Effect of Progesterone on the Stimulation of Phosphatidylinositol Turnover by Epinephrine in Guinea Pig Ductus Deferens

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Abstract—Progesterone enhanced the contractile effect of epinephrine on the ductus deferens of the guinea pig in vitro. In relation to this mechanical phenomenon, we examined the phosphatidylinositol metabolism. In the 3H-myoinositol labeled ductus deferens, radioactivity in phosphatidylinositol bisphosphate was about 2.6 times as high as that in phosphatidylinositol. Phosphatidylinositol and phosphatidylinositol phosphate were not changed by epinephrine (100 μM), but phosphatidylinositol bisphosphate was increased at 10 sec and 1 min after the administration of epinephrine (100 μM). Progesterone (100 μM) added 5 min before the administration of epinephrine increased the stimulatory effect of epinephrine on the phosphatidylinositol bisphosphate metabolism, but had no effect on the phosphatidylinositol and phosphatidylinositol phosphate metabolism. These studies suggest that progesterone expresses its activity not through the cytoplasmic progesterone receptor but through the epinephrine-mediated smooth-muscle contractile mechanism.

The hypothesis of a universal mechanism of steroid hormone action had been proposed by Thompson and Lippman (1). Although steroids have a classical receptor-mediated pathway of hormonal action, it is not sufficient to account for all the known effects of steroids. For example, Baulieu and co-workers (2) have demonstrated that progesterone and other steroid molecules can prompt the maturation of *Xenopus laevis* oocytes, although these cells do not contain steroid receptors. Kaya and Saito (3) have demonstrated that steroids have a direct, non-genomic effect on the erythrocyte membrane. Sanchez-Bueno et al. (4) reported that estradiol increased the chicken liver glycogen phosphorylase activity prior to the increases of protein synthesis. Egana et al. (5) and Diez et al. (6) have found for cortisol and testosterone an early glycojenolytic effect prior to and independent of protein synthesis. Therefore, it seems that in addition to the receptor-mediated mechanism, steroids may also operate through other mechanisms; and in particular, they may act through an effect on the plasma membrane. In our previous report (7), we examined the direct, non-genomic effects of progesterone on the contraction and relaxation of the smooth muscles in relation to the adrenergic mechanism. In the present paper, we examined the direct, non-genomic effect of progesterone on the smooth muscles of the ductus deferens of the guinea pig in relation to the phosphatidylinositol (PI) response.

Materials and Methods

Materials: The following drugs were used: 4-Pregnene-3,20-dione (progesterone), Sigma Chemical Co.; epinephrine, Daiichi Chemical Co.; myo-[2-3H]inositol (specific activity 14 Ci/mmol) and ACSII scintillation mixture, Amersham. All other chemicals were purchased from Wako Pure Chemical Ind. Progesterone dissolved in propylene glycol.
was used. Propylene glycol used as a vehicle had no effect on the PI response. Other drugs were dissolved in distilled water.

Preparation and incubation of ductus deferens. Ductus deferens from Hartley guinea pigs weighing 200–300 g was quickly excised and placed in ice-cold Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4, 0.9% NaCl, 1.15% KCl, 1.22% CaCl$_2$, 2.11% KH$_2$PO$_4$, 3.82% MgSO$_4$·7H$_2$O, 2.6% NaHCO$_3$) containing 10 mM D-glucose. The tissues were trimmed to remove foreign tissues, and ducti were flushed with KRB buffer to wash out the seminal contents. The tissue was cut into transverse slices from 0.5 to 1.0 mm in width.

To label the tissue phospholipids, the portions (about 300 mg wet wt.) were incubated in 1 ml KRB buffer with 10 mM D-glucose that contained 10 μCi of myo-[2-$^3$H]inositol for 60 min at 37°C. At the end of the incubation, the slices were washed three times with 3 ml of ice-cold fresh KRB buffer with 10 mM D-glucose and were reincubated for 30 min at 37°C in 3 ml fresh KRB buffer that contained 10 mM D-glucose to minimize a large pool of labeled myoinositol which might be retained (8). After reincubation, the labeled slices were separated into 6 tubes (in each tube, about 50 mg), then incubated in 3 ml of KRB buffer for 10 sec, 1 min, 5 min and 15 min, respectively, at 37°C in the presence or absence of epinephrine (100 μM). At the end of the incubation, the slices were washed three times with 3 ml of ice-cold 5% trichloroacetic acid, suspended in 2 ml of chloroform/methanol (2:1, v/v), and homogenized in a glass homogenizer. The homogenate was transferred to a test tube and 4 ml of chloroform/methanol was added. To the combined homogenate, 1 ml of 0.5 M KCl was added and mixed. The homogenate was then centrifuged at 2400 g for 20 min and the lower phase was transferred for analysis of polyphosphoinositides (9, 10).

Analysis of polyphosphoinositides: Polyphosphoinositides were analyzed by the method of Takenawa et al. (10, 11). Silica Gel 60-precoated TLC plates (Merck) were used, and a modified developing system was used (chloroform/methanol/28% ammonium hydroxide/water, 21:17:3:2). Each spot was scrapped off and removed to vials. Five ml of ACSII scintillation mixture was added to each vial, and tritium was assayed by measuring the radioactivity of each sample for 10 min in a liquid scintillation spectrometer (ALOKA LSC-900).

Phosphorus content was determined by a Phospholipid B-Test from Wako. The radioactivity was expressed as the radioactivity per mg of lipid phosphorus.

Results

In the $^3$H-inositol labeled ductus deferens, the radioactivity in phosphatidylinositol bisphosphate (TPI) was about 2.6 times as high as that in PI.

After the incubation with epinephrine at a concentration of 100 μM, the labeling of TPI in $^3$H-inositol-labeled ductus deferens was increased significantly at 10 sec and 1 min, but it was reduced to the control level after 5 min (Fig. 1).

There was no significant change in the labeling of PI and phosphatidylinositol phosphate (DPI).

The incubation of the tissues with progesterone (100 μM), which was added before the application of epinephrine, increased the labeling of epinephrine (100 μM)-stimulated TPI. PI and DPI were not significantly changed by the incubation with progesterone (Table 1).

Discussion

The present studies were performed to show the non-genomic effect of progesterone on smooth muscles. We reported (7) that progesterone increased the contractile response of guinea pig smooth muscles (ductus deferens, uterus and taenia caecum) to epinephrine, and on the contrary, progesterone inhibited the contraction of rabbit uterus and ductus deferens caused by epinephrine. Progesterone immediately relaxed the Ca$^{2+}$-induced contraction of the ductus deferens.
and uterus of rabbit and guinea pig maintained in K+-Tyrode's solution (7). Brink et al. (12) reported the similar effects of cortisol in the airway smooth muscle of the guinea pig. The mechanism responsible for the increasing effect on the adrenergic response of smooth muscles, however, remains largely unknown. In the present study, we examined the direct, non-genomic effects of progesterone on the plasma membrane-dependent biochemical phenomena in relation to the epinephrine contraction of smooth muscle in guinea pig.

The contractile mechanism of smooth muscles was profoundly influenced by calcium ions. Then we examined the PI-response that seemed to be substantially related to Ca^{2+} channels (13). We measured the radioactivities of PI, DPI and TPI at 10 sec, 1 min, 5 min and 15 min. However, after the addition of epinephrine, the radioactivity of TPI increased at 10 sec and 1 min, but returned to the control level at 5 min.

The traditional notion of PI-response is that first of all, TPI breakdown occurs, followed by DPI and PI breakdown. In the presence of calcium, inositol tris-phosphate (IP_3) and phosphatic acid carry calcium ions. However, Dixon (14) and Hokin-Neaverson (15) recently re-

### Table 1. Effect of progesterone on epinephrine-stimulated PI, DPI and TPI levels

|                | PI (dpm) | DPI (dpm) | TPI (dpm) |
|----------------|----------|-----------|-----------|
| Epinephrine    |          |           |           |
| 100 μM         | 1641.8±295.0 (100%) | 134.6±32.2 (100%) | 4443.7±1461.7 (100%) |
| Progesterone    |          |           |           |
| + Epinephrine  | 100 μM   | 2272.9±464.0 (138%) | 147.8±22.0 (110%) | 11358.7±3452.8 (256%) |

The ^3H^-inositol labeled ductus deferens was incubated for 5 min at 37°C in the medium containing 100 μM progesterone. Then the tissue was incubated for 10 sec at 37°C in the medium containing 100 μM epinephrine. Results are means±S.E.M. for three separate experiments conducted in triplicate. In parentheses, the results without progesterone are shown as 100 percent. PI: phosphatidylinositol, DPI: phosphatidylinositol phosphate, TPI: phosphatidylinositol bisphosphate. P: lipid phosphorus.

![Fig. 1. Effect of epinephrine on the degradation of PI, DPI and TPI](image)
ported that the metabolism of PI was different from the metabolism of DPI and TPI. Soukup (16) reported that TPI was increased by carbamylcholine. Furthermore, Akhtar (17) reported that the labeling of PI was higher than that of TPI. The present results, however, have shown that the labeling of TPI is higher than that of PI. Recently, the roles of protein kinase C, 1,2-diacylglycerol, IP$_3$ and Ca$^{2+}$ as the intracellular second messengers have been established (18, 19).

TPI acts as a precursor of 1,2-diacylglycerol which activates protein kinase C and opening of the Ca channel (19). Therefore, the remarkable increase in the TPI level in the ductus deferens of guinea pigs after incubation with progesterone suggests that progesterone stimulates the PI turnover in the smooth muscle. Epinephrine acts as a ligand on the adrenergic a$_1$-receptor in the membrane and initiates the PI turnover mechanism. Progesterone increased TPI as a precursor of 1,2-diacylglycerol and finally increased the contractile activity of epinephrine on the guinea pig ductus deferens. However, the direct relationship between the increase of TPI and muscle contraction remains to be studied.

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