Choline acetyltransferase and acetylcholine in *Xenopus* oocytes injected with mRNA from the electric lobe of *Torpedo* (Torpedo brain mRNA/carnitine acetyltransferase)

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Communicated by Bernard Katz, September 14, 1984

**ABSTRACT** Xenopus oocytes were injected with poly(A)+mRNA obtained from the electric lobes of *Torpedo marmorata* and *Torpedo ocellata*, which contain the cell bodies of the neurons that innervate the electric organs. The electric lobe mRNA preparation induces the oocytes to synthesize a catalytically active form of the enzyme choline acetyltransferase (EC 2.3.1.6). Enzymatic activity is found almost exclusively in the cytoplasmic fraction of injected, but not control, oocytes. Evidence is presented that distinguishes between the induced choline acetyltransferase activity and an intrinsic carnitine acetyltransferase activity present in the oocytes. This latter enzyme is associated principally with particulate fractions of the oocyte. The level of acetylcholine, which accumulates in mRNA-injected oocytes, is relatively insensitive to pharmacological manipulations that alter the acetylcholine content of other cells. These results show that *Xenopus* oocytes may be used advantageously to study functional properties of polypeptides associated with presynaptic elements in the nervous system.

Since the work of Gurdon et al. (1), the technique of injecting *Xenopus* oocytes with foreign mRNA has been used to study the synthesis, post-translational processing, and secretion of polypeptides (2, 3). More recently, this technique has been exploited for the study of polypeptides involved in synaptic function (4–8). In this communication we describe the results of experiments in which the injection of oocytes with poly(A)+mRNA from the electric lobe of *Torpedo* induced the oocytes to synthesize a catalytically active form of the enzyme, choline acetyltransferase (CAT; acetyl-CoA:choline O-acetyltransferase, EC 2.3.1.6).

**MATERIALS AND METHODS**

**Isolation of Poly(A)+ mRNA.** As previously described (5, 9), poly(A)+mRNA was isolated from the electric lobes or electroplax of two *Torpedo* species (*Torpedo marmorata* and *Torpedo ocellata*), using phenol/chloroform extraction to obtain RNA followed by chromatography on oligo(dT)-cellulose to select poly(A)+mRNA (10). A poly(A)+mRNA fraction was also obtained from 10 g of defolliculated *Xenopus laevis* oocytes (1-1.3 mm diameter) taken from a single donor.

**Injection of Oocytes.** *Xenopus laevis* oocytes were manually dissected and cultured (11) at 14–16°C in Barth’s medium (in mM: NaCl, 88; KCl, 1; NaHCO3, 2.4; MgSO4, 0.82; Ca(NO3)2, 0.33; CaCl2, 0.41; Tris-HCl, 7.5; pH about 7.2) containing antibiotics (gentamicin at 0.1 mg ml−1 and nystatin at 50 units ml−1). The oocytes were injected usually with 35–45 ng of mRNA and kept in the same culture fluid, with drug additions where indicated, until used for experiments. The culture fluid was replaced at 2-day intervals. The abbrevi-
Other Procedures. (i) Enucleation. Oocytes were enucleated by the procedure of Ford and Gurdon (15) except that defolliculation was done by using collagenase treatment of oocytes (2 mg ml⁻¹ for 1 hr at 20°C).

(ii) Ribonuclease treatment of poly(A)⁺ mRNA. Electric lobe mRNA (10 μg in 10 μl of 20 mM Hepes buffer, pH 7.2) was treated at 30°C with 0.5 unit of ribonuclease A coupled to agarose. After 3 hr the mixture was centrifuged (1 min at 12,000 × g) and the supernatant fluid was used for injection of oocytes.

(iii) Chemicals. The following chemicals came from Sigma: BW284-C51 [1,5-bis(4-allyldimethylammoniumphenyl)-pentan-3-one], actinomycin D, Electrophorus electricus acetylcholinesterase (type V-S), acetyl-CoA, L-carnitine, choline chloride, collagenase (type 1), cycloheximide, physostigmine (eserine) sulfate, and ribonuclease coupled to agarose. Hemicholinium-3 came from Aldrich and was recrystallized from ethanol. [¹⁴C]Acetyl-CoA (53-56 mCi mmol⁻¹; 1 Ci = 37 GBq) was purchased from Amersham. β-Bungarotoxin was the gift of P. N. Strong. (2-Benzoylethyl)-trimethylammonium chloride (BETA) was synthesized as described (16).

RESULTS

CAT Activity and AcCho of Oocytes: Time Course. With standard assay conditions, CAT activity could not be detected in homogenates of control noninjected oocytes. When the number of oocytes was raised to 20–30 per 0.1 ml of the homogenization buffer, choline acetylation was barely detectable at a level of 95 fmol min⁻¹ per oocyte (mean of two experiments). In view of the substantial amount of carnitine acetyltransferase activity of oocytes (see below), it is possible that some of the acetylation of choline was due to carnitine acetyltransferase and not to a low level of CAT activity (cf. ref. 17).

When oocytes were injected with mRNA from Torpedo electric lobe, CAT activity was measurable within 2 days of the mRNA injection (Fig. 1A). However, mRNA derived from Torpedo electroplax did not induce CAT activity in appropriately injected oocytes. Both the specific activity and the temporal pattern of accumulation of CAT activity varied among different batches of oocytes injected with electric lobe mRNA. In the two examples shown in Fig. 1A, CAT activity reached a peak between days 10 and 12 and then declined. We have not systematically examined parameters such as the quantity of mRNA injected or culturing conditions, which should affect the appearance of CAT activity in EL oocytes.

CAT activity was not detected in oocytes injected with mRNA from Torpedo electroplax, even though this mRNA preparation induced functional nicotinic AcCho receptors (5) and acetylcholinesterase in oocytes (unpublished data). Moreover, mRNA preparations derived from rat or chicken brains, which contain appreciable amounts of CAT, did not induce the expression of CAT activity in injected oocytes. Thus, the electric lobe of Torpedo, probably because of its high density of cholinergic neurons, is a rich source of active mRNA coding for CAT.

We measured the AcCho content of single noninjected oocytes, and of oocytes injected with Torpedo electroplax or electric lobe mRNA. AcCho was consistently detected only in the EL oocytes (Fig. 1B). In the example illustrated, the AcCho content reached an apparent peak at day 8 and thereafter it declined gradually. However, at any given period after injection, there was a considerable variation in the AcCho content of individual oocytes, which was reflected in the large standard errors (Fig. 1B). Such variability may be due to a number of causes, including the availability of the substrates, choline and acetyl-CoA, for CAT. The important point is that our results show that the CAT of EL oocytes assayed in vitro (Fig. 1A) is functional in the oocytes and that AcCho can be detected as long as 3 weeks after the injection of the foreign mRNA.

CAT Activity and Carnitine Acetyltransferase Activity of Oocytes. We were concerned that the CAT activity we measured may have been due to carnitine acetyltransferase, which can interfere with CAT measurements (18, 19). Data presented below suggest that the low rate of acetylation of choline by noninjected oocytes may be attributable to carnitine acetyltransferase activity.

![Fig. 1](image-url)

**Fig. 1.** CAT activity and AcCho content of oocytes: Time course. (A) CAT activity was measured in homogenates of two different groups of EL oocytes (□ and ▪) at the days indicated after injection of mRNA. Under similar conditions, CAT activity could not be detected in noninjected control oocytes or in oocytes injected with mRNA from Torpedo electroplax. Points represent the mean of triplicate determinations; SEM was less than 12%. (B) AcCho content of single EL oocytes was measured between days 2 and 21 after mRNA injection. Individual entries give the mean ± SEM of data for 3 or 4 oocytes. AcCho was occasionally detected in the range of 0.1 to 1.0 pmol in noninjected control oocytes or in oocytes injected with mRNA from Torpedo electroplax (see Discussion).
Carnitine acetyltransferase, while the CAT activity of EL oocytes is clearly due to the enzyme CAT, whose synthesis was induced by the foreign mRNA.

First, homogenates of control, noninjected, oocytes had no detectable CAT activity, while homogenates of EL oocytes had CAT activity (Fig. 1 and Table 1). Furthermore, although the rate of acetylation of choline differed between control and EL oocytes, their carnitine acetyltransferase activities were the same (Table 1). Thus, EL oocytes had acquired a capacity to acetylate choline without a concomitant change of carnitine acetyltransferase activity.

Second, we examined the distribution of CAT and carnitine acetyltransferase activities in subcellular fractions of EL oocytes obtained by a procedure similar to that of Ohlsson et al. (20). Yolk platelets were separated from the initial homogenate by centrifugation through a 1.5 M sucrose cushion. The remaining supernatant was centrifuged at high speed to yield a soluble fraction and a membrane pellet. Under these conditions more than 90% of the CAT activity resided in soluble fractions, whereas nearly the same proportion of the recovered carnitine acetyltransferase activity was found in the pellet and was presumably membrane bound. The differential distribution of these two enzyme activities suggests that they are mediated by different polypeptides.

When control oocytes were subjected to the same fractionation scheme, the carnitine acetyltransferase activity was distributed in a pattern very similar to that of EL oocytes (data not shown). However, when the distribution of CAT was assessed in these control oocytes (using 200 oocytes), the choline acetylating activity was found almost exclusively in the high-speed pellet. In one experiment the initial homogenate gave an apparent CAT activity of 92 fmol min⁻¹ per oocyte, while the activity in the high-speed pellet was 61 fmol min⁻¹ per oocyte. This result suggests that the membrane-associated carnitine acetyltransferase may give rise to the apparent CAT activity of native oocytes.

AcCho in Subcellular Fractions of EL Oocytes. Nearly all of the AcCho of EL oocytes was found in the supernatant fraction (Table 2). With the fractionation procedure used, any structures resembling synaptic vesicles would be found in the pellet. Therefore, the experiments suggest that in the injected oocytes the newly synthesized AcCho is not parcelled into membrane-limited compartments, as occurs normally in cholinergic nerve terminals.

**Evidence That CAT Activity of EL Oocytes Arises Directly Via Translation of Injected mRNA.** Several experiments were performed to see if the oocytes were translating Torpedo mRNA that codes for CAT or whether this enzyme activity arose by a more indirect pathway. First, procedures were performed to exclude any short-term participation of the oocyte's own genome in the expression of CAT activity. For instance, treatment of oocytes with actinomycin D, an inhibitor of RNA synthesis (21), did not prevent the appearance of CAT activity or of AcCho in EL oocytes (Table 3). In fact, oocytes kept in actinomycin D showed significantly higher (P < 0.05 by the paired Student's t test) CAT activity than controls. This is in accord with previous experiments (unpublished data), showing that the number of functional nicotinic AcCho receptors induced by mRNA from *Torpedo*

### Table 1. Carnitine acetyltransferase activity and CAT activity of control and injected oocytes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activity, pmol min⁻¹ per oocyte</th>
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</thead>
<tbody>
<tr>
<td>Carnitine acetyltransferase</td>
<td>141 ± 7</td>
</tr>
<tr>
<td>Choline acetyltransferase</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Noninjected oocytes</td>
<td>129 ± 19</td>
</tr>
<tr>
<td>EL oocytes</td>
<td>5.1 ± 1.1</td>
</tr>
</tbody>
</table>

Results are the mean ± SEM of 8–12 determinations in four separate experiments.

### Table 2. Subcellular fractionation of oocytes: enzyme activities and AcCho

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Enzyme activity, pmol min⁻¹ per oocyte</th>
<th>AcCho, pmol per oocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carnitine acetyltransferase CAT</td>
<td></td>
</tr>
<tr>
<td>Original homogenate</td>
<td>135 ± 11</td>
<td>86 ± 12</td>
</tr>
<tr>
<td>Yolk-platelet pellet (a)</td>
<td>11 ± 3</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td>1.5 M sucrose cushion (b)</td>
<td>4 ± 1</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>Soluble (c)</td>
<td>11 ± 3</td>
<td>4.4 ± 0.5</td>
</tr>
<tr>
<td>High-speed pellet (d)</td>
<td>85 ± 9</td>
<td>0.2 ± 0.04</td>
</tr>
<tr>
<td>% membrane bound</td>
<td>86</td>
<td>6 ± 7</td>
</tr>
</tbody>
</table>

EL oocytes were fractionated and either the enzyme activities or the AcCho contents of the fractions were measured. Results are the mean ± SEM of three to six separate experiments using oocytes 8–14 days after injection of mRNA. Percent membrane bound gives the percent of recovered enzyme activity (or AcCho) in the pellet fractions and was computed [(a + d)/(a + b + c + d)] × 100%.

When EL oocytes were cultured in the presence of the protein synthesis inhibitor cycloheximide (2 μg ml⁻¹; at this concentration, cycloheximide inhibits the incorporation of [³H]histidine into oocyte protein by more than 95%; unpublished observations) we detected little or no CAT activity or AcCho in these cells. This suggests that protein synthesis is necessary for the production of CAT and AcCho in EL oocytes. We also found that treatment of the electric-lobespoly(A)⁺ mRNA with ribonuclease destroyed the capacity of this material to induce oocytes to synthesize CAT and AcCho. This indicates that a ribonuclease-sensitive factor is responsible for inducing CAT and AcCho in EL oocytes. Finally, we injected oocytes with poly(A)⁺ mRNA derived from the *Xenopus* oocytes themselves. Injection of this mRNA fraction into oocytes did not cause a detectable expression of CAT activity. This result suggests that the injected EL mRNA does not work by activating an intrinsic, but translationally silent, mRNA of the oocyte, and it supports the earlier observation (Fig. 1) of a tissue specificity in the capacity of mRNA to induce CAT in oocytes.

**Pharmacology.** A preliminary study was made of possible

### Table 3. Effect of actinomycin D and enucleation on the appearance of AcCho and CAT in EL oocytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CAT activity, pmol min⁻¹ per oocyte</th>
<th>AcCho, pmol per oocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 4</td>
<td>Day 8</td>
</tr>
<tr>
<td>(1) Control</td>
<td>2.3 ± 0.6</td>
<td>4.8 ± 1.1</td>
</tr>
<tr>
<td>(2) Actinomycin D</td>
<td>3.0 ± 0.3</td>
<td>7.4 ± 1.8</td>
</tr>
<tr>
<td>(3) Enucleation</td>
<td>1.5 ± 0.2</td>
<td>2.8 ± 0.6</td>
</tr>
</tbody>
</table>

*Xenopus* oocytes were injected with electric lobe mRNA. In entry 3, the cells had been enucleated prior to injection of mRNA. The cells were then cultured in normal Barth's medium with antibiotics. In entry 2 the medium also contained actinomycin D (50 μg ml⁻¹). At days 4 and 8 oocytes were taken to measure CAT activity and AcCho content. Data for AcCho content are the mean ± SEM of 3–10 determinations. CAT activities are the mean ± SEM or range for 2 or 3 experiments.
changes in the AcCho content of EL oocytes caused by various substances that are known to affect AcCho turnover in other tissues. Oocytes were injected with electric lobe mRNA and then bathed for 4 days in Barth's medium with BETA (0.1 mM), an inhibitor of CAT (16); β-bungarotoxins (10 μg/ml−1), a polypeptide neurotoxin that inhibits AcCho release from nerve endings and stimulates AcCho synthesis in rat diaphragm (22, 23); hemicholinium-3 (0.1 mM), a compound that blocks choline transport (see ref. 19); or eserine sulfate (0.1 mM), a cholinesterase inhibitor. Under the conditions tested, none of these compounds produced a significant change of the AcCho content (96 pmol per oocyte) of EL oocytes.

DISCUSSION

Xenopus oocytes injected with mRNA from Torpedo electric lobe contain CAT activity and AcCho. In contrast, noninjected oocytes, or those injected with mRNA from several other sources (e.g., Torpedo electroplax), show very low or undetectable levels of CAT activity and AcCho. The CAT that is found in EL oocytes is distinct from the endogenous carmine acetylcholine transferase activity and several lines of evidence support the contention that EL oocytes translate Torpedo mRNA coding for CAT to generate a catalytically active enzyme. These findings extend the work of Schmid et al. (7) by showing that Xenopus oocytes can express a functional enzyme that is characteristic of cholinergic nerve terminals.

Our evidence shows (Tables 1 and 2) that EL oocytes acquire a significant level of CAT activity that is distinct from the intrinsic carmine acetylcholine transferase activity of these cells. We also attempted to distinguish pharmacologically between these enzyme activities. However, even substances such as BETA, which is a potent and relatively selective inhibitor of mammalian CAT (16), were found to be effective inhibitors of both enzymes in the same concentration range. As a consequence, the subcellular fractionation data provide the clearest differentiation between the soluble CAT activity and the predominantly membrane-associated carmine acetylcholine transferase activity of EL oocytes (Table 2); this enzyme distribution mirrors that found in other tissues (18, 19, 24).

At any time from 2 days onward (Fig. 1) the CAT activity of a typical EL oocyte is sufficient to synthesize the cell's entire store of AcCho in less than 1 hr. However, in none of our experiments did the AcCho content of EL oocytes reach the level that might be expected if CAT were maximally active. This suggests either that there are restraints on CAT activity in situ or that AcCho is lost due to secretion into the medium or hydrolysis by intracellular esterases. In preliminary experiments the AcCho output from EL oocytes was found to be less than 3 pmol hr−1 (unpublished observations). Thus, secretion can be only a minor route for controlling the accumulation of AcCho in the oocyte. Attempts have also been made to halt the degradation of oocyte AcCho by using the cholinesterase inhibitor eserine. Xenopus oocytes are known to have intrinsic acetylcholinesterase activity (8, 17, 25), a large percentage of which is membrane associated (25). However, eserine had no significant effect on oocyte AcCho content. This suggests, first, that the native acetylcholinesterase activity of the oocyte had little access to the AcCho that is synthesized in the cytoplasm and, second, that it is a low rate of synthesis of AcCho, rather than a high rate of secretion or degradation of this amine, that controls its accumulation in EL oocytes. Further study will be necessary to determine what mechanism(s) control the oocyte AcCho level.

Using a large number of gametes, Robbins and Molenar (17) reported a mean AcCho content for a Xenopus egg of about 0.2 pmol. This quantity of AcCho is near the limit of accurate measurement in our experiments in which single oocytes were used. We have not approached this question systematically, but our data for individual oocytes reveal an AcCho content of less than 1 pmol per oocyte. Assuming that the 0.2 pmol value is accurate, then the AcCho concentration of native oocytes (which have a volume of approximately 1 μl) is about 0.2 μM and rises to 0.1–0.2 mM in EL oocytes. This level of AcCho is considerably lower than the 10 mM value estimated for Torpedo nerve terminals (26), which have the advantage, of course, that large amounts of AcCho are stored in synaptic vesicles. By the same token, Dowdall et al. (27) reported a CAT activity for Torpedo electromotor neurons that was equal to 311 pmol min−1 mg−1. Homogenates of EL oocytes can synthesize AcCho at a rate of approximately 5–10 pmol min−1 mg−1, or about 2–3% as rapidly as electromotor neurons.

We thank Margaret Roch, Ruth Booth, and Kathy Rice for assistance with the acetylcholine analyses and the staff of the Stazione Zoologica, Naples, Italy, for help in obtaining the Torpedo. This work was supported by grants from the U.S. National Institutes of Health (MH 17691), the U.K. Medical Research Council, and The Royal Society of London.