Effect of lysophosphatidic acid on the preimplantation development of mouse embryos

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1. Introduction

Lysophosphatidic acid (LPA) is a naturally occurring phospholipid and a key intermediate in the pathway of synthesis of glycerophospholipids in many kinds of animal cells. It is also present as a vasopressor in incubated plasma of mammals in which it is generated from lysophosphatidylcholine by the action of phospholipase D [1].

LPA is known to have other biological activities such as platelet aggregation [2, 3], smooth muscle contraction [4], stimulation of DNA synthesis and proliferation of fibroblasts [5-7] and evocation of an inward Cl⁻ current in Xenopus laevis oocytes [8-10]. Tigyi and Miledi [11] identified albumin-bound LPAs in human serum as active components for induction of Ca²⁺-activated Cl⁻ current in Xenopus laevis oocytes and of neurite retraction in PC 12 cells. Recently the effects of LPA on oocytes were suggested to be mediated by a specific membrane receptor linked to a pertussis toxin (PT) sensitive Gi-protein [10]. However, the influence of LPA on embryo development has not been clarified.

In this study, we examined the effect of LPA on the preimplantation development of ICR x ICR mouse embryos obtained by fertilization in vitro.

2. Materials and methods

2.1. Superovulation and in vitro fertilization

Ovulated eggs were obtained from 3-week-old female ICR strain mice (CD-1, Japan Charles River Co., Yokohama) and epididymal spermatozoa from 8-week-old male ICR mice (CD-1, Japan Charles River). The female mice were induced to superovulate by intraperitoneal injection of 5 IU pregnant mare serum gonadotropin (Teikoku Hormone MFG Co., Tokyo) and 48 h later of 5 IU human chorionic gonadotropin (hCG) (Teikoku Hormone MFG). The oocytes were recovered 15 h after hCG injection and placed in 0.3 ml of Whittingham's T6 medium supplemented with 0.4% bovine serum albumin (Sigma Chemical Co., St. Louis, MO) under paraffin oil. This medium is referred to basic CM. Sperm pellets obtained from the epididymides were placed in 0.3 ml of basic CM under 5% CO₂ in humidified air for 1.5 h. The oocytes were inseminated with motile spermatozoa (5-10 x 10⁶ cells/ml) and cultured in 0.3 ml of basic CM under paraffin oil for 5-6 h. Fertilization was assessed by noting the presence of two pronuclei and a second polar body.

2.2. Effect of LPA on embryo development

A stock solution of 1.0 mM oleoyl-LPA (Sigma) was prepared in phosphate-buffered saline with 1% bovine serum albumin, and kept at -20°C until use.

Mouse embryos were incubated in basic CM for 6 h after insemination. Pronuclear stage embryos were then transferred into fresh basic CM with and without various concentrations of LPA (10⁻⁴, 10⁻³, 10⁻² M) and incubated for 90 h. Development of the preimplantation embryos was examined every 24 h under a dissection microscope. The rates of development from the pronuclear, 2-cell and 4-cell stage, respectively, to blastocysts (blastocyst rates) were compared.

For determination of the relationship between the effect of LPA and the stage of embryonic development, 10⁻³ M of LPA was added to basic CM at the pronuclear stage or the 4-cell stage. Pronuclear stage embryos were selected 6 h after insemination and divided into 3 groups. As a control, the first group was cultured continuously in basic CM under 5% CO₂ in humidified air for 90 h. The second group was cultured in basic CM containing 10⁻³ M of LPA under the same conditions as the first group for 90 h. The third group was cultured in fresh basic CM for 42 h, and then 4-cell embryos were selected, and cultured further in basic CM containing 10⁻³ M of LPA for 48 h. The blastocyst rates of the three groups were then compared.

To demonstrate that a Gi-protein is involved in the embryo development stimulated with LPA, mouse embryos cultured in basic CM for 6 h after insemination were then incubated with (2 groups) or without...
Table 1
Effect of LPA on mouse embryo development

<table>
<thead>
<tr>
<th></th>
<th>No. of pronuclear embryos</th>
<th>No. of 2-cell embryos</th>
<th>No. of embryos developing from 2-cell to 4-cell stage</th>
<th>No. of embryos developing from 2-cell stage to blastocysts</th>
<th>No. of embryos developing from 4-cell stage to blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic CM (control)</td>
<td>115</td>
<td>101</td>
<td>48 (47.5)</td>
<td>17 (16.8)</td>
<td>17 (35.4)</td>
</tr>
<tr>
<td>Basic CM + LPA (M)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>101</td>
<td>93</td>
<td>52 (55.9)</td>
<td>26 (28.0)</td>
<td>26 (50.0)</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>96</td>
<td>88</td>
<td>38 (43.2)</td>
<td>22 (25.0)</td>
<td>22 (37.9)*</td>
</tr>
<tr>
<td>$10^{-6}$</td>
<td>99</td>
<td>92</td>
<td>39 (42.4)</td>
<td>24 (26.1)</td>
<td>24 (61.5)*</td>
</tr>
<tr>
<td>$10^{-7}$</td>
<td>128</td>
<td>117</td>
<td>63 (53.9)</td>
<td>38 (32.5)*</td>
<td>38 (60.3)*</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

*Significantly different from control ($P < 0.01$).

(2 groups) 200 ng/ml of PT (Funakoshi Co., Tokyo) for 18 h. Two-cell embryos were then selected and cultured in droplets of basic CM (control or PT-treated) or basic CM containing $10^{-5}$ M of LPA (LPA-treated or PT+LPA-treated) under 5% CO$_2$ in humidified air for 72 h, and the blastocyst rates of the four groups were compared.

2.3. Statistical analysis

The chi-square test was used for statistical analysis.

3. Results

Mouse embryos were cultured for up to 90 h in basic CM with or without LPA ($10^{-8}$-$10^{-5}$M), and results are shown in Table 1. There was no significant difference in the rates of development of embryos from the pronuclear stage to the 4-cell stage in the five groups. However, 32.5% of the 2-cell stage embryos developed to blastocysts when $10^{-5}$M of LPA was added to the basic CM at the pronuclear stage, whereas only 16.8% did so in the absence of LPA ($P < 0.01$). Furthermore, the rates of development from the 4-cell stage to blastocysts were significantly higher in the presence of LPA ($10^{-5}$, $10^{-6}$ or $10^{-7}$ M) than in its absence ($P < 0.05$).

As shown in Table 2, the blastocyst rate from 4-cell stage embryos was 35.4% in the control group, and addition of LPA ($10^{-5}$ M) at the pronuclear stage increased the rate to 54.3%. However, addition of LPA at the 4-cell stage did not increase the blastocyst rate over that of the control group.

Table 2
Relationship between the embryonic stage and the stimulating effect of LPA

<table>
<thead>
<tr>
<th></th>
<th>No. of 4-cell embryos</th>
<th>No. of embryos developing from 4-cell stage to blastocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic CM (control)</td>
<td>79</td>
<td>28 (35.4)</td>
</tr>
<tr>
<td>Basic CM + LPA (4-cell)</td>
<td>103</td>
<td>39 (37.9)</td>
</tr>
<tr>
<td>Basic CM + LPA (pronuclear)</td>
<td>92</td>
<td>50 (54.3)*</td>
</tr>
</tbody>
</table>

Values in parentheses are percentages.

*Significantly different from control ($P < 0.05$).

When pronuclear stage embryos pretreated with PT were cultured in basic CM containing $10^{-5}$ M of LPA, the blastocyst rates from the 2-cell and 4-cell stages were 16.3% and 38.5%, respectively. These rates were comparable with those of the control group and the group treated with PT alone, but were significantly lower than those of the group treated with LPA alone (Table 3).

4. Discussion

There are several reports of the significance of growth factors such as insulin [12-14] and insulin-like growth factor-1 [12-14] and epidermal growth factor [15] in mouse embryo development. Other as yet unidentified growth factors may also be involved in embryo development. LPA is a newly discovered mitogen for fibroblasts [5-7] and an activator of *Xenopus laevis* oocytes [B-lo]. Recently we found that incubated human follicular fluid contained a considerable amount of LPA (unpublished observation). Therefore, in this study, we examined the effect of LPA on mouse embryo development.

ICR x ICR mouse embryos obtained by fertilization in vitro tend to stop development at the 2-cell stage, a phenomenon named '2-cell-block'. This 2-cell-block is considered to be due to lack of vital components produced by transcription from the embryonic genome or by synthesis from maternal messenger RNA [16]. In this study, we found that about 20% of 2-cell embryos escaped 2-cell-block, developing to blastocysts in basic CM. Addition of LPA to the basic CM at the pronuclear stage significantly increased the blastocyst rate from the 4-cell stage, but not the rate of development from the 2-cell to 4-cell stage. These findings suggest that the stimulating effect of LPA on mouse embryo development is not attributable to an effect in overcoming 2-cell-block. As LPA has been reported to stimulate DNA synthesis and proliferation of fibroblasts, it may act as a growth factor and may stimulate cell division of mouse embryos directly.
The finding that LPA significantly increased the blastocyst rate from the 4-cell stage, but not the rate from 2-cell to 4-cell embryos (Table 1) suggested that it was not essential for the development of embryos from the pronuclear stage to the 4-cell stage. To examine this possibility, we added LPA to the culture medium at the pronuclear stage (Table 2), 2-cell stage (Table 3) and 4-cell stage (Table 2), respectively, and compared the resulting blastocyst rates with that of control embryos.

When LPA was added at the 4-cell stage, about 38% of the 4-cell embryos developed to blastocysts, and this value was comparable with that of the control group. However, when LPA was added for 90 h and 72 h from the pronuclear and 2-cell stage, respectively, the blastocyst rates were significantly higher. These results indicate that the presence of LPA from the 2-cell stage is necessary for its stimulating effect on mouse embryo development to blastocysts.

Both the mitogenic action of LPA on fibroblasts [5] and its activating effect on oocytes [10] are reported to be mediated through G-protein or related PT substrate. In this study, we found that pretreatment of pro-nuclear stage embryos with PT for 18 h resulted in block in a GTP-dependent manner and (iii) activation of a PT-sensitive Gi-protein and considered to be a member of the superfamily of seven-transmembrane-domain receptors [18].

From our results and these reports, we conclude that LPA promotes the preimplantation development of mouse embryos and that its effect is probably mediated by a Gi-protein linked receptor on the surface of mouse embryos.

References