Cortical and zona reactions of heat-activated mouse eggs

A. Komar and M. Kujawa*

Laboratory of Experimental Embryology, Department of Histology and Embryology and *Laboratory of Electron Microscopy, Institute of Biostructure, Medical School of Warsaw, Chatubińskiego 5, 02-004 Warsaw, Poland

Summary. Oocytes collected from superovulated Swiss albino mice were activated by heat-shock and/or fertilized in vitro. Electron microscopy showed that the cortical reaction in heat-treated eggs was incomplete. Digestion of the zona pellucida of untreated, heat-treated and fertilized eggs by pronase showed that zona pellucida hardening did occur but was weaker in heat-treated than in fertilized eggs. Fertilizability of heat-treated eggs decreased 1.5 h after heating. We concluded that, in heat-activated eggs, changes occur at the zona and plasmalemma but they are not identical with those in fertilized eggs.

Introduction

Agents that parthenogenetically activate mammalian eggs, with varied efficacy, also evoke the cortical granule reaction and zona reaction (for review, see Gulyas, 1980). The aim of the present study was to determine whether the cortical granule reaction, and hence the zona reaction, takes place in the heat-treated eggs.

Materials and Methods

Experiments were carried out with oocytes collected from outbred Swiss albino mice induced to superovulate by i.p. injections of 5–7.5 i.u. PMSG (Gestyl, Organon) and 5–7.5 i.u. hCG (Chorulon, Intervet) 48–53 h apart. The oocytes were recovered 16 h after hCG injection and exposed to heat (44.5°C) for 5 min (Komar, 1973). Untreated control and heat-treated eggs were fertilized according to the method of Toyoda, Yokoyama & Hosi (1971).

Transmission electron microscopy. Untreated and heat-treated (1 and 3 h after heat treatment) oocytes (20/group) were prepared for electron microscopy by the method of Konwiński, Abramczuk, Barańska & Szymkowiak (1974).

The oocytes were then embedded in Epon 812 (Glauert, 1974). Ultrathin sections were cut on a Reichert OmU-3 ultramicrotome, stained with uranyl acetate (Watson, 1958) and lead citrate (Venable & Coggeshall, 1965) and examined in a JEM 100 C electron microscope. In each group 100 randomly selected sections (5 from each oocyte) were analysed.

Ultrastructural morphometrical evaluation was based on stereological methods (Weibel, 1969) and done on electronmicrographs prepared at a final magnification of × 17,000.

The results were tested statistically by one-way analysis of variance and values of $P < 0.05$ were considered to be statistically significant.

Enzymic digestion. Experiments were carried out with untreated, heat-treated and fertilized eggs recovered at 19 h after hCG. Untreated and heat-treated eggs were treated with 0.025% hyaluronidase to disperse the follicular cells. Only heat-treated eggs that contained a pronucleus, or pronuclei, or had cleaved were examined.
In all 3 groups the eggs were digested with 0.035% pronase (Sigma) in phosphate buffer, pH 7.2. The pronase was in 0.1 ml drops overlayed with paraffin oil on a heating plate (37°C). Enzyme concentration, temperature and pH at which the experiments were carried out were carefully checked. The eggs were examined every 10 min to determine the time required for digestion of the zona pellucida. The mean time required for dissolution of the zonae was calculated separately for each group of eggs. The statistical significance of mean differences was evaluated by Student’s t test.

**Fertilizability of heat-treated eggs.** Heat-treated zona-intact oocytes were inseminated for up to 1.5 h (Group 1) and for up to 3 h (Group 2) after heating. Oocytes were cultured for 6–7 h at 37.5°C and 5% CO₂ in air. Whole-mount preparations were made and the eggs were examined by light microscopy.

**Results**

**Electron microscopy**

There were many cortical granules, uniformly distributed just beneath the plasma membrane, in untreated eggs which were at 2nd metaphase.

After 1 h after exposure to heat the oocytes were at 2nd telophase and there was a significant decrease in both the volume fraction and number of cortical granules per unit area compared with control eggs (Table 1). Large surface areas were devoid of cortical granules, although other areas contained a few apparently unaltered granules. Migration of granules to the egg centre or extrusion of intact granules into the perivitelline space was not observed.

At 3 h after heat treatment the eggs were at the pronuclear stage and only single cortical granules were found at the egg surface.

The difference in cortical granule numbers in heat-treated eggs at 1 and 3 h was significant (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Volume fraction (Vᵣ %) and mean number of cortical granule profiles per 100µm² surface area (Nₐ) of cytoplasm cross-section in untreated and heat-treated mouse oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oocytes</strong></td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Untreated</td>
</tr>
<tr>
<td>1 h after heating</td>
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<tr>
<td>3 h after heating</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m.

* P < 0.05 compared with value for untreated oocytes.
† P < 0.05 compared with value for oocytes 1 h after heating.

**Enzymic digestion**

Significant differences (P < 0.001) in the solubility of zonae were found; the mean time for removal of the zona was 52.4 ± 0.69 min for untreated eggs (n = 104), 64.3 ± 0.79 for heat-treated eggs (n = 72) and 70.6 ± 0.56 min for fertilized eggs (n = 51).

**Fertilizability of heat-treated eggs**

The results in Table 2 indicate that fewer eggs were fertilized when eggs were inseminated 1.5–3 h after heat treatment. There was no obvious effect on numbers of supernumerary spermatozoa (data not shown).
Table 2. Penetration of zona-intact heat-treated mouse eggs inseminated at two times after heat treatment

<table>
<thead>
<tr>
<th>Time of insemination (h)</th>
<th>Heat-treated eggs</th>
<th>Control eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activated</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of eggs</td>
<td>No. fertilized (%)</td>
</tr>
<tr>
<td>&lt;1-5</td>
<td>446</td>
<td>119* (26-7)</td>
</tr>
<tr>
<td>1-5-3</td>
<td>288</td>
<td>47* (16-3)</td>
</tr>
</tbody>
</table>

* P < 0-01 (χ² test).

Discussion

In heat-treated eggs about one third of the normal number of cortical granules were observed by electron microscopy and we consider that this indicates an incomplete cortical reaction. Artificial activation of mouse eggs by an electric shock (Gulyas, 1976) or osmotic activation with hyaluronidase (Solter, Biczysko, Graham, Pieńkowski & Koprowski, 1974) also gives a partial or incomplete cortical reaction when studied with transmission electron microscopy. However, cortical granules can be effectively extruded from mouse oocytes by calcium-free medium (Whittingham & Siracusa, 1978), injection of free Ca ++ into the oocyte (Fulton & Whittingham, 1978) or the ionophore A 23187 (Gulyas & Schmell, 1980).

The mean time needed for digestion of the zonae of heat-activated eggs, 3 h after treatment was intermediate between that required for fertilized and unfertilized eggs, as also found by Mintz & Gearhart (1973) for mouse parthenogenones. Because Schmell & Gulyas (1980) reported that the zona reaction occurs within 3 h after exposure of mouse eggs to the ionophore A 23187, we conclude that, in heat-activated eggs, hardening of the zona, one indication of the zona reaction, is incomplete.

In zona-intact mouse eggs the block to polyspermy is very fast and develops within 1 min after fertilization (Sato, 1979). Glycoprotein ZP3 has receptor activity and is responsible for the binding of spermatozoa to unfertilized mouse eggs (Bleil & Wassarman, 1980). We observed that spermatozoa bound to the zona and penetrated the cytoplasm of heat-treated eggs up to 3 h after treatment but the fertilizability of treated eggs was reduced after 1-5 h. We therefore might conclude that neither the zona nor the plasmalemma block was fully efficient and that ZP3 was not changed in heat-treated eggs.

The present study indicates that a high temperature (44·5°C) stimulated egg development but this parthenogenetic stimulus could not elicit the whole chain of reactions accomplished by the spermatozoon.

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References


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