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Estrogen Increases Adrenergic- But Not Cholinergic-Mediated Production of Inositol Phosphates in Rabbit Uterus

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SUMMARY

α_1-Adrenergic and muscarinic cholinergic stimuli activate uterine contraction. Estrogen increases adrenergic but not cholinergic sensitivity of rabbit myometrium independent of its effects on adrenoceptor concentration. Since both α_1-adrenergic and muscarinic receptors are coupled to phosphatidylinositol hydrolysis, we tested the hypothesis that estrogen increases adrenergic- but not cholinergic-mediated inositol triphosphate production. We found that maximal production of inositol phosphates stimulated by norepinephrine was increased approximately 3-fold following estrogen treatment. Cholinergic-stimulated production was not increased by estrogen treatment. These results demonstrate that the effect of estrogen to enhance uterine adrenergic sensitivity is associated with an increased post-receptor response. The nature of the selectivity of estrogen for adrenergic versus cholinergic response remains obscure, but the results suggest the presence of parallel pathways for receptor activation of a common post-receptor response.

The rabbit uterus contracts in response to both α_1-adrenergic and cholinergic stimulation (1). Estrogen increases the sensitivity and maximal contractile response to α_1-adrenergic agonists and in pharmacologic doses increases receptor concentration. However, it is without apparent effect on cholinergic response under the same conditions (Table 1) (2, 3). Although the maximal contractile response to NE correlates with the increase in α_1-adrenoceptors, this increase is not causally related to the change in sensitivity for the following reasons. 1) Untreated mature animals with physiologic concentrations of estrogen have an α_1 receptor concentration similar to that in ovariectomized animals yet are 4 times more sensitive to adrenergic agonists. 2) In addition, if rabbits are treated with pharmacologic doses of estrogen, the increased myometrial sensitivity persists after estrogen withdrawal even when α receptor concentrations return to levels present in ovariectomized animals (Table 1). The correlation of maximal response, but not sensitivity, with receptor concentration indicated that maximal response could be influenced by receptor concentration, but that changes in sensitivity would require changes beyond the agonist-receptor complex.

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α_1-Adrenergic and muscarinic cholinergic receptors in several tissues have been demonstrated to activate PIP2-PLC, catalyzing the specific hydrolysis of membrane PIP2 to form IP3 and diacylglycerol (4, 5). IP3 is known to release calcium from intracellular storage sites and is thus thought to be a "second messenger" for calcium-mobilizing responses (6, 7). Diacylglycerol is known to activate cellular Ca^{2+} plus phospholipid-activated protein kinase C, a key enzyme regulating Ca^{2+}-mediated cellular responses (8).

In this study we examined the role of PLC activation and subsequent IP3 generation in the observed increase in adrenergic sensitivity. Our hypothesis was that changes in the ability of α agonists to generate IP3 in the differently treated animals would correlate with α-adrenergic sensitivity. In addition, since our previous studies indicated that estrogen did not increase uterine sensitivity for muscarinic cholinergic contractile response, we studied the effects of muscarinic agonists on IP3 generation to assist in localizing the difference in response to α-adrenergic and muscarinic agonists.

Our findings indicate that both α_1-adrenergic and muscarinic cholinergic receptor activation result in myometrial IP3 generation; however, estrogen increases only the α-adrenergic activation of PIP2-PLC.

Experimental Procedures

Materials. [^{3}H]-*myo*-Inositol (16 Ci/mmol) was obtained from New England Nuclear. Dowex 1X8 (chloride form, 100–200 mesh) was

ABBREVIATIONS: NE, norepinephrine; PIP2-PLC, phosphatidylinositol 4,5-biphosphate-specific phospholipase C; PIP2, phosphatidylinositol 4,5-biphosphate; IP, IP2, and IP3, *myo*-inositol 1-phosphate, 1,4-biphosphate, and 1,4,5-triphosphate; PLC, phospholipase C; IPs, combined pool of IP+IP2+IP3; OVEX, ovariectomized (group); OVEX-E2, estrogen-treated ovariectomized (group); E2-OVEX, estrogen-pretreated ovariectomized (group); PI, phospatidylinositol; ANOVA, analysis of variance; PIP, phosphatidylinositol 4-phosphate or 3-phosphate; [^{125}]-HEAT, [^{125}I]-2-[β-(4-hydroxylphenylethyl)aminoethyl]tetralone.

obtained from Sigma Chemical Co. and converted to formate form by successive washing with 1 M NaOH, H_2O, and 1 M formic acid. NE, carbamylcholine (carbachol), and yohimbine hydrochloride were obtained from Sigma. Prazosin hydrochloride was obtained from Pfizer and phentolamine hydrochloride was from Ciba-Geigy. Drugs were dissolved in either deionized water or 1 mM HCl (NE), and appropriate vehicle controls were used. All other chemicals and solvents were of reagent grade.

Treatment protocols. Female New Zealand White rabbits (1.5–3.5 kg body weight) were used for all studies. Bilateral ovariectomy was performed under ketamine-xylazine anesthesia (9) and a minimum period of 7 days was allowed for recovery from surgery before the animals were used for assay or estrogen treatment. We used four groups of animals: ovariectomized (OVEX), estrogen-treated ovariectomized (OVEX-E2), mature intact rabbits (~3 kg body weight; MATURE), and estrogen-pretreated ovariectomized (E2-OVEX) rabbits. Estrogen treatment was with estradiol benzoate in sesame oil vehicle administered for 4 successive days (50 μg/kg intramuscularly per day), and the animals were sacrificed 24 hr after the last dose by pentobarbital overdose (50 mg/kg, intravenously). The E2-OVEX rabbits were first treated with estradiol benzoate as described, then ovariectomized and used for studies 7–10 days after surgery.

Assay of inositol phosphate production. The conditions used for incubation of uterine tissue were adapted from those of Brown and Brown (10) and Berridge *et al.* (11). Uteri were rapidly removed from rabbits and maintained under continuous aeration in Kreb's solution (pH 7.4) on ice (4°). Kreb's solution (12) contained the following salts (all in mM concentration): NaCl 118, KCl 4.7, $MgSO_4$ 1.18, KH_2PO_4 1.17, glucose 11.1, $NaHCO_3$ 25, and $CaCl_2$ 2.5.

The uterine horns were opened longitudinally to form a flat strip, then minced using a McIlwain tissue chopper (Brinkmann Instruments), producing pieces roughly 1 × 1 × 5 mm in size. The minced tissue (100 mg wet weight) was transferred to Kreb's solution (200 μl final volume), [^{3}H]-*myo*-inositol (16 μCi/ml) was added to start the reaction, and the tubes were aerated, stoppered, and incubated for 60 min at 37° in a shaking water bath (80 oscillations/min). At the end of the incubation period the tubes were kept on ice until the tissue was transferred to nonradioactive medium for agonist stimulation.

Agonist stimulation of IPs production was carried out in a final volume of 200 μl of Kreb's solution containing (final concentrations): LiCl (10 mM), cocaine hydrochloride (20 μM to inhibit catecholamine reuptake), and agonist or vehicle. The tissue from the first incubations was blotted and then transferred to this medium, aerated, stoppered, and incubated for 5 min at 37° to facilitate LiCl uptake. Agonists or vehicles were then added to the tubes, which were aerated, stoppered, and incubated for 30 min at 37°. At the end of the incubation period, the reaction was stopped by the addition of 1 ml of ice-cold $CHCl_3$/methanol (1:2, v/v), and the tubes were stored on ice or at −20° until further processed.

Ion exchange chromatography of inositol phosphates. The method of Berridge (4) was used without modification for separation of uterine [^{3}H]inositol phosphates. The aqueous extract, prepared as described in Ref. 4, was loaded onto columns (Bio-Rad) of Dowex 1X8 formate form (1.2 ml, approximately 0.8 × 3 cm). The columns were washed with 10 ml of deionized H_2O to elute [^{3}H]inositol (the bulk of the ^{3}H in the sample), followed by 6 ml of 5 mM $NaBO_4$/60 mM NaCOOH to elute glycerophosphoinositol. The inositol phosphates were routinely eluted as a pool (IP, IP2, and IP3 = IPs) using 7 ml of 1 M NH_4COOH/0.1 M HCOOH. Individual inositol phosphates were sequentially eluted using increasing concentrations of NH_4COOH in HCOOH as described in the legend to Fig. 1 (see Results). The elution pattern for PI metabolites in rabbit uterus was similar to that observed for several different tissues by other investigators (10, 11), and is consistent with the preferential production of IP3 in response to agonists followed by rapid degradation to IP2 and IP. Radioactivity was determined by counting 0.5 ml of eluate in 3.8 ml of scintillant (Safety Solve, National Diagnostics) at 34% efficiency.

The data are presented as mean values ± standard errors. Statistical significance of differences was tested by the unpaired *t* test or, when more than two comparisons were made, by ANOVA. A confidence level of 95% was the criterion for significance.

Results

Separation of IP, IP2, and IP3 by ion exchange chromatography. NE was used to stimulate PIP2-PLC activity in uterine minces from estrogen-treated rabbits, and the water-soluble [^{3}H]inositol phosphates were extracted and separated by ion exchange as shown in Fig. 1. Inositol 1-phosphatase activity was inhibited with 10 mM LiCl, causing the accumulation of metabolized IP3 as a mixture of the three metabolites. Because the IP3 peak is a minor portion of the total inositol phosphate pool as a result of the rapid dephosphorylation of IP3 and IP2 that occurs in intact tissue, we routinely assayed the pooled mixture of IP, IP2, and IP3 (IPs) as the agonist-stimulated response (see Experimental Procedures). It is possible that some of the IP2 and IP is produced by direct hydrolysis of PIP and PI; thus, the measured response may not reflect solely PIP2 hydrolysis (see below).

Kinetics of [^{3}H]inositol uptake and agonist-stimulated PI hydrolysis. Fig. 2a shows the time course for labeling of uterine PI pools *in vitro*. We chose a 60-min loading period for the present study. The time course for accumulation of [^{3}H]-IPs is shown in Fig. 2b. Production was linear for at least 40 min once detectable levels were achieved. A 30-min accumulation was used as the agonist response for these studies. The relative proportions of the different inositol phosphates produced during the incubations is shown in Fig. 2c. Since the relative amounts of IP2 and IP were nearly equivalent, direct hydrolysis of PIP and PI (respectively), in addition to PIP2, probably accounts for some of the agonist response.

Dose dependency and characterization of uterine IPs

TABLE 1

The effect of estrogen on rabbit uterine contractile response to NE and α_1-adrenoceptor concentration

The EC_{50} values were obtained from cumulative dose response studies conducted *in vitro* in Kreb's solution. The maximal contractile response of uterine strips to NE and to 70 mM KCl was determined as integrated area (g × 90 sec), and the ratio of the two responses was determined for individual strips. α_1 Receptor concentration was determined by saturation analysis using [^{125}I]HEAT. The data are means ± standard errors for (*n*) rabbits as reported in Ref. 3.

	Treatment			
	OVEX	OVEX-E2	Mature	E2-OVEX
EC_{50} (nM)	746 ± 139[a] (7)	164 ± 16 (9)	176 ± 18 (5)	131 ± 12 (7)
Maximal response	1.49 ± 0.07 (5)	2.48 ± 0.10[a] (5)	1.5 ± 0.2 (3)	1.48 ± 0.12 (5)
α_1 Receptors (fmol/mg)	46 ± 14 (5)	99 ± 16[b] (8)	46 ± 7 (14)	46 ± 10 (8)

[a] Significantly different from all other treatments, $p < 0.001$ by one-way ANOVA.
[b] Significantly different from all other treatments, $p < 0.01$ by one way ANOVA.

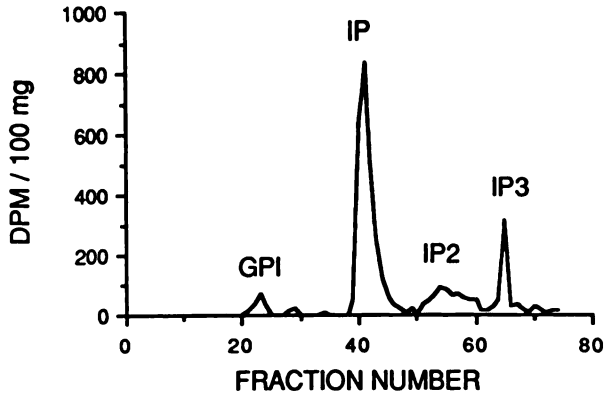

Fig. 1. Separation of IPs by ion exchange chromatography. The stimulation of [^{3}H]inositol phosphate production by 10 μM NE for 30 min at 37° is plotted as the difference from a vehicle control without agonist for each fraction (negative values converted to zero), expressed as DPM of ^{3}H/100 mg tissue wet weight. The water-soluble tissue extracts were applied to columns containing Dowex 1X8 anion exchange resin (formate form). Fractions (0.5 ml) were collected beginning immediately after the extract volume had eluted. The eluting reagents were: H_2O (10 ml) to elute [^{3}H]inositol, 5 mM $NaBO_4$/60 mM NaCOOH (6 ml) to elute glycerophospho-^{3}H-inositol (*GPI*), 0.2 M NH_4COOH/0.1 M HCOOH (6 ml) to elute IP, 0.4 M NH_4COOH/0.1 M HCOOH (6 ml) to elute IP2, and 1 M NH_4COOH/0.1 M HCOOH (7 ml) to elute IP3. The highest values for basal production (i.e., no agonist present) in the experiment shown were (in DPM/100 mg): IP, 198; IP2, 80; and IP3, 62.

production. A graded increase in the production of IPs with increasing concentration was observed for both NE and carbachol (Fig. 3). The EC_{50} for NE stimulation was 4.4 ± 0.8 μM (n = 6). For carbachol stimulation, the EC_{50} was 11.9 ± 3.3 μM (n = 4). The subtype-selective antagonists prazosin and yohimbine were used to determine the relative contributions of α_1 and α_2 receptors, respectively, to the NE response, since both receptors are present in the uterus. The data in Fig. 4 demonstrate that this response is mediated by α-1-adrenoceptors, which is consistent with a causal role in uterine contraction. The response to carbachol was inhibited by atropine (41.5 ± 11% inhibition by 10 μM atropine at 1 mM carbachol, n = 5), indicating that it was mediated by muscarinic receptors.

Effect of estrogen on adrenergic and cholinergic stimulation of uterine IPs production. In uteri from estrogen-treated rabbits (OVEX-E2), maximal NE-stimulated production of IPs was approximately 3 times that of OVEX uteri (Fig. 5). Mature rabbits exposed only to physiologic concentrations of estrogen also had a significantly greater production of uterine IPs than did the estrogen-withdrawn (OVEX) group. Rabbits pretreated with estrogen prior to ovariectomy (E2-OVEX) also exhibited enhanced adrenergic contractile sensitivity despite the return of α_1 receptor concentration to levels present in estrogen-withdrawn (OVEX) rabbits. In the E2-OVEX treatment group, NE stimulated uterine IPs production to the same extent as for estrogen-treated rabbits. We did not observe any effect of estrogen on the adrenergic sensitivity (EC_{50}) for IPs production [e.g., EC_{50} OVEX-E2, 4.4 ± 0.8 μM (n = 6); OVEX, 1.99 (3.3 and 0.56); and E2-OVEX, 4.1 ± 0.9 μM (n = 3)].

Cholinergic IPs response was not significantly different in any of the four treatment groups (Fig. 6) when analyzed by one-way ANOVA. In order to assess the relative responses to adrenergic and cholinergic stimulation, the data depicted in Figs. 5 and 6 were analyzed by two-way ANOVA. This analysis revealed a significant difference in response to the two agonists (p = 0.002). Thus, maximal IPs production in response to cholinergic stimulation was less than that elicited by adrenergic stimulation (except for OVEX rabbits, where cholinergic stimulation was greater than adrenergic: compare Figs. 5 and 6).

The effect of estrogen on uterine production of IP3 in response to adrenergic and cholinergic stimulation was also determined (Fig. 7). Compared with uteri from OVEX rabbits, OVEX-E2-treated uteri exhibited an increase in IP3 production in response to NE. Thus, as suggested by the IPs production data shown above, at least part of the effect of estrogen is to enhance PIP2 breakdown in response to α_1-adrenergic stimulation.

The differential effects of estrogen on PIP2-PLC activation by these two uterine autonomic receptors suggest that they use separate but parallel receptor-effector pathways. If this is the case, then it would be expected that the IPs production in response to one agonist would be additive to that produced in response to the other. When the maximal responses of NE (100 μM) and carbachol (1 mM) together were compared with the response of each agonist alone, we found that the responses are simply additive (109 ± 3%, n = 3, of the sum of the separate responses).

Discussion

These studies demonstrate that rabbit uterine inositol phosphate production is stimulated by two agonists known to cause uterine contraction. We chose to study intact uterine tissue in order to examine the activation and regulation of this response within as normal a physiologic setting as was feasible. This approach revealed contrasting effects of estrogen on uterine adrenergic and muscarinic activation of PIP2-PLC activity.

The effects of estrogen on uterine adrenergic contractile response are 2-fold: an increase in contractile sensitivity (i.e., a reduction in the EC_{50} for NE-stimulated contraction), and an increase in maximal contractile response (developed tension) (3). The former occurs without a change in α_1-adrenoceptor concentration, while the latter is dependent on an increase in receptors (see Table 1). In the present study, we found that α-$_1$-stimulated IPs production increased in parallel with contractile sensitivity under the influence of estrogen. As with contractile sensitivity, the increase in IPs production occurs independent of a 2-fold change in α_1 receptor concentration, since uteri from mature and E2-OVEX rabbits have increased IPs production without an increase in α_1-adrenoceptor concentration. Also, uterine sensitivity to adrenergic stimulation of IPs production (i.e., EC_{50}) was not affected by estrogen, even though α_1 receptor concentration was increased. These observations support the concept that uterine PIP2-PLC activation acts as a mediator of signals initiated through α_1 receptors in the plasma membrane and is a target for estrogen to alter adrenergic contractile sensitivity. However, estrogen has many effects on the uterus, including increases in myofibrillar and other protein content accompanying cellular hypertrophy. We have previously demonstrated that estrogen treatment does not affect cholinergic contractile sensitivity (3), suggesting a selective effect on the adrenergic response pathway. The effect of estrogen to increase adrenergic IPs response while not changing cholinergic response helps to localize this effect to PIP2-PLC activation by the receptors. Thus, it is unlikely that the effect of estrogen is to increase the uptake of [^{3}H]inositol, alter the

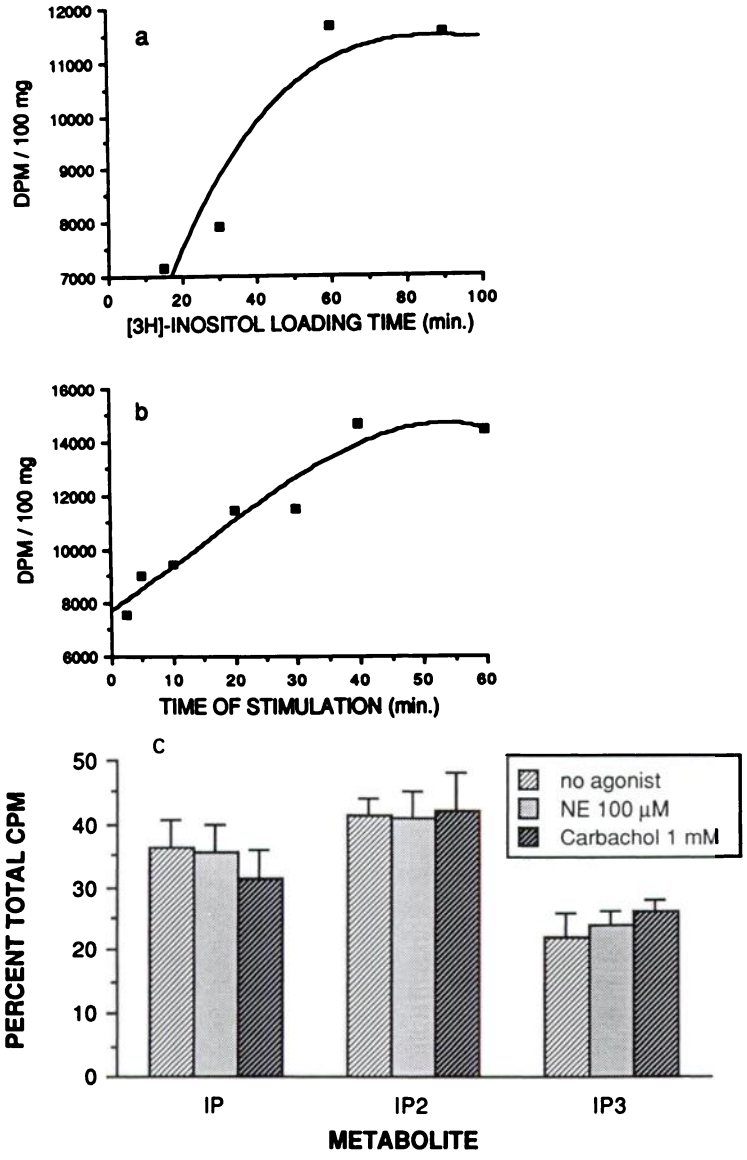

Fig. 2. Time course of [^{3}H]inositol uptake and IPs production. Uterine minces were incubated with [^{3}H]inositol at 37° for the times indicated (a) or for 90 min (b), and then transferred to fresh medium without [^{3}H]inositol but containing NE (10 μM) and incubated for either 30 min (a) or the indicated times (b). The data plotted in these representative curves are the total IPs production for either 30 min (a) or the indicated times (b) determined in duplicate. All data shown in this representative experiment were obtained from the same uterus. The time course for IPs production was verified in two other experiments yielding similar results. In c, the relative proportions of three major PI metabolites produced after stimulation for 30 min with 100 μM NE (n = 5) or 1 mM carbachol (n = 3) are shown. The data are means ± standard errors.

pool size of PIP2, or nonselectively increase the activity of PIP2-PLC.

The lack of similar effects on cholinergic and adrenergic IPs response supports the concept of parallel but independently regulated pathways for agonist activation of PIP2-PLC. Adrenergic IPs production (i.e., maximal response) was increased in all groups manifesting increased contractile sensitivity. Cholinergic stimulation of uterine IPs production was not changed by treatment, nor is contractile sensitivity affected. Thus, the effect of estrogen to increase adrenergic IPs response is consistent with its enhancement of adrenergic contractile sensitivity among the different treatment groups and is also consistent with its apparent lack of ability to increase cholinergic response. The apparently additive interaction between adrenergic and cholinergic stimulation of uterine IPs production also supports the presence of parallel agonist response mechanisms.

The dose response relations for adrenergic and cholinergic stimulation of uterine IPs production were right-shifted compared with those for uterine contraction (i.e., EC_{50} of NE: contraction = 0.17 μM, IPs production = 4.4 μM). A similar discrepancy for the IPs dose response to NE and vasopressin compared to a physiologic endpoint (Ca^{2+} mobilization) in isolated hepatocytes has been reported (13). Lynch *et al.* (13) concluded that the degree of discrepancy between the two dose response curves was proportional to the relative concentration of membrane receptors: with a higher receptor concentration, there was less discrepancy between the dose response curves for the two endpoints. Our results do not support this type of relationship, since we found that changes in uterine α_1-adrenoceptor concentration did not affect the discrepancy between the relative dose response curves for contraction and IPs production. For example, E2-OVEX rabbits had the same EC_{50} for IPs production as estrogen-treated rabbits (4.1 and 4.4 μM, respectively), as was also true for the EC_{50} for contraction, yet the E2-OVEX uteri had one-half as many α_1 receptors as the OVEX-E2 uteri (Table 1). Thus, a change in receptor concentration did not result in a change in the discrepancy between relative EC_{50} values for the two types of responses.

One problem with the use of intact tissue was the need for long agonist exposure periods to achieve a sufficient IPs signal.

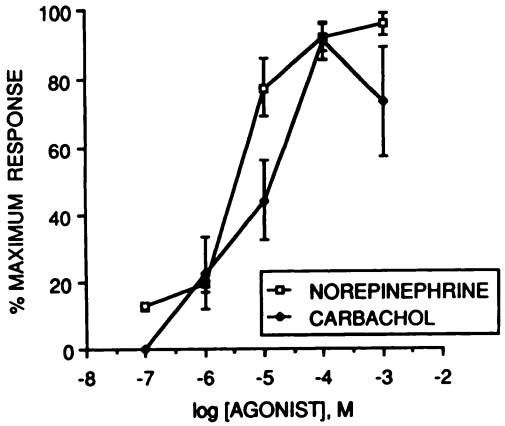

Fig. 3. Dose response curves for agonist-stimulated IPs production in rabbit uterine minces. Shown are dose response curves for IPs production in uteri from estradiol-treated rabbits for NE and carbachol. The data are the mean ± standard error of IPs production for a 30-min period determined in duplicate in the presence of the agonist concentration indicated, expressed as per cent of maximum response. For NE, the data are the mean ± standard error for responses from six rabbits, and the EC_{50} was 4.4 ± 0.8 μM (maximal stimulation was 175 ± 20% above control). The carbachol response is from four rabbit uteri, and the EC_{50} was 11.9 ± 3.3 μM (maximal stimulation was 69 ± 6% above control).

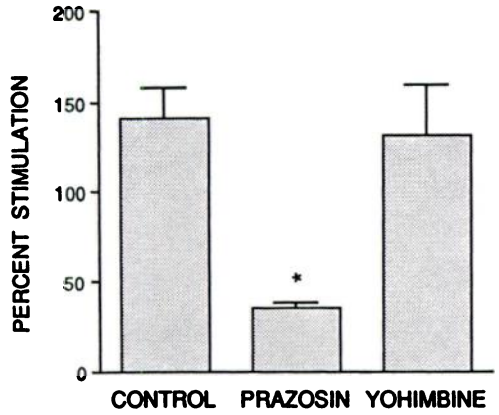

Fig. 4. α-Adrenergic selectivity of IPs production. IPs accumulation stimulated by 10 μM NE or vehicle for 30 min was determined in duplicate in uterine minces from estrogen-treated rabbits. Values are means ± standard errors for five rabbits. At the antagonist concentrations used, prazosin (0.1 μM) is selective for α_1-adrenoceptors, and yohimbine (0.2 μM) is selective for α_2-adrenoceptors.

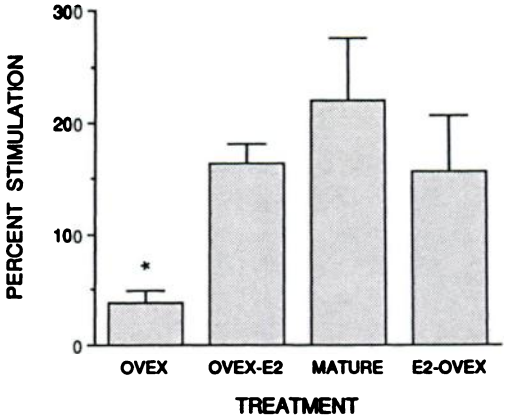

Fig. 5. Effect of estrogen treatment on NE-stimulated uterine IPs production. Uterine minces from rabbits treated as indicated were used to measure IPs production *in vitro*. Values are means ± standard errors of IPs production stimulated by 100 μM NE (30 min accumulation) determined in duplicate. The number of rabbits represented in the graph are: *OVEX*, 6; *OVEX-E2*, 11; *MATURE*, 3; and *E2-OVEX*, 6. *, $p < 0.05$ by one-way ANOVA for difference from all other groups. E2-OVEX rabbits were treated with estrogen, ovariectomized, and withdrawn from estrogen 7–10 days before sacrifice.

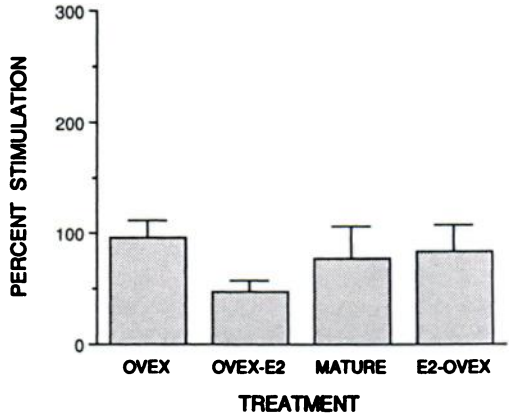

Fig. 6. Effect of estrogen treatment on carbachol-stimulated IPs production. Uterine minces from rabbits treated as indicated were used to measure IPs production *in vitro*. Values are means ± standard errors of IPs production stimulated by 1 mM carbachol (30 min accumulation) determined in duplicate. The number of rabbits represented in the graph are: *OVEX*, 4; *OVEX-E2*, 9; *MATURE*, 5; and *E2-OVEX*, 4. The OVEX-E2-treated group is significantly different from OVEX by unpaired comparison ($p = 0.034$), but not different by one-way ANOVA of all four groups. Treatments are as described in the legend for Fig. 5.

This is not likely to have contributed to the apparent insensitivity of uterine IPs production stimulated by NE and carbachol. IPs production was linear with time once detectable levels were reached. Thus, it is likely that the production at earlier times had the same dose response relation. Recently, it has become apparent that IP3 exists as two major isomeric forms, 1,3,4- and 1,4,5-phosphate (14), the latter of which is known to be biologically active (15). The function of inositol 1,3,4-phosphate is currently unknown, but it is probably produced with a different time course than the 1,4,5 isomer (14, 16). For example, in guinea pig hepatocytes (16) the 1,4,5 isomer is produced with no measurable latency following angiotensin II stimulation, peak production is attained within 20 sec, and production declines thereafter. Production of the 1,3,4 isomer exhibits a latency of about 10 sec in these cells but, thereafter, increases rapidly and is maintained for at least 30 min. Thus, by 5–10 min, inositol 1,3,4-phosphate accounts for more than 90% of the IP3 produced by these cells. In view of this, it is conceivable that independent measurement of the 1,4,5 isomer at early times in the uterus could reveal a different dose response relation.

In interpreting the results of the present study, it is important to remember that all studies to date support the concept that 1,4,5 IP3 is the major precursor of 1,3,4 IP3, and of the IP2 and IP which accumulate initially in the presence of LiCl. The data in Fig. 7 support the conclusion that the effect of estrogen is to increase the amount of IP3 made in response to adrenergic stimulation. However, the data in Fig. 2c suggest that some IP2 comes directly from PIP and/or that some IP comes directly from a pool of PI having a lower specific radioactivity than PIP or PIP2. Thus, these studies suggest that estrogen can increase the amount of IP3 made in response to adrenergic stimuli, but they do not allow complete delineation of the lipid precursors of the metabolites produced during long incubations with agonists.

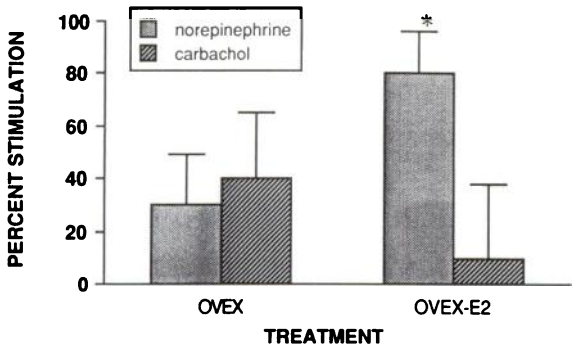

Fig. 7. Effect of estrogen treatment on uterine production of IP3. Uterine minces from rabbits treated as indicated were incubated with either NE (100 μM) or carbachol (1 mM) for 30 min *in vitro*, and the IP3 fractions were eluted from Dowex colums and counted. The values are means ± standard errors of IP3 production for five (NE) or three (carbachol) rabbits, expressed as per cent stimulation relative to control incubations conducted without agonist. Estrogen treatment increased IP3 production in response to NE ($p = 0.036$, one-tailed t test) but did not change the response to carbachol ($p = 0.43$, two-tailed t test).

In our studies, the low levels of IP3 production at early times precluded rigorous testing of the role of IP3 in myometrial contraction (a very rapid response). Nonetheless, the apparent insensitivity of response does not obviate a "second messenger" role for IP3 in uterine activation, since sufficient (but undetectable) levels of IP3 may be produced at times early enough to comprise the initial agonist signal. Rapid production of 1,4,5 IP3 in amounts sufficient to have a causal role in the initiation of cellular responses has clearly been demonstrated in several different tissues (5, 17). Furthermore, the correlation of changes in uterine IP3 production with changes in adrenergic sensitivity upon hormonal manipulation supports a causal role in response.

The recognition that, in some tissues, cellular 1,4,5 IP3 increases and decreases rapidly has resolved one of the puzzling problems with the candidacy of IP3 as a second messenger, since the transient nature of intracellular calcium release appeared inconsistent with the continued presence of high levels of IP3 with maintained agonist exposure. If inositol 1,3,4-phosphate is indeed the predominant IP3 produced at the time point used in our studies, the observed correlation of hormonal effects on contractile sensitivity with IP3 generation is consistent with a functional, albeit unknown, role for this isomer.

The nature of the apparent selectivity of estrogen for adrenergic response remains to be elucidated. The lack of dependence on changes in α_1-adrenoceptor concentration localizes the estrogen effect as post-receptor. The coupling of both adrenergic and cholinergic responses to IPs production further localizes the critical events to PIP2-PLC activation, rather than subsequent cellular events. The differential regulation of response to muscarinic and α_1 receptor activation may implicate separate receptor-PLC complexes and/or PI substrate domains. The simple additivity between adrenergic and muscarinic activation of PI-PLC supports the presence of parallel pathways. Both types of receptors are thought to exist on the same myometrial cells, but we know of no direct evidence for this. Alternatively, estrogen could alter cellular levels of a specific PI-kinase (in a manner analogous to the effect of platelet-derived growth factor) (18), which could increase the available substrate (PIP2) for PIP2-PLC activated by α_1 but conceivably not by muscarinic receptors. However, we are not aware of any direct evidence for separate, receptor-specific pools of PIP2 in myometrium. Perhaps a more reasonable explanation would be an estrogen effect directed toward a coupling protein acting between the receptor and PIP2-PLC. Guanyl nucleotide-regulatory proteins have been implicated as transducers for agonist-stimulated PIP2-PLC activation in several tissues (19–21). A selective effect of estrogen on the transducer for α_1 but not muscarinic PIP2-PLC activation would provide a basis for differential hormone targeting (as would different transducers).

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