Bioactivity of various forms of GnRH in relation to their resistance to degradation at the pituitary level in the rainbow trout, Oncorhynchus mykiss

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HAL Id: hal-02779234
https://hal.inrae.fr/hal-02779234v1

Submitted on 4 Jun 2020

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
criterium for successful maturation
- germinal vesicle breakdown (GVBD).

Materials and Methods

Actively reproducing fish were
kept at a temperature of 26°C under
a photoperiod (14 h light - 10 h
dark) (Iwamatsu, 1974). Inorder 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
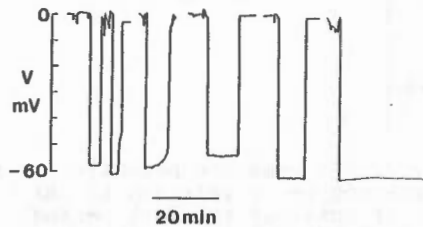than eggs and the recordings were
more stable (Fig. 1).

Fig. 1. Recordings of the membrane
potential in six different immature
oocytes of medaka.

Membrane potentials were also much
more negative in the immature than
the mature oocyte (Fig. 1). A sample
of 29 immature oocytes had a mean
membrane potential of -60.7 ± 3.0 mV
(SD) compared with -28.0 ± 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).
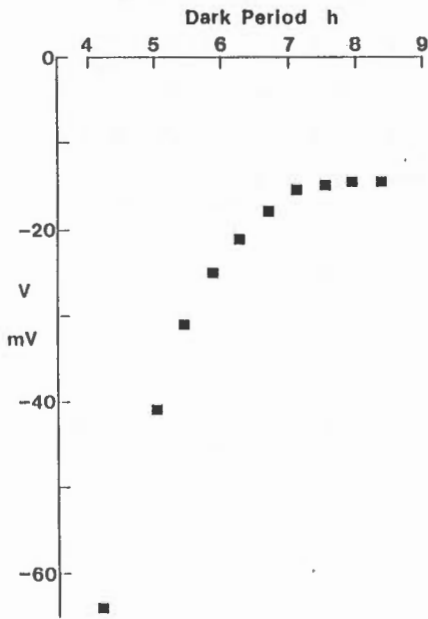
Fig. 2. The membrane potential of a single oocyte in relation to the time of onset of the dark period. The individual points were taken from a continuous recording.

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.

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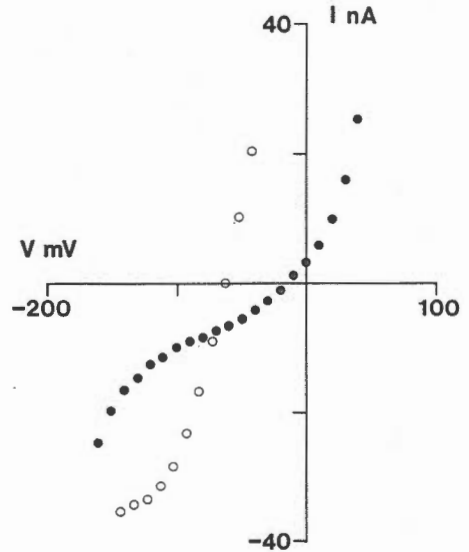
Fig. 3. Comparison of the current/voltage relationships of the immature(o) and mature(•) medaka oocyte. A two electrode voltage clamp was used, both microelectrodes remaining inserted in the oocyte throughout maturation.

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.
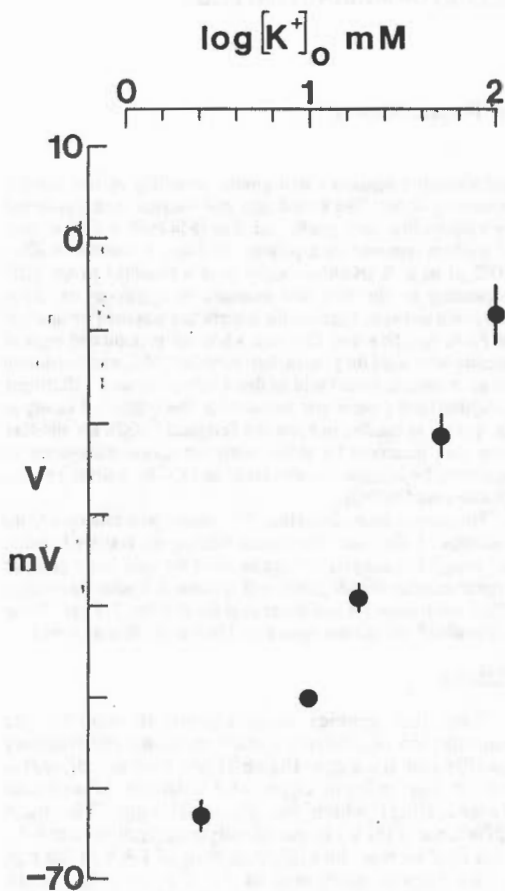
310

log $[K^+]_o$ mM

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 depolarises 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 -46 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

Iwamatsu, I. 1974. Studies on oocyte maturation of the Medaka, *Oryzias latipes*. Annot. zool. Japon. 47: 30-42

Marshall, W.S., Habibi, H.R. and C.A. Lessman, 1985. Electrophysiology of oocytes during meiotic maturation and after ovulation in brook trout (*Salvelinus fontinalis*). Can. J. Zool. 63: 1904-1908.

Nuccitelli, R. 1980. The electrical changes accompanying fertilization and cortical vesicle secretion in the medaka egg. Dev. Biol. 76: 483-498.

Yamamoto, T. 1941. The osmotic properties of the egg of fresh water fish, *Oryzias latipes*. J. Fac. Sci. Tokyo Univ. IV 5: 461-472.

Proceedings

of the

Fourth International Symposium

on the

Reproductive Physiology of Fish

University of East Anglia, Norwich, U. K., 7-12 July 1991

Edited by: A. P. Scott, J. P. Sumpter, D. E. Kime, and M. S. Rolfe

Published by FishSymp 91, Sheffield.

Proceedings of the Fourth International Symposium on the Reproductive Physiology of Fish, University of East Anglia, Norwich, U. K., 7-12 July 1991

A. P. Scott, J. P. Sumpter, D. E. Kime and M. S. Rolfe (Editors)