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MEMBRANE CONDUCTANCE CHANGES DURING OOCYTE MATURATION IN THE TELEOST ORYZIAS LATIPES

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Summary

Oocyte maturation in a number of species is accompanied by changes in the membrane potential. This seems to be due to modification of the membrane conductance to particular ions. Apart from a study of denuded oocytes in the brook trout Salvelinus fontinalis (Marshall, et al., 1985) there is a paucity of such knowledge in fish. This report concerns oocyte maturation in the medaka, Oryzias latipes. These preliminary results from follicular cell enclosed oocytes indicate that the membrane depolarizes during maturation due at least in part to a decrease in the membrane conductance to K⁺.

Introduction

At the end of vitellogenesis when the growth phase of oogenesis is complete, oocytes remain blocked in meiosis. Follicular cells surrounding oocytes are stimulated at the appropriate moment to produce a hormone which acts directly on the oocyte and triggers maturation and the reinitiation of meiosis. One of the rare studies of the electrical properties of fish oocyte during maturation was carried out in the brook trout, oocytes having had their follicular cell layers removed (Marshall et al. 1985). In this present study certain aspects of the membrane electrical properties of medaka oocytes have been investigated. The medaka offers many advantages for such studies. The small fish are easy to keep and by controlling the photoperiod and temperature, oocytes and eggs can be produced by the same fish every day throughout the year. It is also easy to observe the major morphological criterion for successful maturation — germinal vesicle breakdown (GVBD).

Materials and Methods

Actively reproducing fish were kept at a temperature of 28°C under a photoperiod (14 h light - 10 h dark) (Iwamatsu, 1974). In order to obtain unfertilized eggs, reproducing female fish were isolated from males the day before they were needed in a beaker which was returned to float in the same aquarium. This prevented male behaviour from stimulating oviposition at daybreak and subsequent fertilization. Fish were decapitated, the ovaries removed and eggs and growing oocytes isolated in modified Yamamoto Ringer (YR) (pH 7.3 buffered with 10 mM Hepes) (Yamamoto, 1941). Oocytes were mounted in a small plexiglass chamber with a coverslip base fixed to which was a grid of fine gold wires. Standard electrophysiological equipment was used to record membrane potentials and to voltage clamp oocytes.

Results

Generally, immature medaka oocytes were much easier to impale than eggs and the recordings were more stable (Fig. 1).

![Fig. 1. Recordings of the membrane potential in six different immature oocytes of medaka.](image)

Membrane potentials were also much more negative in the immature than the mature oocyte (Fig. 1). A sample of 26 immature oocytes had a mean membrane potential of −60.7 ± 3.0 mV (SD) compared with −26.0 ± 9.9 mV in 30 mature oocytes.
Immature oocytes were almost exclusively isolated from ovaries removed from fish at different times during the light period. The mean diameters of the groups of oocytes isolated each day ranged from 1.06 to 1.24 mm but there was no apparent difference in membrane potential related to the time of isolation and thus the size of the oocytes.

The stable recordings permitted the membrane potential to be monitored continuously over a long period and would remain close to the mean value for many hours if oocytes were removed well before the start of the dark period. Such oocytes remained immature. However, oocytes removed later would gradually undergo membrane depolarization (Fig. 2).

Some immature oocytes were impaled by a second microelectrode allowing current to be injected to modify the membrane potential and enable the membrane resistance to be determined. Current/voltage relationships of an oocyte before and after maturation have been plotted in Fig. 3 and represent the typical differences found between immature and mature oocytes.

Fig. 2. The membrane potential of a single oocyte in relation to the time of onset of the dark period. The individual points were taken from a continuous recording.

This oocyte was isolated just at the beginning of the 10 h dark period and impaled 2 h later. At the end of recording the oocyte had undergone GVBD and subsequently ovulated. In some cases oocytes ovulated during recording without manifesting any marked modification of the membrane potential.

Fig. 3. Comparison of the current/voltage relationships of the immature (o) and mature (e) medaka oocyte. A two electrode voltage clamp was used, both microelectrodes remaining inserted in the oocyte throughout maturation.

The immature oocyte revealed a steeper linear relationship indicating a relatively smaller membrane resistance (1 Megaohm) and thus greater membrane conductance than in the mature oocyte (12.5 Megaohms). Thus maturation of the medaka oocyte leads to a decrease in membrane conductance. The two traces intercept the zero current axis at different points on the voltage axis which indicate the different membrane potentials of the immature and mature oocytes.
Fig. 4. The relationship between the membrane potential of 3 immature medaka oocytes and the external $[K^+]$. Bars indicate ±SD.

Variation in the concentration of $K^+ ([K^+])$ led to a 43 mV change in membrane potential for a 10 fold change in $[K^+]$ (range 10 mM to 100 mM) for 3 immature oocytes (Fig. 4).

Discussion

Our investigation shows that the medaka oocyte membrane depolarizes dramatically during maturation due to a decrease in membrane conductance. At least part of this decrease involves a reduction of the membrane conductance to $K^+$ from a 43 mV/10 fold change in the $[K^+]$ in the immature oocyte to about 7 mV in the ovulated egg.

In the brook trout, using denuded oocytes (Marshall et al., 1985), meiotic maturation was accompanied by a much smaller membrane depolarization from -48 to -38 mV than in the medaka (-60 to -28 mV) in which the follicular layer remained intact. The reduction in $K^+$ conductance, from 29 mV/10 fold change, in the denuded trout oocytes to 7 mV was also less than in the medaka. The ovulated eggs of both species had similar relationships between membrane potential and $[K^+]$ (see also Nuccitelli, 1980). As suggested by the authors of the study on brook trout, removal of the follicular layer may modify the membrane electrical properties of the oocyte. The differences in membrane resistance between the two species and their increases during maturation have not been too closely compared owing to the presence of the follicular layer in the medaka.

This preliminary study will now be extended to the defolliculated medaka oocyte which will address the possible contribution of the follicular cells to the changes in membrane conductance during maturation. However, the relative contribution of each cell type to the membrane ionic conductance changes will require closer study of the time course of these events and the role of ions in addition to $K^+$.

References


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