

The Stimulatory Effect of Human Chorionic Gonadotropin on Amino Acid Uptake by Amphibian Follicles

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Human chorionic gonadotropin (hCG) stimulates the uptake of eight different amino acids and four nucleosides by *Xenopus laevis* ovarian follicles. This hormone also stimulates amino acid uptake in the follicles of another amphibian, *Callyptocephallela caudiverbera*. The stimulation of uptake is due to a reduction in the amino acid concentration required for half-maximal uptake velocity and not to an increment in V_{max} . The effect of hCG does not require protein synthesis but requires physiological conditions of temperature and pH. Incorporation of radioactive exogenous amino acid into proteins is also stimulated by the hormone, but high-resolution electrophoresis shows that there are no drastic qualitative changes in the pattern of proteins synthesized at early times after hCG treatment. The effect of hCG on the uptake of exogenous amino acids does not appear to be required for oocyte maturation because other hormones such as progesterone and testosterone which induce maturation do not increase amino acid uptake. Also the concentration of hCG required for oocyte maturation is significantly lower than that required for an effect on amino acid transport. Inhibitors of oocyte maturation such as theophylline and cycloheximide do not inhibit the action of hCG on amino acid uptake by the amphibian follicles.

INTRODUCTION

Amphibian oocytes have been used by a number of investigators as model systems to study the complex maturation process through which the full-grown germ cell completes meiosis and acquires the capacity to be fertilized. These giant cells can be easily dissected out from the amphibian ovary as follicles in which the oocyte is surrounded by several layers of follicle cells. The isolated follicles have the attractive feature that they can be induced to mature *in vitro* under the stimulus of hormones such as human chorionic gonadotropin (hCG), progesterone, and testosterone or by treatment with nonphysiological reagents (Merriam, 1972; Masui, 1973; Schuetz, 1967; Brachet *et al.*, 1975; Was-

serman and Masui, 1975; Schorderet-Slatkine *et al.*, 1976). The work of Smith and Ecker (1971) and Masui (1973) has established that progesterone and hCG differ in the manner in which they induce oocyte maturation in that progesterone has the capacity to induce maturation in oocytes that have been denuded of follicle cells, while hCG requires the presence of these cell layers to trigger the maturation process.

Recently Hallberg and Smith (1976) have reported that treatment of *Xenopus laevis* ovarian follicles with hCG causes a rapid increase in the uptake of [³H]leucine resulting in stimulation of the incorporation of this amino acid into oocyte proteins. In these experiments it was clearly shown that hCG requires the presence of follicle cells to exert its effect but that the resulting increment in leucine uptake is due to an increased inflow of exogenous amino

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acid into the oocyte and not into the surrounding layer of follicle cells.

The experiments presented in this report analyze the effect of hCG on the mechanism of uptake of exogenous nutrients by *X. laevis* ovarian follicles and asks how this effect is related to the oocyte maturation phenomena. The results obtained indicate that the hCG effect on ovarian follicle uptake is a general one since the hormone causes increments in the entrance of all eight amino acids tested as well as that of four nucleosides. A similar response is observed with follicles from another amphibian species. The hCG effect is shown to be a result of an increase in the affinity of the nutrient-transport system rather than an increase in the maximum velocity of entry. In addition, the present report describes the conditions of temperature and pH required for the hormonal effect on uptake and establishes that protein synthesis is not essential for its triggering. However, no direct correlation between the hCG effect on the follicle transport of nutrients and its capacity to induce amphibian oocyte maturation has been found in these studies using known inhibitors of this process.

MATERIALS AND METHODS

Adult female *Xenopus laevis* were obtained from the South African Snake Farm, Cape Province, South Africa.

Callyptocephallela caudiverbera, a Chilean frog, was captured in the vicinity of Santiago.

The animals were anesthetized in ice water and a piece of ovary was removed and placed in an amphibian saline solution that contained 63 mM NaCl, 1 mM KCl, 0.5 mM CaCl₂, 1 mM MgCl₂, 20 mM Tris-HCl, pH 7.4, and 10 μg/ml each of streptomycin sulfate and penicillin. Cells were dissected out using watchmaker's forceps and selected by size. These cells which contain a surrounding layer of follicle cells will be referred to as follicles. In all experiments the *Xenopus laevis* oocytes

or follicles had a diameters between 1.1 and 1.3 mm. Thus the *X. laevis* follicles correspond to stage VI cells (Dumont, 1972).

The assays for the uptake of exogenous radioactive amino acids or nucleosides and those for the incorporation of amino acid into protein have been described previously (Bravo *et al.*, 1976). Essentially, the follicles were incubated in amphibian saline solution with the added labeled compound at the concentration and time indicated and then were washed extensively with the same saline solution and transferred to a glass fiber filter. The cells were squashed on the filter, dried, and counted in a liquid scintillation system ("uptake" assay). In all cases, the values expressed are averages of triplicate groups of five follicles each. Protein synthesis was measured in the same manner except that, at the end of the incubation cells were homogenized in the presence of 5% TCA, filtered, dried, and counted.

For the assay of oocyte maturation, groups of approximately 200 follicles were incubated with the hormones or maturation-inducing compounds for 18 hr in amphibian saline at 20–22°C. Groups of 100 follicles were then examined microscopically to observe germinal vesicle breakdown which was taken as a criterium of maturation (Masui, 1973).

Radioactive proteins synthesized by the ovarian follicles were prepared by incubating 30 follicles for 3 hr in amphibian saline containing 20 μM [³⁵S]methionine (specific activity, 11,000 mCi/mmole) at 21°C. Subsequently the follicles were homogenized in 100 μl of a buffer containing 10 mM Tris-HCl, pH 7.4, 5 mM MgCl₂, 25 μg/ml of pancreatic RNase, and 25 μg/ml of DNase I. The homogenate was incubated for 30 min at 0°C and for 15 min at 25°C before addition of NaCl to a final concentration of 75 mM. The mixture was then centrifugated at 2000 rpm for 5 min and the supernatant was used for gel analysis. Control experiments in which the protease

inhibitor *para*-methylsulfonyl fluoride was added to the homogenizing medium at a concentration of 0.25 mg/ml did not significantly alter the electrophoretic patterns obtained.

Analysis of proteins synthesized by the ovarian follicles was carried out by the method of O'Farrell (1975) as modified by Gurdon *et al.* (1976) which includes a first dimension of isoelectric focusing gel fractionation and a second dimension of electrophoresis in a 15% SDS-polyacrylamide gel slab. The dried gels were developed by the fluorographic method of Bonner and Laskey (1974) and Laskey and Mills (1975).

Radioactive amino acids and nucleosides were purchased from New England Nuclear Corp. Progesterone and testosterone were purchased from Calbiochem. The human chorionic gonadotropin used in most experiments was purchased from Sigma Chemical Co. but in some experiments chromatographically pure hCG obtained from Dr. S. Koide of the Biomedical Division of the Population Council or from the National Pituitary Agency, University of Maryland, School of Medicine, was employed.

RESULTS

Characteristics of hCG Stimulation of Uptake of Different Amino Acids

The results presented in Table 1 show

the stimulatory effect of hCG on the uptake of eight different amino acids. The amino acids tested represent three of the four specific groups of transport systems that were previously detected in *Xenopus* follicles (Bravo *et al.*, 1976) since they include aromatic, basic, and aliphatic amino acids. It is clear that hCG stimulates significantly all the amino acids tested.

The results presented in Fig. 1 show a double-reciprocal plot of the effect of the concentration of four amino acids, lysine, alanine, phenylalanine, and glycine, on their uptake velocity in follicles that have been treated with hCG and in untreated controls. It is evident that the stimulus seen with hormone is a result of a change in the K_T for those amino acids and that the V_{max} of the reaction is not altered.

Table 2 summarizes the changes of K_T observed for these four different amino acids after hormonal stimulation of the follicles. The stimulation of uptake is due to K_T decreases that range from 2- to 5.3-fold.

Table 3 shows that hCG also stimulates the uptake of the four nucleosides tested, adenosine being the nucleoside most efficiently taken up by the oocyte. The stimulation of the uptake of these compounds is lower than that observed with amino acids, but points to the fact that the hor-

TABLE 1

THE EFFECT OF hCG ON THE UPTAKE OF DIFFERENT AMINO ACIDS BY *Xenopus laevis* FOLLICLES^a

Amino acid	Specific activity (mCi/mmmole)	Radioactivity uptake (cpm/follicle)		Stimulation (%)
		Control cells	hCG-treated cells	
[¹⁴ C]Alanine	154	4,866	8,302	71
[¹⁴ C]Arginine	220	5,217	10,095	94
[¹⁴ C]Glycine	102	1,379	2,606	89
[¹⁴ C]Isoleucine	171	3,645	5,502	51
[¹⁴ C]Lysine	324	19,019	31,136	64
[³⁵ S]Methionine	1,100	7,172	13,487	88
[¹⁴ C]Phenylalanine	413	7,562	11,178	48
[¹⁴ C]Serine	155	7,861	12,111	54

^a Full-grown follicles were incubated for 2 hr with 20 μ M concentrations of each radioactive amino acid in amphibian saline and the total uptake of radioactivity was measured as described under Materials and Methods. Where indicated, follicles were incubated with 60 units/ml of hCG in amphibian saline for 30 min before addition of the radioactive amino acid.

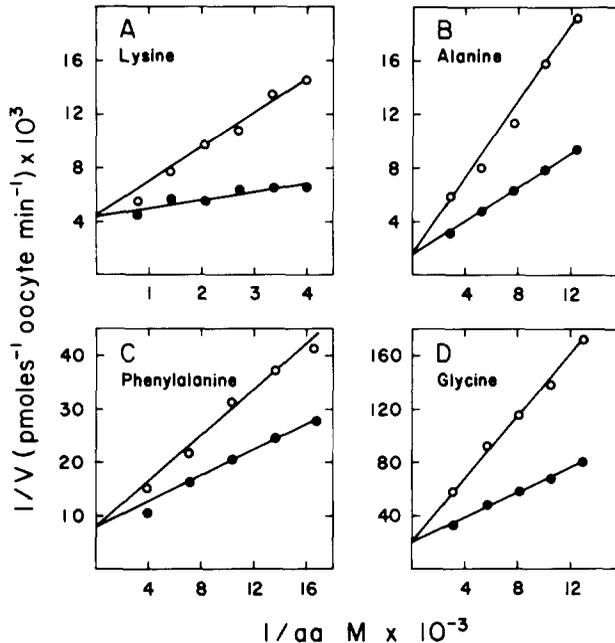


FIG. 1. Double-reciprocal graphs of the effect of concentration of amino acid on the velocity of uptake by *X. laevis* follicles. The uptake velocity of the radioactive amino acids present in the exogenous medium at different concentrations was measured after 2 hr of incubation with untreated *X. laevis* follicles (○—○) and with follicles from the same animal that had been pretreated with hCG at 60 units/ml for 30 min (●—●). The specific activities used in millicuries per millimole were: (A) lysine, 281; (B) alanine, 154; (C) phenylalanine, 413; and (D) glycine, 120.

TABLE 2
DECREASE IN K_T OF AMINO ACIDS AFTER
TREATMENT OF *Xenopus laevis* FOLLICLES WITH
hCG^a

Amino acid	K_T (M)		Factor of decrease in K_T
	Control cells	hCG-treated cells	
Alanine	10×10^{-4}	4.2×10^{-4}	2.4
Glycine	5.9×10^{-4}	2.3×10^{-4}	2.6
Lysine	5.8×10^{-4}	1.1×10^{-4}	5.3
Phenylalanine	2.8×10^{-4}	1.4×10^{-4}	2.0

^a The K_T values for the different amino acids were determined from the graphs shown in Fig. 1 by the standard procedure (Bravo *et al.*, 1976).

monal effect on membrane permeability is rather general.

As shown by Hallberg and Smith (1976), it has been observed that the surrounding follicular layer is required for the hCG on amino acid permeability since oocytes denuded of these cells by Pronase treatment do not respond to the hormone. A similar

TABLE 3
EFFECT OF hCG ON THE UPTAKE OF RADIOACTIVE
NUCLEOSIDES BY *Xenopus laevis* FOLLICLES^a

Nucleoside	Radioactivity uptake (cpm/follicle)		Stimulation (%)
	Control cells	hCG-treated cell	
[³ H]Adenosine	1462	2191	50
[³ H]Guanosine	986	1372	39
[³ H]Cytidine	328	442	36
[³ H]Uridine	272	342	26

^a Full-grown follicles were incubated with $10 \mu M$ concentrations labeled nucleosides for 1 hr in amphibian saline as described under Materials and Methods. The hCG-treated follicles were preincubated with 100 units/ml of hormone for 30 min before addition of the ³H-labeled nucleoside. All nucleosides used were of a specific activity of 3000 mCi/mmole.

result has been reported for the action of hCG on oocyte maturation (Masui, 1973; Schuetz, 1967; Smith and Ecker, 1971). The radioactivity present in the oocyte-

soluble fraction and in the membrane fractions that include the intact layer of follicle cells was measured after hormone treatment by the technique of squashing the follicle under a coverslip as described by Hallberg and Smith (1976). The amount of labeled amino acid taken up by the oocyte constitutes more than 95% of the total, confirming the observation of Hallberg and Smith (1976). Thus the measurements carried out with follicles essentially constitute determinations of oocyte uptake and protein synthesis.

Effect of hCG Uptake Stimulation on the Incorporation of Exogenous Amino Acid into Proteins

The stimulation of radioactive amino acid uptake caused by the hormone results, as would be anticipated, in an increment in the incorporation of label into the proteins being synthesized by the oocyte since it increases the specific activity of the intracellular precursor pools.

However, hormone treatment generally increases exogenous amino acid incorporation into protein more than it increases its uptake into cells. This is observed in Fig. 2 which shows that, while amino acid uptake is stimulated less than twofold, incorporation into protein is increased 3.5 fold. The measurements were carried out with 30-min pulses of [14 C]arginine using follicles that had been preincubated with hCG for the times indicated on the abscissa. Similar results were reported by Hallberg and Smith (1976) but the present work differs from theirs in the time of hormone treatment required to observe maximum effects on amino acid uptake and incorporation into protein. While our data show that 15 min and 1 hr of hCG pretreatment are sufficient to observe optimal stimulation of these processes, respectively, the results by these authors (Hallberg and Smith, 1976) indicate that 3 and 6 hr are needed for equivalent effects. This apparent discrepancy may be a result of their using stage IV follicles in their assays

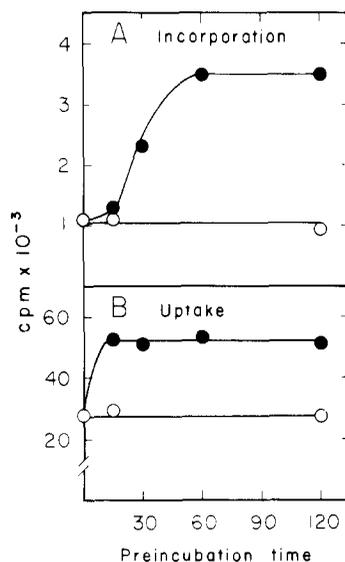


FIG. 2. The effect of preincubation time with hCG on amino acid uptake and incorporation into proteins. Follicles were incubated with 20 μ M [14 C]arginine for 30 min in amphibian saline. In A the radioactivity incorporated into protein and, in B, the total radioactivity taken up by the cell were measured as described under Materials and Methods. Prior to the addition of [14 C]arginine the follicles were preincubated in amphibian saline for the specified times in the presence (●—●) or absence (○—○) of 60 units/ml of hCG.

while we used stage VI in the present work. Only stage VI follicles are capable of undergoing the maturation process (Dumont, 1972).

In an effort to determine whether the hCG stimulation of amino acid incorporation caused qualitative differences in the proteins synthesized by these cells, analysis of radioactive proteins was carried out by the high-resolution technique described by O'Farrell (1975). This technique which combines isoelectric focusing and electrophoresis in a two-dimensional polyacrylamide gel is highly reproducible and can separate thousands of different proteins synthesized by *X. laevis* oocytes (Gurdon *et al.*, 1976). The results shown in Fig. 3 demonstrate that after 3 hr of hCG treatment there is no substantial qualitative change in the pattern of proteins synthesized by the ovarian follicles although there is a significant stimulation of amino

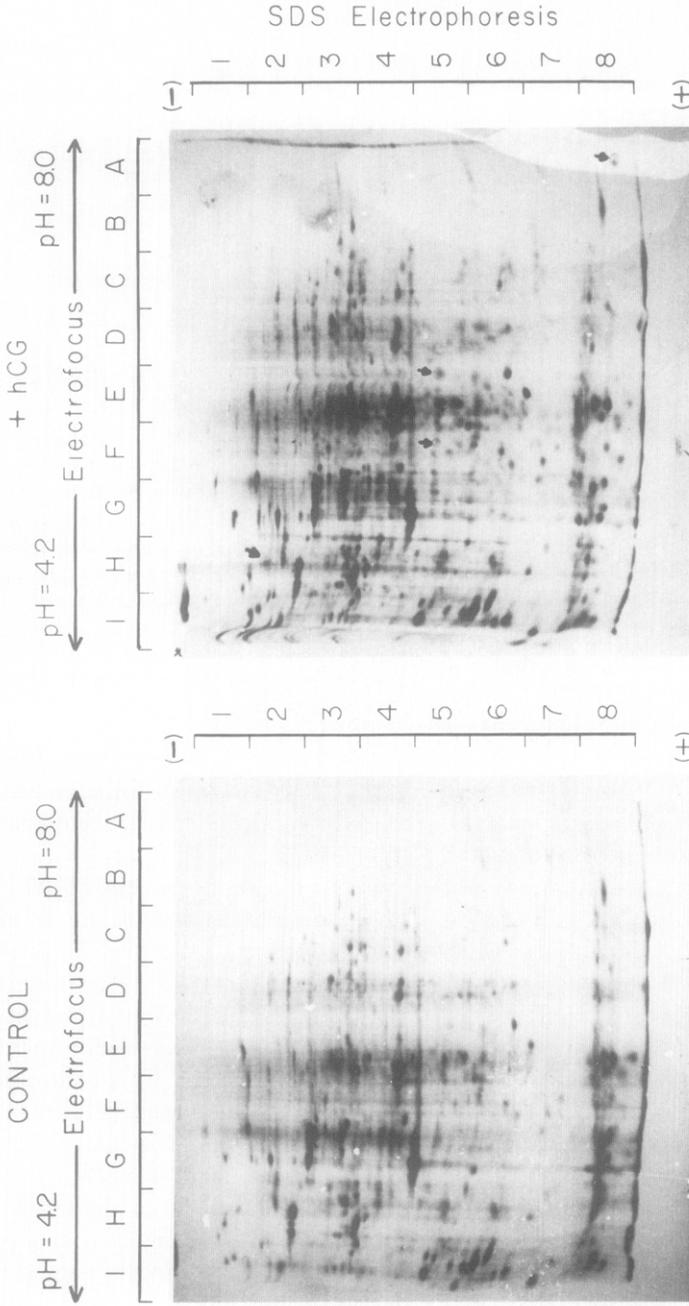


FIG. 3. Fractionation of proteins synthesized by control and hCG-treated follicles on bidimensional gel electrophoresis. In each group 20 follicles were incubated for 3 hr with 20 μM [^{35}S]methionine and the total soluble proteins synthesized were analyzed by electrophoresis and fluorography as detailed under Materials and Methods. The left side corresponds to proteins of control oocytes, and the right side, to the proteins of cells treated with 60 units/ml of hCG for 1 hr prior to the addition of [^{35}S]methionine. The arrows point to new spots in the hormone-treated cells.

acid incorporation in the hormone-treated cells. Some new minor components appear after hCG treatment and are indicated by arrows. It has not been determined whether these putative new proteins belong to the follicle cells or to the oocytes.

Requirements for the hCG Effect on Amino Acid Uptake

There is general agreement that protein synthesis is required for oocyte maturation induced by either progesterone or hCG (Smith and Ecker, 1971). Figure 4, however, shows that cycloheximide, which is a very efficient inhibitor of amphibian oocyte protein synthesis (Bravo and Allende, 1976), does not block the stimulation of amino acid uptake brought about by hCG although it inhibits more than 90% of the protein synthesis in the hormone-treated cells. Similar results, not shown, have been obtained using puromycin at a concentration of 5×10^{-4} M.

Another factor which notably affects the biosynthetic function of amphibian follicles is temperature. It has been shown (Bravo and Allende, 1976) that both protein synthesis and amino acid uptake by

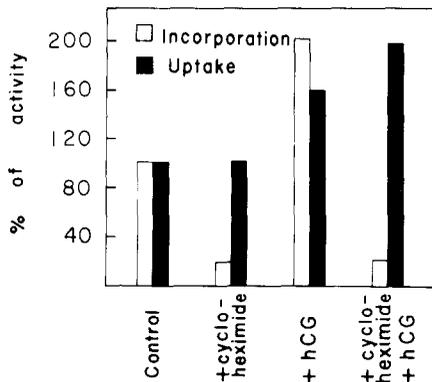


FIG. 4. The effect of the inhibition of protein synthesis on the stimulation of amino acid uptake caused by hCG. Follicles were incubated for 1 hr with $20 \mu\text{M}$ [^{14}C]lysine (specific activity, 324 mCi/mmole) and the amount of radioactivity incorporated into protein (empty bars) and taken up by the cell (full bars) was measured as described under Materials and Methods. Where indicated, follicles were preincubated with 0.1 mM cycloheximide for 30 min and with 60 units/ml of hCG for 1 hr.

amphibian oocytes are optimal at about 20°C , a temperature considerably below that usually seen with bacteria and warm-blooded animals. Figure 5 shows that the hCG stimulation of [^{14}C]alanine uptake and incorporation into proteins has a narrow optimum at approximately 20°C , with little or no stimulation being observed at 10 and 30°C .

A similar experiment was carried out in which the hormonal effect on protein synthesis and amino acid uptake was tested on follicles that were incubated at different pHs. The results obtained (not shown) indicate that maximal hormonal effect occurred at pH 7.5, whereas no stimulation could be observed at pH values below 6.5 and above 8.5.

Relationship between hCG Stimulation of Amino Acid Uptake by Ovarian Follicles and the Oocyte Maturation Process

The effect of factors that induce oocyte maturation on amino acid uptake by ovarian follicles was tested in order to study the possible relationship between the two phenomena caused by hCG.

The data presented in Fig. 6 were obtained using different concentrations of chromatographically pure hCG to test its effect on the uptake of [^{14}C]leucine by *X. laevis* follicles and the effect of these concentrations on oocyte maturation measured by germinal vesicle breakdown. It is clear that the two effects have different concentration requirements as evidenced by the fact that at 4 units/ml of hCG there is no stimulation of uptake but over 40% maturation. A concentration of 10 units/ml give 100% maturation while 30 units/ml are required for the maximum uptake effect. These results obtained with chromatographically pure hormone also demonstrate that the effects observed are not due to contaminants present in the less pure hCG preparations utilized in most of the other experiments.

Table 4 shows that progesterone and testosterone which can induce maturation

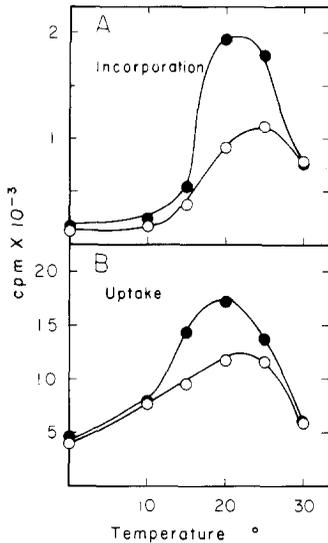


FIG. 5. The effect of temperature on the hCG stimulation of amino acid uptake and incorporation into proteins of *X. laevis* follicles. Follicles were incubated for 90 min with 20 μ M [¹⁴C]alanine (specific activity 136 mCi/mole) in amphibian saline and the radioactivity incorporated into proteins (A) or taken up by the cell (B) was measured as described under Materials and Methods. Follicles were placed at the temperatures indicated 30 min before addition of the radioactive amino acid. Parallel determinations were made with control follicles (○—○) and with follicles that had been exposed to 60 units/ml of hCG 30 min prior to the addition of the radioactive amino acid (●—●).

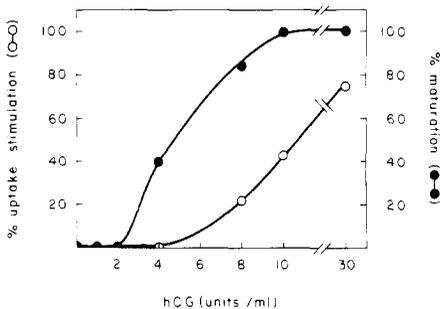


FIG. 6. Effect of hCG concentration on *X. laevis* follicle maturation and amino acid uptake. Follicle maturation was measured as described under Materials and Methods, after incubation of the cells for 18 hr in the presence of the indicated concentrations of chromatographically pure hCG. Stimulation of [¹⁴C]leucine (specific activity, 330 mCi/mole) uptake was measured in oocytes that had been preincubated for 30 min with the indicated hCG concentrations prior to a 90-min incubation with 20 μ M radioactive amino acid. Uptake was measured as described under Materials and Methods.

very efficiently have no effect on amino acid uptake. Lanthanum ion which causes oocyte maturation (Schorderet-Slatkine *et al.*, 1976) inhibits amino acid uptake.

Similar negative results were obtained with progesterone after treatment of the oocytes with this hormone for different times throughout the whole 24-hr maturation period (data not shown). Hallberg and Smith (1976) also report that progesterone does not stimulate amino acid uptake.

Table 4 shows further that hCG, but not progesterone, stimulates amino acid uptake by other amphibian follicles, the very large cells from the Chilean frog *Callyptocephallela caudiverbera*.

Effect of Drugs That Inhibit cAMP Phosphodiesterase on Oocyte Uptake and Protein Synthesis

Recent results from this laboratory (unpublished results), have established that changes in cAMP levels occur in *Xenopus* oocytes after treatment with hormones that induce maturation. It has also been shown that theophylline, a known inhibitor of cAMP phosphodiesterase, blocks oocyte maturation (O'Connor and Smith, 1976).

Figure 7 shows that theophylline at 10⁻³ M, a concentration which completely inhibits oocyte maturation, has an inhibitory effect on oocyte protein synthesis but does not block significantly the stimulation of amino acid uptake induced by hCG.

DISCUSSION

The results presented above demonstrate that hCG has an effect on the uptake systems of at least two species of amphibian follicles resulting in an important increase in the transport of all amino acids and nucleosides tested. The stimulation of uptake of the exogenous nutrients is a result of a decrease in the concentrations required to attain half-maximal velocity of entry, K_T , and not an increase in the maximal velocity of the process. In simple terms this may be interpreted as

TABLE 4

EFFECT OF DIFFERENT COMPOUNDS THAT INDUCE AMPHIBIAN OOCYTE MATURATION ON THE UPTAKE OF [³⁵S]METHIONINE BY FOLLICLES from *Xenopus laevis* and *C. caudiverbera* FROM *Xenopus laevis* AND *Callyptocephallela caudiverbera*^a

Oocyte origin	Agent	[³⁵ S]Methionine uptake (cpm/follicle)	Control (%)	Oocyte maturation (%)
<i>X. laevis</i>	None	5,217	100	0
	Progesterone	5,478	105	97
	hCG	10,121	194	98
	Testosterone	5,060	97	94
	Lanthanum ion	3,704	71	89
<i>C. caudiverbera</i>	None	10,717	100	—
	Progesterone	10,325	96	—
	hCH	15,327	143	—

^a The [³⁵S]methionine taken up per follicle was calculated from triplicate groups of five follicles each for *X. laevis* and three follicles each for *C. caudiverbera* which were incubated for 1 hr with a 20 μ M concentration of the radioactive amino acid in amphibian saline. Where indicated, the groups of follicles were preincubated for 1 hr with different agents before addition of [³⁵S]methionine. The concentrations used were: progesterone, 1 μ M; hCG, 60 units/ml; testosterone, 1 μ M; and lanthanum chloride 10 mM. For the assay of *X. laevis* follicle maturation, groups of approximately 100 follicles were incubated with each agent for 18 hr in amphibian saline and, subsequently, the follicles were examined for germinal vesicle breakdown.

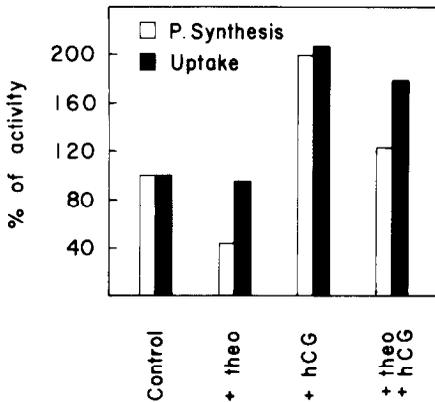


FIG. 7. The effect of theophylline on the hCG stimulation of amino acid uptake and incorporation into proteins by *X. laevis* follicles. In all cases the uptake and incorporation of [³⁵S]methionine into proteins were measured after 1 hr of incubation with a 20 μ M concentration of the amino acid in amphibian saline as described under Materials and Methods. The empty bars indicate incorporation of radioactivity into proteins, and the shaded bars, radioactivity uptake by the follicles. Follicles had been preincubated with 1 mM theophylline for 2 hr and with 60 units/ml of hCG for 1 hr.

an increase in the affinity of the transport components for their specific nutrients but not an increase in the number of the

transport systems themselves.

The study of the requirements for the hormonal stimulation of uptake by follicles leads to the conclusion that the cells must be under physiologically optimal conditions of temperature and pH to respond to the stimulus but do not require the biosynthesis of new proteins. The maturation process on the other hand is blocked by the addition of inhibitors of protein synthesis (Smith and Ecker, 1971). The important stimulation of the incorporation of exogenous amino acids into oocyte nascent proteins observed with hCG appears to be largely a quantitative rather than a qualitative phenomenon. This is indicated by the fact that, after 3 hr of hormone treatment, two-dimensional analysis of proteins synthesized shows the appearance of only three to four new minor components among hundreds of proteins resolved by this method. Hallberg and Smith (1976) have presented evidence for a stimulatory effect of hCG on the assembly of ribosomal particles. Unfortunately most eucaryotic ribosomal proteins are very basic and are not fractionated by the

gels used in this report. For this reason it is not possible to determine whether hCG stimulates specifically the biosynthesis of these proteins.

The lack of a direct correlation between the hCG effect on the uptake of exogenous nutrients by ovarian follicles and its effect on maturation is a very noteworthy observation in that the hormone's action on these cells can be separated into two distinct processes. Low concentrations of hCG (8-10 units/ml) give full effect on oocyte maturation while the effect on amino acid uptake requires considerably higher hormone concentration. Also it was found that cycloheximide and theophylline, which are efficient inhibitors of oocyte maturation, do not block the action of hCG on the follicle transport systems. On the other hand, other hormones or potent inducers of oocyte maturation such as progesterone, testosterone, and lanthanum ions do not affect the transport of exogenous nutrients into the oocytes of ovarian follicles as does hCG. It may not be concluded, however, that the hCG effect on the permeability of the oocyte in the ovarian follicles is not important for the process that accompanies the physiological maturation of amphibian oocytes. It is possible that this effect on the membrane transport systems may play a key role in preparing the germ cell for fertilization and early embryogenesis.

The results of Hallberg and Smith (1976) showed that the effect of hCG on oocyte amino acid uptake requires the presence of the follicle cells surrounding the oocyte. However, it is clear that this action of hCG is not mediated in a simple fashion by endogenous progesterone synthesis triggered by hCG because the steroid hormone itself does not elicit the effect on the amino acid transport of the germ cell. It would seem, therefore, that follicle cells may be playing other roles in addition to the accepted idea that they produce progesterone (Fortune and Blackler, 1974) in response to hCG.

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