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Debilitating Interaction of Adrenalectomy and Intrahypothalamic Implants of Prostaglandin E_2 Upon Open-Field Activity Levels and Sexual Receptivity in Estrogen-Primed Ovariectomized Rats[1]

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HALL, N. R. AND W. G. LUTTGE. *Debilitating interaction of adrenalectomy and intrahypothalamic implants of prostaglandin E_2 upon open-field activity levels and sexual receptivity in estrogen-primed ovariectomized rats.* PHARMAC. BIOCHEM. BEHAV. 6(6) 677–681, 1977. – A group of estrogen-primed, ovariectomized rats was adrenalectomized and tested for sexual receptivity following hypothalamic implantations of PGE_2. The combination of PGE_2 and adrenalectomy led to severe debilitation as manifested by greatly reduced open-field activity scores and inhibition of estrogen and progesterone induced sexual receptivity. Neither exogenous progesterone nor corticosterone was able to restore these behaviors to normal levels. A mechanism involving PGE_2 and adrenalectomy-induced transient ischemia was discussed as a possible cause of the debilitation.

Prostaglandin E_2 Estradiol Sexual receptivity Open-field activity Adrenalectomy Cerebral blood flow Hypothalamus

IN PREVIOUS studies we have shown that both intraventricular [10,11] and intracerebral [9] implants of microgram quantities of prostaglandin E_2 (PGE_2) can markedly facilitate the display of sexual receptivity in estrogen-primed ovariectomized rats. A detailed regional analysis of basal diencephalic and mesencephalic sites indicated that unilateral PGE_2 implants localized in basal anterior-hypothalamic and preoptic regions produced the greatest stimulation of receptivity [9]. Luteinizing hormone releasing hormone (LHRH), which is released by administration of PGE_2 [1, 4, 12, 23, 24], stimulates sexual receptivity in estrogen-primed ovariectomized, ovariectomized-adrenalectomized and ovariectomized-hypophysectomized rats [18–22, 25, 28]. Furthermore since anterior hypothalamic and preoptic brain regions have recently been shown to be the sites for the necessary actions of estradiol in the induction of receptivity [2, 3, 15–17], we have speculated that reproductive behavior in female rats may be mediated via an estrogen + progesterone → PGE_2 → LHRH → receptivity mechanism localized in these same brain regions [9–11].

Alternatively it is possible that PGE_2 could exert its facilitatory effects upon sexual behavior via its stimulation of adrenal progesterone release. In our first study with intraventricular PGE_2 implants [10], we tested the possibility of pituitary-adrenal involvement by treating the ovariectomized rats with a dose of dexamethasone sufficient to inhibit the release of ACTH. Since high levels of sexual receptivity were observed in the presence of this synthetic glucocorticoid, we concluded that the PGE_2 effects on behavior were probably not mediated via ACTH stimulated adrenal progesterone. However, a conclusive test ascertaining the role played by adrenal progesterone would have to incorporate adrenalectomized control animals. The

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present study reports some unexpected results using this paradigm.

METHOD

Adult female CD rats (Charles Rivers Labs, Wilmington, MA) were bilaterally ovariectomized under ether anesthesia and housed individually with food and water available at all times. The animal colony lights were left on from 2100 to 0900 hr. One week later all females were anesthetized with sodium pentobarbital (50 mg/kg) and stereotaxically implanted with 22 g stainless steel outer wall guide tubes in the calvarium directly dorsal to the preoptic area and anterior hypothalamus. The 5 mm long guide cannulas were rigidly held in place with wire, skull screws and dental cement. Approximately one week later the guide tubes were cleared with a 30 g needle and plugged with short, empty, L-shaped 27 g cannulas. Previous work using this same implantation procedure has revealed that there is minimal histological damage after repeated implantation of longer L-shaped hormone- or drug-containing cannulas through the permanent guide tubes [9, 10, 11, 17]. These longer 28 g cannulas were exchanged with the plug cannulas (and vice versa) while the rats were unanesthetized and, after sufficient handling, with apparently little stress to the rats. Cannulas were loaded by tapping them 20 times into small stainless steel pans containing crystalline PGE_2. Weighing these cannulas on a Cahn G–2 electrobalance before and after filling indicated that they each contained a mean ± SEM of 4 ± 1 μg PGE_2. Visual inspection of the cannulas at the end of the behavioral tests revealed that all or most of the PGE_2 had dissolved out. At the conclusion of the experiment all females were perfused with 10% Formalin-saline, the brains serially sectioned at 75 μm and the sections stained with hematoxylin and eosin to aid in the confirmation of the implantation sites.

Beginning at least one week after stereotaxic surgery females were started on a series of weekly tests for sexual receptivity. Each test consisted of placing the female into a 30 × 30 × 60–90 cm clear Plexiglas testing arena containing a sexually vigorous stud male. Males were permitted to mount the females 10 times and the lordosis quotient (L/M × 100) was computed for each female. Only mounts with pelvic thrusting were counted. If the male failed to either initiate or continue mounting, the female was moved to another testing arena containing a different stud male. All testing was conducted under dim red-light illumination. The purpose of these preliminary tests was to give all the females sexual experience and to establish the threshold doses of estradiol benzoate (E_2B) for each female. This threshold dose was defined as the maximum dose which, when given SC (0.1 cc peanut oil) 48 hr prior to testing, would not stimulate receptivity (i.e., L/M ≤ 20) unless an sc injection of progesterone (500 μg in 0.1 cc peanut oil) was given 3–5 hr before the test. Threshold doses for E_2B were found to range from 4–8 μg/rat by this procedure.

After at least four weeks of preliminary testing females were given a threshold injection of E_2B and 48 hr later an implant of PGE_2 was lowered into the basal anterior hypothalamic-preoptic area. Three hr later all implanted females were tested for sexual receptivity by the same procedure as that employed in the preliminary tests. After these tests, activity levels were measured for each implanted female. The rat to be tested was placed into the center of a 91.4 cm clear Plexiglas chamber divided into 16 equal sized squares and the total number of squares crossed during the 5 min test was used as the index of activity. A square crossing was recorded when three of the rat's feet had crossed a line into a square. All PGE_2 cannulas were removed and the short plug cannulas reinserted after this test.

Six rats, having shown L/M scores of 50 or higher following intracerebral PGE_2 were subjected to bilateral adrenalectomy while under ether anesthesia. These rats were subsequently housed with food and 0.9% NaCl available at all times. Over the next seven weeks these rats received repeated sex and activity tests following a variety of treatment paradigms summarized in Table 1. The first postadrenalectomy test was conducted after threshold SC E_2B priming and intracerebral PGE_2 (E + PG–1) by the same procedure as that employed in the last preadrenalectomy test. All of the rats were then tested with SC E_2B and progesterone (E + PR–1) and then one more time with SC E_2B and intracerebral PGE_2 (E + PG–2). Test 4 after adrenalectomy was conducted after the standard SC E_2B and progesterone paradigm, but in addition, the animals received intracerebral implants of PGE_2 three hr prior to the tests (i.e., concurrently with the progesterone injections) (E + PR + PG). On Test 5 the rats were tested again with SC E_2B and progesterone, but without a PGE_2 implant (E + PR – 2). On Test 6, the animals were primed with SC E_2B and 48 hr later implanted with PGE_2. Thirty minutes prior to the implants and again one hr before the tests, the animals received SC injections of corticosterone (250 μg/rat suspended on 0.1 cc saline) (E + CS + PG). A final test was carried out following the standard SC E_2B and progesterone paradigm (E + PR – 3).

RESULTS

Adrenalectomy was found to block PGE_2-facilitated sexual receptivity in estrogen-primed ovariectomized rats (Fig. 1). A single factor analysis of variance for repeated measures followed by a Newman-Keuls a posteriori test [29] revealed the following group differences in L/M scores: (E + PR–1), (E + PR–2), (E + PR–3) > (E + CS + PG), (E + PG–1), (E + PG–2), (E + PR + PG), (E) ($p<0.01$). Although PGE_2 stimulated high levels of receptivity when the animals had their adrenal glands (i.e., E + PG–ad), the mean lordosis quotient was significantly less than the L/M scores for the E + PR conditions following adrenalectomy ($p<0.05$).

Correlated with the decreased receptivity scores was decreased activity when the adrenalectomized animals were implanted with PGE_2 (Fig. 2). A single factor analysis of variance for repeated measures followed by a Newman-Keuls test revealed that females implanted with PGE_2 prior to adrenalectomy (E + PG-ad) had higher open-field activity scores ($p<0.05$) than during any of the postadrenalectomy tests, although moderate to high levels of activity were also displayed during the standard SC E_2B plus progesterone priming conditions. Systemic injections of either progesterone (E + PR + PG) or corticosterone (E + CS + PG) were unable to overcome the PGE_2 inhibition ($p<0.01$) of both receptivity and activity.

With the exception of the inactivity following intracerebral PGE_2 implants, all of the animals appeared in good health following all of the treatments. The reversibility of this inactivity was apparent by the high levels of both

TABLE 1

INJECTION AND TESTING PARADIGM

	Code	Treatment
(Preadrenalectomy)	E	E_2B*—(48 hr)—Sex Test
(Preadrenalectomy)	E+PG-ad	E_2B—(48 hr)—PGE_2 Implant—(3 hr)—S and A Tests†
Test 1	E+PG-1	E_2B—(48 hr)—PGE_2 Implant—(3 hr)—S and A Tests
Test 2	E+PR-1	E_2B—(48 hr)—500 μg Progesterone—(3 hr)— S and A Tests
Test 3	E+PG-2	E_2B—(48 hr)—PGE_2 Implant—(3 hr)—S and A Tests
Test 4	E+PR+PG	E_2B—(48 hr)—500 μg Progesterone: PGE_2 Implant—(3 hr)—S and A Tests
Test 5	E+PR-2	E_2B—(48 hr)—500 μg Progesterone—(3 hr)—S and A Tests
Test 6	E+CS+PG	E_2B—(48 hr)—250 μg Corticosterone—(30 min)—PGE_2 Implant—(2 hr)—250 μg Corticosterone—(1 hr)—S and A Tests
Test 7	E+PR-3	E_2B—(48 hr)—500 μg Progesterone—(3 hr)—S and A Tests

*Doses varied between 4 μg and 8 μg depending upon individual thresholds.
†Sex and Activity Tests.

activity and sexual receptivity typically displayed during subsequent tests without PGE_2 implants.

DISCUSSION

Stimulation of adrenal progesterone release has been shown to facilitate the induction of sexual receptivity in estrogen-primed ovariectomized rats [5]. ACTH secretion (and presumably adrenal progesterone) has further been shown to increase following injection 0.5 to 1.0 μg PG into the medial basal hypothalamus [13]. Although the sites for maximal PGE_2-facilitation of sexual receptivity found in our earlier studies were located several millimeters away from those implants shown to release ACTH, it is possible

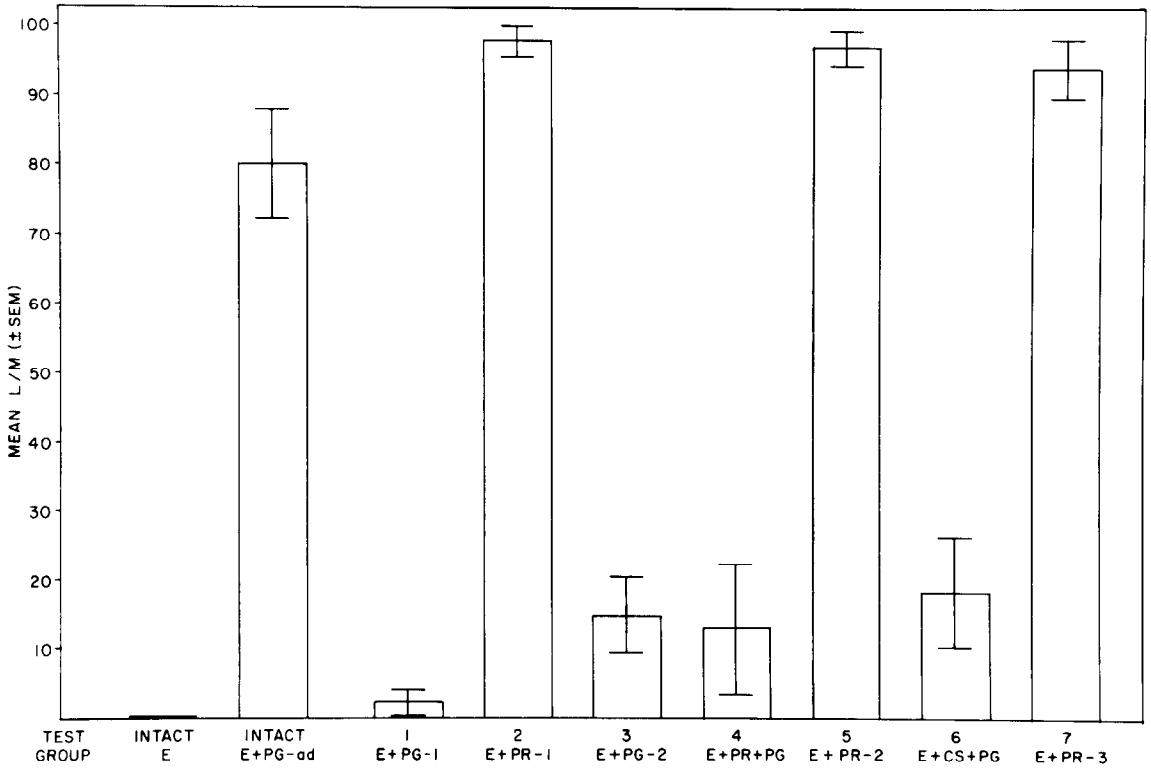

FIG. 1. Receptivity scores of ovariectomized and adrenalectomized rats tested at 7 weekly intervals with SC estrogen and PGE_2, progesterone and/or corticosterone. See text and Table 1 for explanation of the codes.

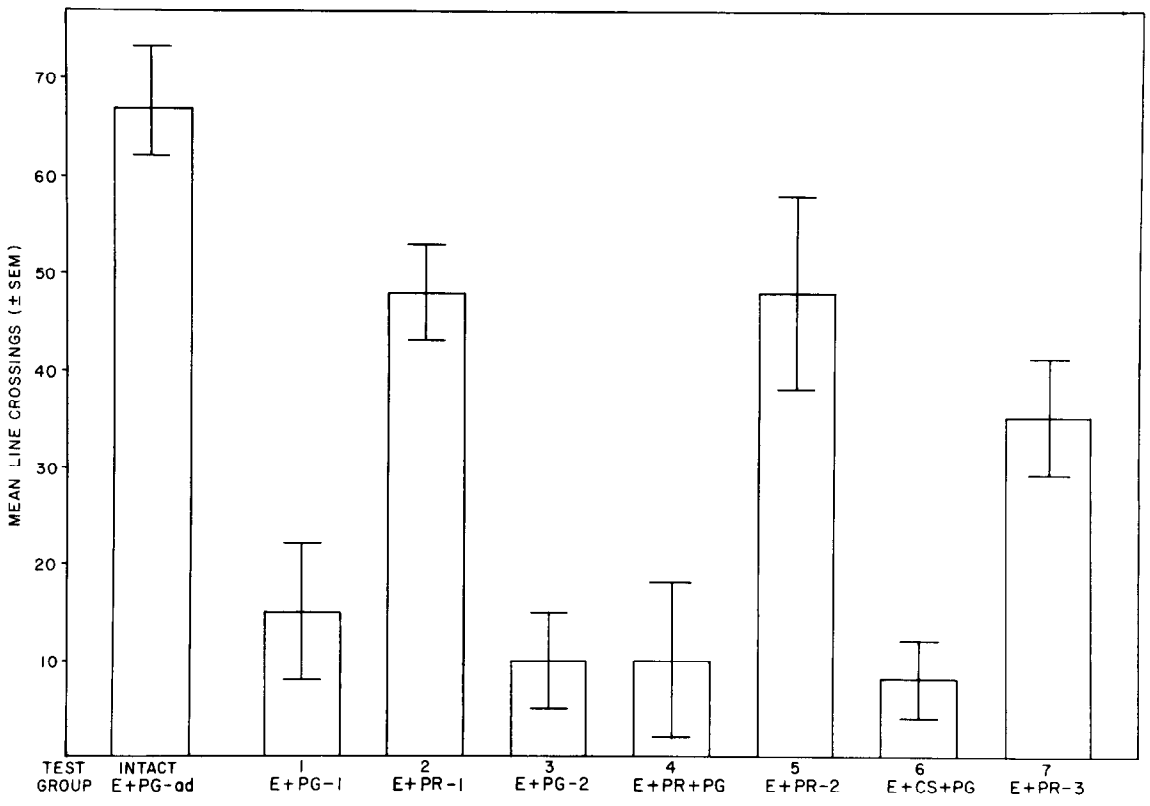

FIG. 2. Open-field activity scores of ovariectomized and adrenalectomized rats tested at 7 weekly intervals with SC estrogen and PGE_2, progesterone and/or corticosterone. See text and Table 1 for explanation of the codes.

that sufficient leakage could have occurred to permit the PGE_2 used in our previous studies to stimulate the release of ACTH and subsequently, adrenal progesterone.

The present study was designed as a direct test of this possibility and it revealed that adrenalectomy does indeed abolish intracerebral PGE_2-facilitated sexual receptivity. However, the present study suggested that this block was not due solely to the elimination of adrenal progesterone. Open-field activity levels were also severely depressed. Adrenalectomy by itself did not result in appreciable decreases in activity, nor did implantation of PGE_2 into the basal anterior hypothalamus and preoptic area of animals with their adrenals intact. But the combination of adrenalectomy and intracerebral PGE_2 implants led to a dramatic decrease in open-field activity scores. This inhibition of activity was sufficient to block the display of sexual receptivity even when the females were primed with systemic E_2B and progesterone. Without the PGE_2 implant, E_2B and progesterone elicited lordosis quotients that were even higher than scores elicited following this paradigm in females with their adrenals (Fig. 1). The combination of adrenalectomy and intracerebral PGE_2 could have decreased the effectiveness of estrogen and progesterone. This possibility is extremely remote, however, since either manipulation by itself has a facilitatory effect on steroid induced sexual receptivity [9, 10, 11] (Fig. 1).

Lethargy has been reported as a behavioral consequence of PGE_2 administration [6, 14, 26]; however, in all of our previous work [9, 10, 11] as well as in the present study, unilateral intracerebral implants of PGE_2 have failed to produce any signs of reduced activity and lethargy. Consequently, it appears that intra-hypothalamic PGE_2 and adrenalectomy interact to cause severe, but reversible inactivity. Our earlier demonstration that dexamethasone-inhibition of the pituitary-adrenal axis failed to block intraventricular PGE_2-induced facilitation of receptivity in estrogen-primed ovariectomized rats [9,10], further supports the contention that receptivity was inhibited as a consequence of decreased activity levels in adrenalectomized, PGE_2 implanted rats.

The mechanism by which intracerebral PGE_2 and adrenalectomy resulted in the debilitation observed in the present study probably involves decreased cerebral blood flow. Adrenalectomy results in a lack of mineralocorticoids, acidosis and decreased cardiac output [27]. Adrenalectomy can also result in elevated levels of MSH [8] which can reduce blood flow to specific brain regions [7]. Similarly, PGE_2 can reduce blood flow by causing vasoconstriction, platelet aggregation or hypocapnia [30]. Consequently, it is hypothesized that the PGE_2-induced vasoconstriction, coupled with the decreased blood flow resulting from adrenalectomy, precipitated a transitory ischemia in certain brain regions. Neuronal reactions to this ischemia resulted in the observed reduction of open-field activity levels. Whether the behavior deficits were due to lethargy and decreased levels of arousal or to dysfunction of the motor system cannot be answered in the absence of electrophysiological measures.

Regardless of the etiology of the debilitation, the implications of these findings in the context of behavioral experiments are important. Testing drugs and hormones in the presence and absence of the adrenal glands is a frequent control procedure in studies of reproductive behavior. This is because the adrenals provide a source of progesterone sufficient to stimulate sexual receptivity in ovariectomized rats [5]. If the drug or hormone being tested were capable of reducing cerebral blood flow, inhibition of the behavior in question could well be due to ischemia and subsequent neuronal damage – not to direct modulation of brain regions subserving reproductive behavior.

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