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NATURE OF PROGESTERONE ACTION ON AMINO ACID UPTAKE BY ISOLATED FULL-GROWN OOCYTE OF *XENOPUS LAEVIS*

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SUMMARY

L-leucine uptake into full-grown oocytes of *Xenopus laevis* is a saturable process which is Na^+ dependent and presumably coupled to Na^+ gradient Our results indicate that progesterone (10^{-6} M) blocks abruptly, around the germinal vesicle breakdown, the saturable transport of L-leucine *p*-Chloromercuribenzoate (10^{-4} M) induces maturation and after a short lag of time strongly inhibits L-leucine uptake Cycloheximide prevents progesterone-induced maturation and permeability changes

INTRODUCTION

Progesterone is the main steroid synthesized within the follicle cells of the *Xenopus* ovarian follicles, this steroid acts directly on the oocyte to induce maturation (Fouchet, C , Mulner, O and Ozon, R , unpublished) The molecular changes which lead to oocyte maturation are still unknown, however, recent researches have defined many events associated with maturation in more precise terms [1, 2] Among the first well defined events associated with progesterone-induced maturation there is a marked permeability change [3], very recently changes in permeability of the *Xenopus* oocyte membrane to amino acid have been established [4]

In this report we describe the general characteristics of the uptake of L-leucine by the full-grown *Xenopus* oocyte and we presented results on the effects of progesterone on changes of permeability of the oocyte membrane

MATERIALS AND METHODS

Animals *Xenopus laevis* females, from South Africa (De Rover, Holland), were bred and maintained under laboratory conditions

Oocyte preparation All experiments were conducted under in vitro conditions on isolated full-grown oocytes (Stage 6 [5]) Animals were anaesthetized with MS 222 (Sandoz) 1g/l, ovaries were removed and oocytes defolliculated by enzymatic treat-

ment with collagenase [6] Isolated oocytes were equilibrated for 1 h in Merriam's solution [7] containing NaCl, 88 mM, KCl, 1 mM, $Ca(NO_3)_2$, 0 33 mM, $CaCl_2$, 0 41 mM, $MgSO_4$, 0 82 mM, Tris, 2 mM Penicillin (100 I U /ml) was added to incubation flasks Full-grown oocytes (diameter 1 1–1 2 mm, Stage 6 [5] were selected under stereotaxic microscope

Oocyte maturation Isolated oocytes were incubated with progesterone 10^{-6} M (progesterone purchased from Sigma was chromatographically pure) until maturation The criterion for maturation was breakdown of the germinal vesicle judged by the appearance of a white spot surrounded by pigment at the animal pole, during the first hours (2–10) of hormonal treatment Some oocytes were heated at 100 °C for 10 min, the absence of the germinal vesicle being ascertained by dissection In all uptake experiments, the rate of maturation under progesterone action was determined in parallel and expressed in percent of germinal vesicle breakdown as a function of time, since it varies for the oocytes from one female to another

Leucine uptake determination Oocytes were incubated with L-[4-^{3}H]-leucine (25 or 50 Ci/mmol obtained from the Commissariat à l'Energie Atomique) at a concentration of 10 μCi/ml and unlabelled L-leucine (Merck) such that the final concentrations of leucine were from 10^{-6} to 10^{-2} M as indicated in the experiments Incubations in 5 ml or 0 5 ml were stopped at the indicated times and groups of 10 oocytes were washed three times in 20 ml of cold solution It has been demonstrated that there was no back flow of amino acid from the oocytes into the medium during washing procedure Pulse experiments were conducted routinely during 25 min with groups of 10 oocytes

Determination of saturable leucine uptake Non-saturable uptake was subtracted from total entry to obtain saturable uptake Estimation of non-saturable uptake was performed in the presence of an excess of unlabelled leucine (10^{-2} M) This procedure is comparable to the method described by Akedo and Christensen [8]

Measurement of radioactivity Ten oocytes were treated with 1 ml of NCS tissue solubilizer (Amersham Searle) at 60 °C for 20 min Radioactivity was determined in a Packard Tri-Carb liquid scintillation spectrometer model 3320 with external standard after addition of 10 ml of scintillation mixture containing 2,5-diphenyloxazole (PPO), 5 5 g, and 1,4-*bis*(2,5-phenyloxazolyl)benzene (POPOP), 100 mg, in Triton X-100, 333 ml, and toluene, 667 ml Efficiency was 30–35 %

RESULTS

Leucine uptake in full-grown oocytes

The kinetic of uptake of labelled leucine by the separated oocytes (Stage 6) was measured Fig 1 shows that L-leucine uptake proceeds linearly for the first h of incubation when the concentration of leucine in medium was 5 10^{-5} M When leucine concentrations varied from 10^{-6} M to 10^{-2} M, similar linear uptakes were always obtained The kinetic pattern of uptake was also entirely similar among oocytes of the same stage from different animals, though some variations existed in the total amount of [^{3}H]leucine incorporated per oocyte per h

Fig 2 indicates that leucine uptake is a saturable process, in each experiment non-saturable entry was subtracted from total uptake (see Materials and Methods) The kinetic analysis of leucine entry into oocyte shows an apparent K_m around

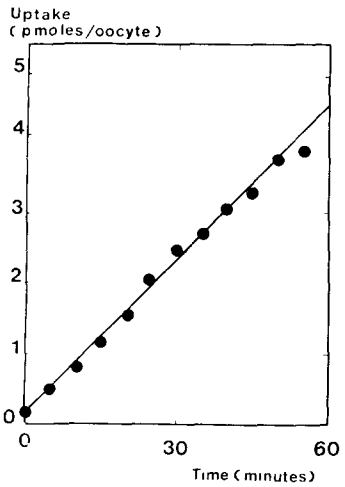

Fig 1 L-Leucine ($5\ 10^{-5}$ M) uptake in full-grown oocytes of *Xenopus laevis* (Stage 6) Oocytes were incubated in a volume of 5 ml and groups of ten oocytes were removed at different times Results are expressed in pmol L-leucine incorporated per oocyte

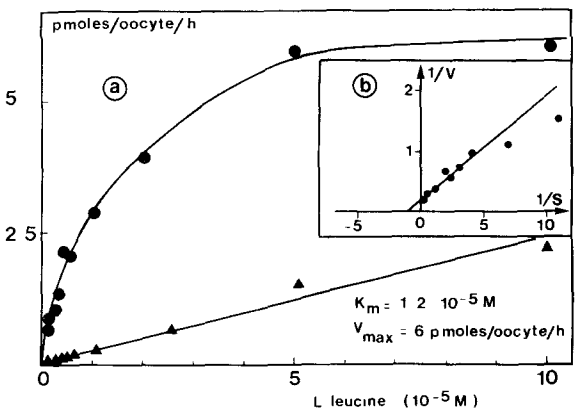

Fig 2 L-Leucine uptake by full-grown oocytes isolated from the same female, as a function of amino acid concentration (a) Groups of ten oocytes were incubated 25 min in 0 5 ml of solution containing L-leucine (10^{-6}–10^{-2} M) Results are expressed in pmol/oocyte per h (●) Saturable uptake into full-grown oocytes (▲) Uptake into mature full-grown oocytes previously treated with progesterone (10^{-6} M), experiment 2 h after germinal vesicle breakdown (b) Lineweaver-Burk [9] plot of saturable uptake

10^{-5} M Similar results were obtained in three different experiments in which K_m was found to be 6, 8 and 12 10^{-6} M, with corresponding V values of 3 6, 1 3 and 6 0 pmol/oocyte per h

Replacing the Na^+ of the incubation buffer with $choline^+$ resulted in lower uptakes of leucine (Fig 3) On the other hand, increasing the Na^+ concentration (132 mequiv /l) increased the amino acid uptake It can be noted that hypertonicity did not modify significantly the amino acid transport when the leucine level was $2\ 10^{-5}$ M At higher Na^+ level (176 mequiv /l) inhibition occurred When oocytes

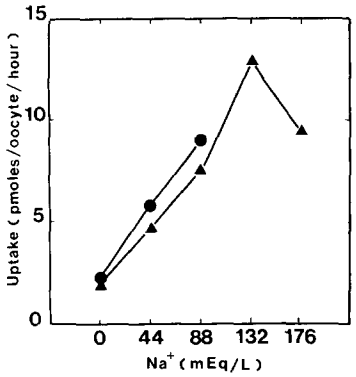

Fig 3 Total L-leucine uptake at different Na^+ levels Groups of ten oocytes were incubated in L-leucine (2 10^{-5} M) for 25 min in different modified buffers (●) uptake in 183 mOsM buffer (▲) Uptake in 359 mosM buffer Osmolarity was maintained constant by choline chloride Uptake is expressed in pmol/oocyte per h

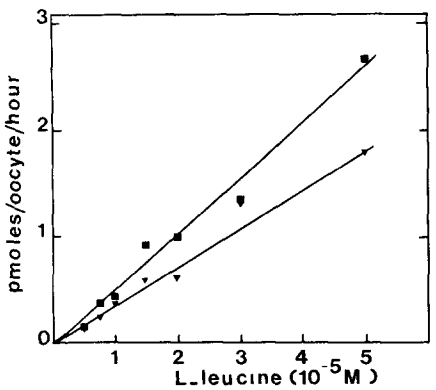

Fig 4 Total L-leucine uptake as a function of amino acid concentration (■) in Na^+-free buffer, and (▼) in buffer containing *p*-chloromercuribenzoic acid (*p*Cl-HgBzO$^-$) 10^{-4} M

were incubated in a Na^+-free buffer, the saturable entry was virtually abolished Fig 4 shows that in these conditions only non-saturable entry can be measured

Incubation of oocytes in a K^+-free buffer did not influence the uptake of leucine when Na^+ level was 88 mequiv /l Ouabain at 5 10^{-5} and 5 10^{-4} M did not inhibit leucine entry into oocyte Ouabain at a concentration of 5 10^{-3} M inhibited 38 % of saturable leucine uptake Leucine uptake is a temperature dependant process, at 4 °C saturable entry is totally stopped *p*Cl-HgBzO$^-$ is a strong inhibitor of leucine entry into full-grown oocytes (Fig 4) Saturable entry is abolished in presence of *p*Cl-HgBzO$^-$(10^{-4} M) This inhibition was already complete in 1 h (Fig 5)

Effect of progesterone on leucine transport

Progesterone induces maturation, i e germinal vesicle breakdown, in full-grown *Xenopus* oocytes The speed at which maturation proceeded in different oocytes (and from different females) varied considerably In a typical experiment, oocytes were

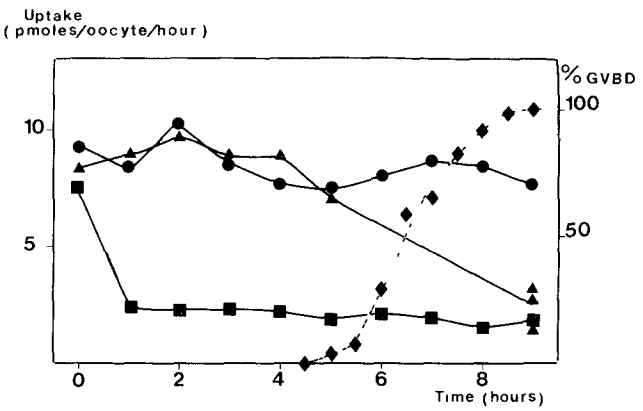

Fig 5 L-leucine uptake during the course of maturation Groups of ten oocytes were incubated indicated times in 0 5 ml of buffer containing L-leucine (5 10^{-5} M) (●) Control uptake by full-grown oocytes, (▲) uptake in the presence of progesterone (10^{-6} M), (■) uptake in the presence of *p*-chloromercuribenzoic acid (10^{-4} M), (◆) represents the % of germinal vesicle breakdown (GVBD) as a function of time measured in parallel

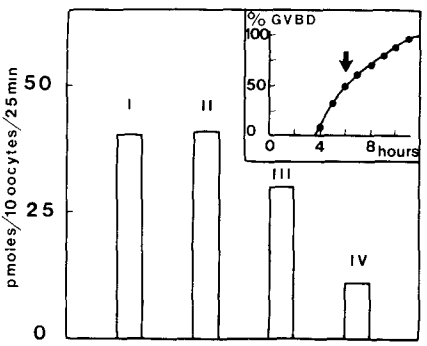

Fig 6 Total L-leucine uptake of oocytes incubated with 10^{-6} M progesterone for 6 h (50 % of germinal vesicle breakdown (GVBD)) I, control oocytes without hormone, II, non-matured oocytes III, oocytes maturing (white spot appearing), IV, matured oocytes (white spot present 1 h) All oocytes were from the same female Groups of ten oocytes were incubated 25 min in 5 10^{-5}M leucine

exposed for 25-min pulses in [H^3]leucine from the time of initial exposure to progesterone (hormone continuously applied 10^{-6} M) until the appearance of a light area surrounded by pigment at the animal pole (the external criterion of maturation) The results presented in Fig 5 indicate that leucine uptake was not modified until germinal vesicle breakdown occurred In this experiment, 9 h after progesterone exposure, 100 % maturation was achieved, when mature oocytes were incubated for about this period, during 25 min the total leucine uptake was abruptly decreased (see Fig 5) Kinetic studies reported in Fig 1 show that saturable leucine entry is completely suppressed 2 h after progesterone-induced maturation

Changes of permeability to leucine occur in all experiments and can be estimated at the time germinal vesicle breakdown is first observed (Fig 6) It does not

depend on time between progesterone exposure and germinal vesicle breakdown For example, in some cases germinal vesicle breakdown occurred in 2 h, change of permeability occurred around the same time When germinal vesicle breakdown was achieved in 10 h, change of permeability also appeared around 10 h

It is well established that inhibitors of protein synthesis block oocyte maturation after progesterone treatment When cycloheximide was added to the incubation medium (100 μg/ml), progesterone-induced maturation was blocked, protein synthesis was inhibited (99 % inhibition) The leucine uptake was not modified during an 8-h exposure to the inhibitor in presence of progesterone

DISCUSSION

These results clearly demonstrate that leucine uptake into full-grown oocytes of *Xenopus laevis* is a saturable process which is Na^+ dependent and presumably coupled to Na^+ gradient Progesterone blocks the saturable transport of leucine abruptly, at the time around maturation, without affecting significantly the uptake not subject to saturation

The active transport systems for amino acids in animal cells show a requirement for Na^+ and require metabolic energy, they appear to be constitutive and firmly bound to the membrane [10] The transport of leucine into *Xenopus laevis* oocytes described in this report is essentially of the same nature It is a saturable process (K_m around 10^{-5} M) which exhibits an absolute requirement for Na^+ and a partial inhibition by ouabain (5 10^{-3} M) The coupling of the leucine entry to metabolic energy cannot be demonstrated for ouabain levels below 10^{-3} M However, ouabain, at concentrations between 10^{-5} and 10^{-4} M modifies ionic transport in amphibian oocytes and we recently observed that ouabain facilitates progesterone-induced maturation at concentration around 10^{-4} M (Ozon, R and Ravel, N , unpublished) The sensitivity of leucine uptake to ouabain, in our experimental conditions, is therefore not strictly linked to $(Na^+ + K^+)$ATPases

Apparent K_m values are similar in three different females, however, the kinetic studies indicate a 5-fold range in comparing the V (pmol leucine/oocyte per h) of leucine uptake into oocytes from different females

*p*Cl-HgBzO$^-$, in contrast with progesterone, initiates an immediate abrupt decrease of leucine entry (Fig 5), the inhibition of transport by a sulfhydryl reagent suggests that the carrier system contains sulfhydryl groups *p*Cl-HgBzO$^-$ is not an inhibitor of progesterone-induced maturation, moreover, and unexpectedly, *p*Cl-HgBzO$^-$ has been reported recently to induce a maturation apparently similar to progesterone-induced maturation in *Xenopus* [11, 12], we also found that this organomercurial (10^{-4} M) induces germinal vesicle breakdown in *Xenopus* oocytes It remains to be demonstrated, however, that *p*Cl-HgBzO$^-$-induced maturation allows eventual further development

Progesterone, the main physiological steroid released directly into *Xenopus* oocytes (Fouchet, C , Mulner, O and Ozon, R , unpublished), induces maturation after a lag of several hours (2–10 h) and around the same time induces a total inhibition of saturable leucine entry, change of permeability is one of the consequences of progesterone action This change does not occur before germinal vesicle breakdown and does not seem to be a prerequisite for progesterone-induced germinal vesicle

breakdown Inhibitors of transport have been demonstrated to interfere with progesterone action, ouabain, an inhibitor of membrane ATPases, facilitates progesterone action on oocytes (Ozon, R and Ravel, N , unpublished), *p*Cl–HgBzO$^-$, a sulfhydryl reagent, at least blocking L-leucine saturable transport (Figs 5 and 6) apparently mimicks progesterone action on oocytes [11, 12] The resulting alterations in membrane permeability modified the subsequent events of oocyte maturation The exact relationship between membrane permeability and progesterone-induced maturation requires further investigation

It has been shown that a factor arising during the course of progesterone-induced maturation in amphibian oocytes is capable of inducing maturation when injected back into non-treated recipient oocytes [2, 6] The maturation-promoting factor appears in progesterone-treated oocytes around 1 h before germinal vesicle breakdown It does not need protein synthesis to induce germinal vesicle breakdown in non-treated oocytes [13] But experiments with cycloheximide demonstrated that protein synthesis is necessary for the appearance of the maturation-promoting factor [2, 6, 12] In our experiments we demonstrated that cycloheximide prevents progesterone inhibition of leucine-saturable uptake into oocytes, as well as germinal vesicle breakdown, the maturation-promoting factor could be an intermediate step in permeability changes induced by progesterone

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