DECREASED UPTAKE OF [^{3}H]LEUCINE DURING PROGESTERONE INDUCED MATURATION OF *XENOPUS LAEVIS* OOCYTES

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1. Introduction

There is much evidence that full grown amphibian oocytes, arrested at the late diplotene stage of meiosis, are triggered by progesterone or a progesterone-like hormone (following a hypophyseal gonadotropin event) in order to undergo maturation. Of all compounds which have been tested, progesterone is the most potent in producing maturation in vitro, followed by deoxycorticosterone and cortisol [1]. Recently a demonstration has been obtained that 'maturation' induced in vitro by progesterone is pertinent to the physiological phenomenon, and allows eventual further development [2].

Changes in permeability of the *Xenopus* oocyte membrane have been much studied. Smith and Ecker [3] and Ecker and Smith [4], using *Rana pipiens* oocytes, indicated a change of permeability, as one of the consequences of hormone activity, in relation to the processes of maturation and ovulation. They were able to show that the maturing oocyte undergoes a marked change in permeability to exogenous materials such as puromycin, beginning with germinal vesicles dissolution Morrill [5] and Morrill, Rosenthal and Watson [6] have suggested that the hormones which induce ovulation in amphibians may also alter the ion permeability of the egg membrane. Very recently, Schuetz, Wallace and Dumont [7] indicate a deoxycorticosterone acetate induced inhibition of protein ([^{3}H]vitellogenin) incorporation by isolated *Rana pipiens* oocytes in vitro.

In this report, it is shown that progesterone can be implicated in an early and profond decrease of radioactive leucine uptake by oocytes. The basic technique compares labelled proteins obtained after exposure to ^{14}C- or ^{3}H-labelled amino acid, either present in incubation medium or injected into the cytoplasm, of non-treated and progesterone pre-incubated oocytes, respectively. Some preliminary considerations aimed at relating protein synthesis to the appearance of a 'maturation promoting factor' are also reported, especially in view of previous discussions by Smith and Ecker [3], Schorderet-Slatkine and Drury [8] and Baltus et al. [9].

2. Material and methods

2.1. *Collection of oocytes*

Ovarian tissues are removed from adult *Xenopus laevis* females through a small incision made on the lateral belly wall. Oocytes are defolliculated by collagenase [8] and, in a given experiment, are all obtained from the same animal.

2.2. *Incubation of oocytes*

Oocytes (100 in most experiments) are incubated for 6 hr in Barth buffer, at room temperature. The

rate of maturation under progesterone action is determined in parallel and expressed in percent of germinal vesicle breakdown (GVBD) as a function of time, since it varies for the oocytes from one female to another. Progesterone (10 μM) is eventually applied by preincubation (15 min at room temperature) and oocytes are then washed three times with the buffer before transfer to the incubation medium.

For labelling, oocytes are exposed for 2 hr (in most cases) at room temperature in 0.3 ml of Barth buffer containing 0.1 mCi of [^{14}C]leucine/ml (CEA, 270 mCi/mmole) or [^{3}H]leucine 0.5 mCi/ml (CEA, 35 Ci/mmole), plus cold leucine in order to have similar amino acid concentration in treated and non-treated oocyte incubation medium.

2.3. *Injection of oocytes*

Oocytes are injected with Barth buffer containing either [^{3}H]leucine, 1 mCi/ml, in the case of progesterone exposed oocyte, or [^{14}C]leucine, 0.1 mCi/ml in the case of untreated oocytes are then left for 2 hr in Barth buffer at room temperature.

2.4. *Homogenization*

At the end of incubation, untreated and treated oocytes are homogenized in 2 ml of buffer containing 0.25 M sucrose. Thereafter a 600 X 15 *g* X min centrifugation eliminates mostly vitellus and some pigments which sediment. The supernatant is further centrifuged, 12 000 X 20 *g* X min, and more pigments and mitochondria are pelleted down. The supernatant is then centrifuged for 90 min at 105 000 *g*, eliminating the 'microsomes'.

It has been difficult to study the first 600 *g* pellet which is protein-rich (approx. 30 mg/200 oocytes) but does not contain much radioactivity. The other fractions have been processed as follows. The 12 000 *g* and 105 000 pellets and 1 ml of the 105 000 *g* supernatant are treated separately with 2 ml of Na-phosphate 0.01 M, pH 7.0 buffer containing SDS 1% w/v. After several strokes, the various preparations are left overnight at 37°C. The 12 000 *g* extract is recentrifuged for 10 min at 6 000 rev/min in a Heraeus centrifuge, the supernatant is collected and, as well as the two other preparations, is then concentrated by dialysis against $NaHPO_4$ 0.01 M, SDS 0.1%, pH 7.0 until representing approximately 200 μl.

2.5. *Electrophoresis*

100 μl of extracts, mixed with 20 μl of 20% Ficoll solution containing some Bromophenol Blue, are used for electrophoresis through 9 cm polyacrylamide 6% gels (87.3%, 2.7% bisacrylamide) in the presence of 0.1% SDS containing Na-phosphate 0.1 M, pH 7.0 buffer. Polymerisation is obtained by mixing 5.7 vol of acrylamide solution, 0.3 vol of ammonium persulfate (15 mg/ml) and 0.01 vol of *NNN'N'*-tetramethylenediamine. Electrophoresis is performed under 2 mA/tube for 20 min and then 8 mA/tube. Gels are then transferred into acetic acid 7% and left overnight. A 60 min electrophoresis in 7% acetic acid, (5 mA/tube) is finally performed in order to chase free amino acids. Gels are frozen and cut in 1.3 mm slices which are introduced into glass vials of a scintillation counter. Soluene 1 ml is added and the suspension is left for 3 hr at 60°C. Proteins are completely eluted from the gels under these conditions, Finally 10 ml of Omnifluor (98% PPO, 2% bis-MSB) 4 g/l of toluene solution are added to each vial. Quenching is constant as indicated by external standard. Counting efficiency gives a cpm $^{3}H/^{14}C$ ratio of 6.6 when the dpm $^{3}H/^{14}C$ ratio is 10.

3. Results

The results are reported in the form of polyacrylamide electrophoresis radioactive patterns of sodium dodecyl sulfate-treated proteins. Only 105 000 *g* supernatant proteins (fig.1, see Material and methods) will be considered in detail.

3.1. *Incubation with radioactive leucine*

In a control test (fig.2 (6)), the extract from 100 untreated oocytes incubated for 2 hr with [^{3}H]leucine and the extract of 100 untreated oocytes incubated with [^{14}C]leucine were mixed together, and the $^{3}H/^{14}C$ ratios of each of the protein fractions gave a horizontal line on the graph, with a value of 6.9 to which the $^{3}H/^{14}C$ ratio obtained by mixing extracts from treated and untreated oocytes was compared. If [^{3}H]leucine is given to progesterone exposed oocytes, the $^{3}H/^{14}C$ is 4.4 for the 0–2 hr period (fig.1 and fig.2 (1)). When the radioisotope-labelled amino acid has been introduced only between the

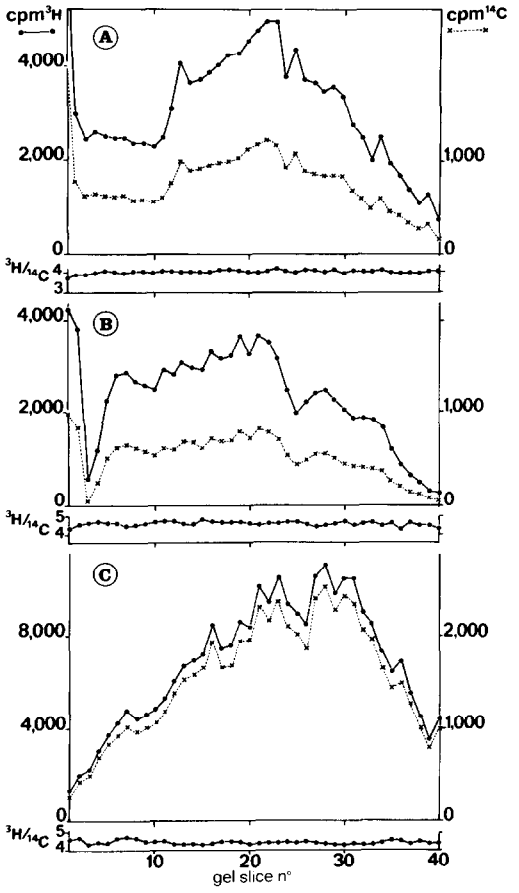

Fig.1. Proteins of progesterone exposed and untreated oocyte homogenate fractions. (A) 12 000 *g* pellet; (B) 105 000 *g* pellet; (C) 105 000 *g* supernatant. 100 progesterone exposed oocytes and 100 untreated oocytes have been incubated 2 hr with [^{3}H]Leu and [^{14}C]Leu, respectively. Homogenate, fractionation and electrophoresis were performed as indicated under experimental procedure. The ^{3}H/^{14}C ratio of (C) is also seen in fig.2 (1).

2nd and the 4th hour after the progesterone treatment, the ratio was 1.3 (fig.2 (2)), and finally it was 0.7 when labelling was performed between 4 hr and 6 hr (fig.2 (3)). Therefore there is a progressive decrease of the ^{3}H/^{14}C ratio of the proteins with time.

The possibility that some proteolysis progressing with maturation is involved has been considered. However, an incubation with [^{3}H]leucine for 4 hr between the 2nd and the 6th hour after progesterone treatment, vs the corresponding untreated oocyte ^{14}C-extract, gave a ^{3}H/^{14}C ratio of 2.3 (fig.2 (4)) which, compared to the results obtained by labelling between 2 and 4 hr, is not in favor of proteolysis.

3.2. *Injection of radioactive leucine*

After injection of radioactive leucine into oocytes, performed 4 hr after progesterone and followed by 2 additional hours of incubation in Barth medium (fig.2 (5) and fig.3), there is not only a general ^{3}H/^{14}C ratio very similar to the control but also two peaks of higher ratio (see below). This result may indicate that there is no inhibition of protein synthesis, but a decreased entry of [^{3}H]leucine into progesterone treated oocytes.

This decreased entry seems to parallel the yield of germinal vesicle breakdown (GVBD). This is suggested also by an experiment where the ratio ^{3}H/^{14}C was decreased to a lesser extent between 0 and 2 hr and 2 and 4 hr than usual, while there was a lower percent of maturation. However between 4 and 6 hr, the

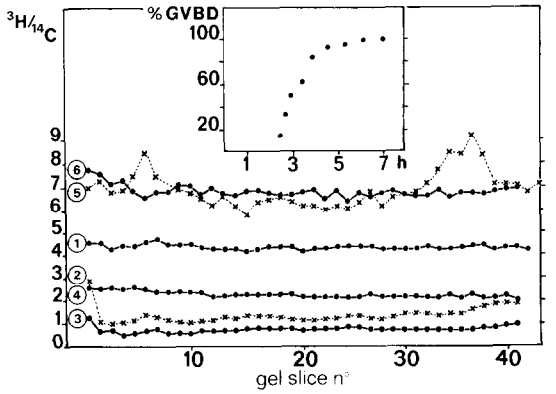

Fig.2. Decreased uptake of [^{3}H]leucine by oocytes during progesterone induced maturation as shown by the evolution of the labelling of 105 000 *g* supernatant proteins. (1) Progesterone exposed oocytes are incubated 2 hr with [^{3}H]Leu; (2) 2 hr after exposure to progesterone, oocytes are incubated 2 hr with [^{3}H]Leu; (3) 4 hr after exposure to progesterone, oocytes are incubated 2 hr with [^{3}H]Leu; (4) 2 hr after exposure to progesterone, oocytes are incubated 4 hr with [^{3}H]Leu; (5) 4 hr after exposure to progesterone, oocytes are injected with [^{3}H]Leu; (6) Untreated oocytes are incubated 2 hr with [^{3}H]Leu. In all cases, untreated oocytes are exposed to [^{14}C]Leu either by incubation (1,2,3,4,6) or by injection (5). *Insert*: maturation, as expressed by GVBD, is reported as a function of time after progesterone exposure.

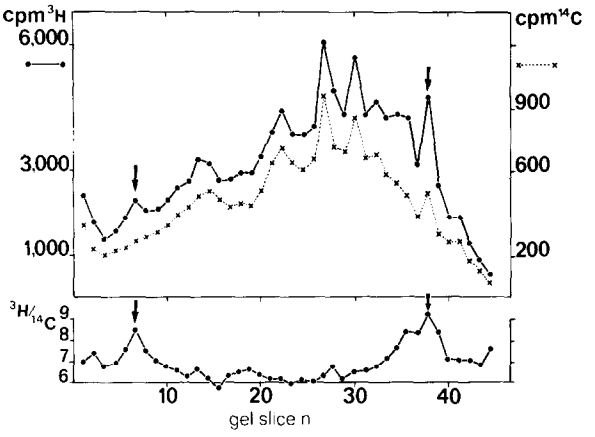

Fig.3. Induced proteins during oocyte maturation. 4 hr after exposure to progesterone, 100 oocytes are injected with [^{3}H]Leu. 100 untreated oocytes are injected with [^{14}C]Leu. Protein patterns correspond to fig.2.5.

percent of maturation was 98% and the decreased $^3H/^{14}C$ ratio as low as usual.

3.3. *Induced proteins*

Considering the possible existence of a 'maturation promoting factor' (MPF), the purpose of protein synthesis experiments using progesterone treated oocytes has been also to see if it would be possible to physically detect such a protein factor using double isotopic labelling. A similar method has been successfully used by Notides and Gorski [10] in early protein synthesis induced in the uterus by estradiol (IP: Induced Protein). The question of a 'Key Intermediary Protein' (KIP), which has been discussed in the early events of hormone action [11], may have some relevance to the production of the MPF. There was no peak during incubation experiments, but the defect of entry of leucine into oocytes leading to a low $^3H/^{14}C$ ratio was not favorable for the detection of such induced protein(s). However, when radioactive leucine was injected at 4 hr after progesterone, two protein peaks became visible above the background in the 105 000 *g* supernatant (fig.3). No other extract contained such induced protein peaks.

In another experiment where radioactive leucine was injected either just after, 2 hr after, or 4 hr after progesterone treatment, and protein synthesis was permitted to proceed for 2 hr in each case, there was again no change of the overall $^3H/^{14}C$ ratio of proteins, and only the first of the two peaks in the 105 000 *g* supernatant was observed 4 hr after progesterone. However in this case, the percentage of maturation was inferior to the one observed in the previous experiment and it is possible that a later injection of radioactive leucine could have led to the detection of the second induced protein peak.

4. Discussion

4.1. *Progesterone induced change of permeability to leucine*

It is proposed that, among the first well-defined events associated with hormone induced oocyte maturation, there is a marked permeability change which is clearly shown by an abrubt decrease of the $^3H/^{14}C$ ratio of uptaken leucine. Because oocytes do not mature synchronously, these experiments are not able to evaluate precisely at which time changes in the membrane permeability take place. However a parallelism with the percentage of GVBD has been noted. Gurdon [12] has already shown a difference between oocytes and fertilized eggs in *Xenopus* regarding amino acid uptake but it was not related to progesterone maturation. In references cited in the Introduction, results refer to the period at or following germinal vesicle dissolution [7]. However no attempt was made to detect an immediate effect brought about by the steroid itself. In other results [13] permeability was related to ionic transport which should not be confused with the process of pinocytosis seen by Schuetz [7].

The present study stresses the appearance of a permeability barrier resulting from the action of a steroid during the process of oocyte maturation of *Xenopus laevis*. It is not yet known how the change of permeability and the overall process of maturation are related one to another, nor, if there is any connection between the change of permeability to small molecules and the inhibition of the micropinocytotic process which, according to the report of Schuetz et al. [7], may be operating more slowly than the change of leucine uptake. However, the stage 6 oocyte of *Xenopus laevis* has been shown not to actively incorporate protein by pinocytosis [14], while remaining permeable to smaller molecules [15].

4.2. *Protein synthesis and 'maturation promoting factor'*

Smith and Ecker [16] found an increase of the rate of overall protein synthesis around GVBD [17]. The present results do not indicate a significant general increase in the synthesis taking place during the period preceding GVBD. However, two distinct peaks of induced proteins are observed. Even though such labelled fractions may be due to other mechanisms (for instance protein translocation), they still suggest selective changes in the pattern of protein synthesis under hormone action. Considering previous experiments where a 'factor' was found to be capable of inducing maturation when serially injected into oocytes [18–20], it is pertinent to consider whether one (or both) of these induced proteins is relevant to the activity of such a cytoplasmic factor. As yet, the validity of such hypothesis has not been assessed.

Acknowledgements

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