the horizontal crossing task was used as per Frye et al. (2000). Rats were placed in a $39 \times 39 \times 30$ cm Digiscan Optical Animal Activity Monitor (Accuscan Instruments, Columbus, OH) that mechanically recorded the number of beam breaks that occurred during a 5-min period.

2.6. Statistical analyses

One-way analyses of variance (ANOVAs) were utilized to determine if there were differences among SERMs' effects on behavior in the forced swim or horizontal crossing test. The α level for statistical significance was $P < .05$. Where appropriate, Fisher's post hoc tests were used to determine group differences.

3. Results

3.1. Forced swim test (see Fig. 1 and Table 1)

17β-E_2, ERβ-selective SERMs, and desipramine (when administered both 30-min and 48-h before testing) regimen had antidepressant effects in the forced swim test compared to vehicle (Fig. 1 and Table 1). There were differences among groups in the time spent immobile

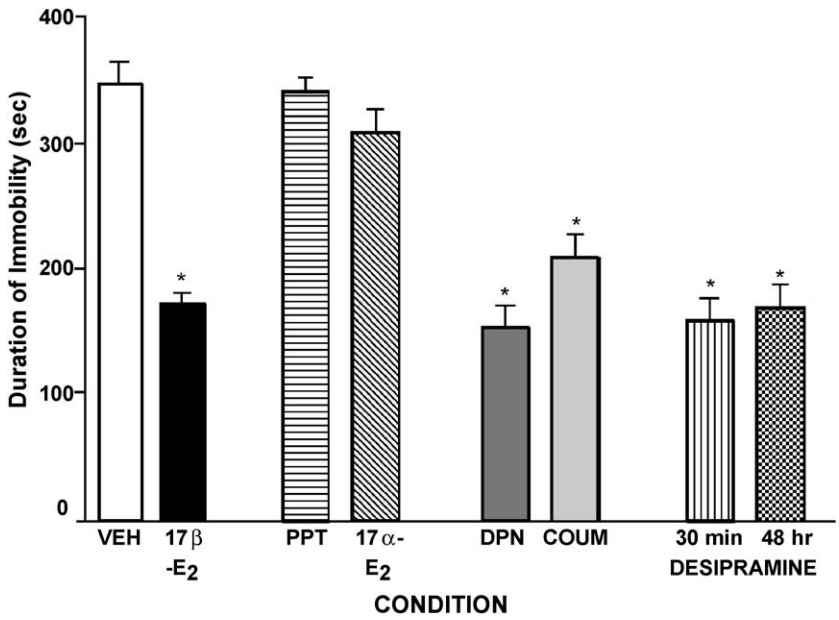

Fig. 1. The mean (± S.E.M.) duration spent immobile in the forced swim test of ovx rats administered vehicle, 17β-E_2, PPT, 17α-E_2, DPN, coumestrol (COUM), or desipramine 30 min or 48 h before testing. * Indicates a significant difference from vehicle, PPT, and 17α-E_2 ($P < .05$).

Table 1
Duration of struggling and swimming (± S.E.M.) in the forced swim test ($n = 14$)

	Duration of struggling	Duration of swimming
SERM (10 μg)		
Vehicle	106.4 ± 7.1	143.0 ± 11.9
17β-E_2	167.9 ± 16.2 *	250.4 ± 14.5 *
PPT	134.6 ± 8.5	121.3 ± 6.0
17α-E_2	153.6 ± 12.2	131.4 ± 14.0
DPN	177.9 ± 9.2 *	252.4 ± 13.7 *
Coumestrol	178.7 ± 10.4 *	220.7 ± 9.1 *
Desipramine (30 mg/kg)		
30 min	215.1 ± 14.6 *	200.0 ± 13.6 *
48 h	222.9 ± 12.0 *	186.1 ± 11.1 *

* Indicates a significant difference from vehicle, PPT, and 17α-E_2 ($P < .05$).

[$F(7,104) = 51.635$, $P < .0001$], struggling [$F(7,104) = 11.519$, $P < .00014$], and swimming [$F(7,104) = 18.662$, $P < .0001$]. Post hoc tests revealed that 17β-E_2, DPN, or coumestrol (which all bind ERβ) reduced duration of time spent immobile and increased the duration of time spent struggling or swimming compared to rats administered vehicle, PPT, or 17α-E_2. Administration of desipramine 30 min or 48 h before testing similarly reduced time spent immobile and increased time spent struggling and swimming compared to vehicle, PPT, and 17α-E_2.

3.2. Horizontal crossing task (see Fig. 2)

There were no significant differences in the number of beam breaks of rats administered vehicle, 17β-E_2, ERβ-selective SERMs, ERα-selective SERMs, or desipramine 30 min or 48 h prior to testing ($P > .6373$) (Fig. 2).

4. Discussion

Results of the present experiment support the hypothesis that E_2's effects for antidepressant behavior involve selective actions at ERβ. Rats administered 17β-E_2, DPN, or coumestrol, all with actions at ERβ, exhibited similar antidepressive behavior as rats administered the tricyclic antidepressant, desipramine, and less depressive behavior than did rats administered ERα-selective SERMs (PPT or 17α-E_2) or vehicle. Administration of vehicle, SERMs, or desipramine did not significantly alter motor behavior in the horizontal crossing task. Together, these data suggest that E_2's effects to mediate antidepressive behavior in the forced swim test may involve actions at ERβ.

The effects of E_2, compared to vehicle, to decrease immobility in the forced swim test are consistent with previous reports of E_2's antidepressant effects. Proestrous rats, which have higher endogenous E_2 levels, demonstrate less depressive behavior in the forced swim test than do diestrous or male rats, with relatively lower E_2 levels (Frye and Walf, 2002; Frye and Wawrzycki, 2003). Ovx increases depressive behavior of female rodents in the tail suspension and forced swim test and E_2 administration attenuates these effects (Bernardi et al., 1989; Estrada-Camarena et al., 2003; Frye and Wawrzycki, 2003; Hilakivi-Clarke, 1996; Okada et al., 1997; Rachman et al., 1998; Walf and Frye, submitted for publication). These previous reports of E_2's ability to reduce depressive behavior in the forced swim test have used various E_2 regimen, including 5 or 10 μg E_2 subcutaneous injections for 48 h (Estrada-Camarena et al., 2003; Walf and Frye, submitted for publication), 5 μg E_2 injections for 22 and 44 h (Frye and Wawrzycki, 2003), 10 μg E_2 subcutaneous injections daily for 7 days (Rachman et al.,

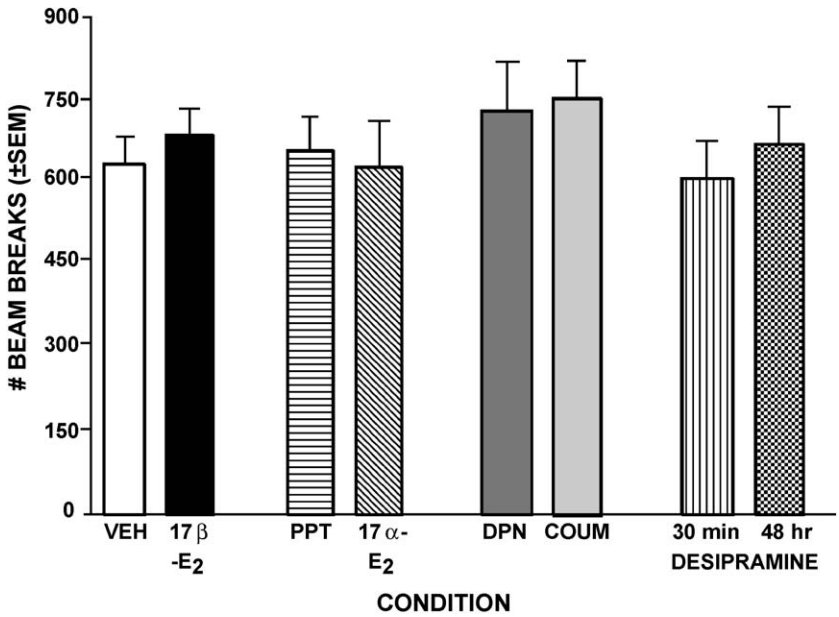

Fig. 2. The mean (± S.E.M.) number of beam breaks made in the horizontal crossing task of ovx rats administered vehicle, 17β-E_2, PPT, 17α-E_2, DPN, coumestrol (COUM), or desipramine 30 min or 48 h before testing.

1998), and subcutaneous E_2 pellets for 60 days (Hilakivi-Clarke, 1996) before testing. Although these reports demonstrate that various E_2 regimen produce antidepressive effects in the forced swim test, not all studies have reported this, suggesting that E_2's effects to reduce depressive behavior in this task may depend upon regimen utilized. For instance, administration of lower (Estrada-Camarena et al., 2003; Walf and Frye, submitted for publication) and higher dosages of E_2 does not reduce immobility of ovx rats (Stoffel and Craft, 2003; Walf and Frye, submitted for publication). It may be that regimen that produce physiological levels of E_2 may be necessary to produce antidepressant effects.

Effectiveness of E_2 or antidepressant treatment to reduce depressive behavior may be due in part to duration of treatment. There is evidence for antidepressive effects of E_2 with acute administration (22–48 h before testing; Estrada-Camarena et al., 2003; Frye and Wawrzycki, 2003; Walf and Frye, submitted for publication). However, some studies have found that chronic administration of E_2, which likely produces prolonged supraphysiological or physiological plasma E_2 levels, does not decrease depressive behavior in the forced swim test of ovx rodents (Galea et al., 2002; Okada et al., 1997), but this effect has not been observed in all studies (Hilakivi-Clarke, 1996). Alternatively, antidepressant treatments are typically chronic. Among people, reductions in depressive symptomology are typically delayed 4–6 weeks after chronic antidepressant treatment is initiated (Montgomery, 1999). Antidepressants are typically administered chronically in animal studies as well. However, the present results extend previous findings of reduced depressive behavior in the forced swim test following a single, acute (30 mg/kg, 30 min before testing; Kitamura et al., 2002) desipramine regimen in rats.

Investigators have been trying to find novel compounds that produce antidepressant effects more rapidly than classical antidepressants. Thus, the present findings, that single, acute treatments with 17β-E_2 or SERMs with actions at ERβ produce similar effects as are observed with desipramine administration reduce depressive behavior, may have great clinical significance. For instance, it may be possible that these SERMs could be used as adjunctive therapy in the weeks prior to the onset of antidepresant action, when an acute treatment is imperative. In addition to a shorter onset of action, another benefit of the use of SERMs may be that they could minimize the sexual side effects associated with some antidepressant therapies (Lane, 1997). As well, studies examining distribution of ERα and ERβ indicate that the uterus and breast epithelium lack ERβ (see Gustafsson, 2003 for review). Given that a significant concern about hormone-replacement therapy is the negative, proliferative effects of E_2 on these tissues, it is possible that ERβ-selective SERMs may reduce these side-effects.

That ERβ-selective SERMs had antidepressant effects similar to E_2 extends previous findings regarding tamoxifen's and ERβ-active SERMs' effects on mood. Tamoxifen, a compound with antiestrogenic effects in the nanomolar range, is commonly used to treat women with breast cancer. Tamoxifen has been reported to increase the prevalence of depression in some (Breuer and Anderson, 2000; Shariff et al., 1995), but not all studies (Nystedt et al., 2003; Day et al., 2001). To date, there are no studies of tamoxifen on affective behavior of rodents that we are aware of, but it has been shown to increase stress responses of female rats (Young et al., 2001). Second, although we are unaware of any other reports of SERMs' effects on depressive behavior in rodents, ERβ-selective SERMs may alter anxiety behavior in the elevated plus maze. Male and female rats fed a diet rich in phytoestrogens, with ERβ selectivity, made more entries onto, and spent more time on the open arms of the elevated plus maze than did rats fed a phytoestrogen-free diet (Lund and Lephart, 2001). These data, together with our present results, suggest that E_2's ability to modulate affective processes may involve actions at ERβ.

Although E_2, SERMs with activity at ERβ, and desipramine reduced immobility, these effects were not independent of changes in struggling and swimming, compared to rats administered vehicle or SERMs with activity at ERα. E_2's ability to increase running-wheel and open-field activity of rodents is well known (Morgan and Pfaff, 2001, 2002). However, our present results using the horizontal crossing task do not reveal robust differences between groups on motor behavior in this task. Thus, these results suggest that all differences in the forced swim test between groups observed may not be entirely due to differences in motor behavior.

Although data from the present study are exciting, as they suggest a potentially beneficial effect of actions at ERβ on depressive behavior, they are not without limitations. First, effects of only a single 10-μg dosage of each SERM was investigated. This regimen was utilized because 10 μg E_2 is sufficient to produce physiological E_2 concentrations and facilitate sex behavior (Frye et al., 1998; Vongher and Frye, 1999). Given that some SERMs have greater activity at ERα or ERβ, than E_2, one concern might be that a 10-μg dosage produces high enough concentrations such that selectivity for ERs is obviated. However, the pattern of the SERMs' antidepressant effects followed their reported activity at ERβ. Rats administered DPN, which is the most selective for ERβ, have the shortest duration of immobility, followed by rats administered 17β-E_2 < coumestrol < 17α-E_2 < PPT < vehicle. Second, in the present study, we used only one highly selective and one less-selective SERM. The data suggest that a high level of activity at ERβ is not necessary, as coumestrol, which is only seven times more active at ERβ than ERα, was effective at decreasing depressive behavior. Investigating effects of other SERMs with different levels of activity at ERβ (and/or ERα) may reveal the threshold amount of activity at these substrates that is necessary for behavioral effects. Third, although the present results do not support a role of ERα, we cannot rule out its involvement in these processes. Indeed, evidence

suggests that ERβ may have a modulatory role on ERα by inhibiting ERα-mediated gene transcription and/or partially replacing ERα in its absence (Lindberg et al., 2003). Finally, our interpretation of the present data is based upon the selectivity of the SERMs at ERα and ERβ. However, it is possible that the ability of the SERMs to affect other receptors, processes, and/or systems may mediate some of their effects on antidepressant behavior (Kian Tee et al., 2004). For these reasons, it is necessary to expand the current investigation to more dosages of these and other SERMs to determine how they may modulate affective processes and activity at ERα and ERβ.

In summary, these data suggest that E_2's antidepressive effects may be due in part to actions at ERβ. Further investigation is needed to increase our understanding of E_2's role and mechanisms for affect. Such information is critical so that women may make more informed choices about existing therapies and to enable the development of more selective therapies with fewer side effects.

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