Comparative Endocrinology. - Hormonal stimulation of the ovarian adenyl cyclase in the rat and the teleost (Carassius auratus): the effect of adenosine triphosphate concentration.

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COMPARATIVE ENDOCRINOLOGY. - Hormonal stimulation of the ovarian adenyl cyclase in the rat and the teleost (Carassius auratus): the effect of adenosine triphosphate concentration. Brief paper (A) by Mr. Christian Salmon, Mrs. Nadine Delerue-LeBelle and Mr. Yves-Alain Fontaine, presented by Mr. Maurice Fontaine.

In the range of ATP concentrations we considered, hormone stimulation of ovarian adenyl cyclase in vitro is mainly obtained by increasing the $S_{\text{max}}$ in the case of the rat, and by decreasing the $K_m$ relative to the ATP in the case of a fish, the carp.

A single gonadotropin (GTH), different from both the FSH and LH of mammals, was clearly shown both in the carp (Cyprinus carpio) $\left(1, 2\right)$ and the Pacific salmon (Oncorhynchus tshawytscha)$^{(3)}$. Very likely parallel differences exist at the target tissue level of the GTH's, not only as regards the nature of the hormone receptors but also as regards certain mechanisms of the GTH-induced stimulation. Carp gonadotropin (cGTH) $^{(4)}$, and also mammals' LH's and FSH's $\left(5, 6, 7\right)$ are capable of stimulating adenyl cyclase (AC) activity in the homogenates of carp (Carassius auratus) ovaries and the ovaries of various mammals, respectively. Some similarities in chemical properties $^{(2)}$ and action on ovarian follicle growth between the cGTH and the FSH led us to compare the latter's action in the rat to the cGTH's action in the carp. The findings presented here have to do with the effect of adenosine triphosphate (ATP) concentration on the AC and its hormonal stimulation.

Material and methods. - Purified ovine FSH (CNRS - FSH with specific activity = 6 to 8 x NIH - FSH - SI) and highly purified cGTH were provided respectively by Mr. Jutisz and E. Burzawa-Gérard. The carp (10-30 g) are kept at 20 °C, subjected to 8 hours of light daily, and fed twice a week. In these conditions the gonosomatic ratio remains in the 2% - 5% range. Wistar rats are raised in the laboratory (22±2°C) and used 21 - 23 days after birth. The ovaries are homogenized, as previously described $^{(9)}$, in a 2 to 3 volume solution of sucrose. After incubation (cf. the key to the figures), reaction is stopped $^{(9)}$, the AMPc is purified according to the method...
used by Krishna and collaborators\(^\text{10}\) and its radioactivity measured. A "white" is performed for each radioactive ATP concentration by arresting the reaction immediately after adding the homogenate. The results were expressed by stating the accumulation of AMPc(V), in relation to ATP (S) concentration, or the ratio S/V as a function of S. This latter transformation results in a right angle if Michael's kinetics are found; the S\(_{\text{Max}}\) is given by the inverse of the slope of this right angle, while the abscissa at its origin is equal to \(-\mathrm{K}_m\). The equations for the right angles \(q/v = \ln(S)\) have been calculated by the least square method. The protein concentration of the homogenates was determined by Lowry and collaborators'\(^\text{11}\) method as modified by Fiszer\(^\text{12}\).

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**Fig. 1:**\(V = \text{AMPc formed (p mols/mn/mg of protein)}\)

**Fig. 2:**\(V = \text{AMPc formed (p mols/mn/ mg of protein)}\)

**Fig. 1 and 2.** Effect of ATP concentration on AC activity in a homogenate of carp ovaries (Fig. 1) or rat ovaries (Fig. 2) in the presence (\(\circ\)) or absence of gonadotropin (\(\bullet\)). The incubation medium (total volume 0.06 ml) contains the following: Tris HCl pH 7.4: 0.029 M buffer; \(32\) P\text{ATP: 0.00025 to 0.005 M and 10 to 50 cpm/picomol generally;} \(\mathrm{MgCl}_2: 0.006 \text{ M;} \text{AMPc: 0.002 M;} \text{ bovine albumen serum: 1 mg/ml;} \text{ sucrose 0.1 M; ovarian proteins: 4 to 8 mg/ml (rat) or 13 to 22 mg/ml (carp); eventually PSH (rat) or cGTH (carp): 83 \mu g/ml. The 2 mn pre-incubation, and the 4 mn (rat) or 16 mn (carp) incubation, are performed at 37\(^\circ\)C (rat) or 20\(^\circ\)C (carp); in these conditions AC activity is seen to be proportional to the length of the incubation period (unpublished data).

**Results.** Figure 1 shows the results obtained experimentally on the carp ovary. AC activity - either in the absence or presence of a cGTH concentration providing maximum stimulation\(^\text{4}\), increases first with the added ATP concentration, then decreases when the latter goes beyond 5 mM approximately. Only results for ATP concentrations less than or equal to 4 mM were used to estimate the apparent \(\mathrm{K}_m\) and the \(S_{\text{Max}}\). In this experience the cGTH reduces
the Km (from 1.25 to 0.80 mM) and increases the $S_{\text{Max}}$ slightly (from 10.2 to 12.7 picomols AMPc/mn/mg of protein). Figure 2 shows the results of an experiment on rat ovaries. We observe a self-inhibition index for added ATP concentrations greater than 2 mM, and only the results for concentrations smaller or equal to this value were used to determine the apparent Km and the $S_{\text{Max}}$. The FSH at a concentration providing for maximum stimulation (9) increases the $S_{\text{Max}}$ considerably (from 14.6 to 53.1 picomols AMPc/mn/mg of protein) and decreases the Km (from 1.01 to 0.42 mM). The table summarizes all of the data obtained. In the case of the carp, the cGTH clearly reduces the apparent Km, while its action on the $S_{\text{Max}}$ is slight and variable. In the case of the rat, the FSH reduces the apparent Km, but increases the $S_{\text{Max}}$ considerably.

Table

Effect of gonadotropic hormone (cGTH or FSH) on the apparent Km and maximum speed of adenyl cyclase activity in homogenates of carp or rat ovaries.

<table>
<thead>
<tr>
<th></th>
<th>Km(B)</th>
<th>Km(H)</th>
<th>$S_{\text{Max}}$ with hormone</th>
<th>$S_{\text{Max}}$ without hormone</th>
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<tbody>
<tr>
<td></td>
<td>(mM)</td>
<td>(mM)</td>
<td>Km(H)</td>
<td>Km(H)</td>
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<tr>
<td>Carp (4 experiments)</td>
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<td>Rat (4 experiments)</td>
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The conditions under which the experiments were performed are described in the key to the figures. Averages are given along with the standard error of the mean.

Discussion. - Ovary homogenates contain, in all probability, a certain concentration (2) of endogenous ATP. The figures show that the $S_{\text{Max}}$'s, estimated as mentioned above, are equal to the actual $S_{\text{Max}}$'s; on the other hand, the apparent Km's are overestimated by 2. The ratios: Km basal/Km in the presence of hormones, as calculated, are minimal limits. Studies on other tissues suggest that the AC's true substratum is the ATP-Mg complex \[\text{(13), (14), (15)}\]. In conditions used to determine the apparent Km relative to the ATP (ATP concentrations on the order of $10^{-3}$ M and less than the Mg concentration), the ATP appears to be equal to the Km relative to the ATP-Mg complex.
In the case of the rat, certain data suggest the existence of two different Km activities relative to the ATP. We have considered here only experiments in which a single apparent Km is evident.

In the range of ATP concentrations that we have considered, the hormone has an essential action on the carp by reducing the apparent Km and the ATP concentration coefficient, thus the intensity of the hormonal stimulation; such a phenomenon, if effected in vivo, is likely to help in finely regulating the stimulation of various ovarian tissues. In the case of the rat, on the contrary, the hormone acts mainly on the AC's S_max. This difference is not due to the difference in the chosen incubation temperatures (20°C for the carp, 37°C for the rat); in effect, even at 20°C, the FSH acts on rat ovary's AC, especially on the S_max (unpublished data).

In various other tissues of mammals, it has also been shown that hormonal stimulation occurs essentially through increasing the AC's S_max [\( S_{\text{Max}} \)] \(^{(13)}\), \(^{(14)}\). On the other hand, in the case of an amphibian, oxytocin stimulates the bladder's epithelial cells, by both increasing the S_max and decreasing the Km \(^{(15)}\).

In the carp ovary, only the Km is clearly modified by the cGTH; other tissues in various fish must be studied to determine whether this peculiarity is connected with this animal's phylogenetic situation.