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Properties of acetylcholine receptors translated by cat muscle mRNA in *Xenopus* oocytes

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Poly(A)$^+$ mRNA, extracted from denervated skeletal muscles of the cat, directs the synthesis of acetylcholine receptors in *Xenopus laevis* oocytes. The receptors are inserted in the oocyte membrane where they form acetylcholine receptor-channel complexes which have properties like those of the native receptors in the muscle membrane.

Key words: acetylcholine receptors/cat muscle mRNA/membrane channels/*Xenopus* oocytes

Introduction

The plasma membrane of *Xenopus* oocytes contains receptors for some of the substances which act as transmitters in the nervous system. Among the neurotransmitter receptors that have been found in the oocytes are one or more types of muscarinic acetylcholine (ACh) receptors, but no nicotinic ACh-receptors (Kusano *et al.*, 1977, 1982). However, when poly(A)$^+$ mRNA extracted from the electric organ of *Torpedo* is injected into *Xenopus* oocytes this mRNA directs the synthesis of nicotinic ACh-receptors (Sumikawa *et al.*, 1981) which are inserted into the oocyte membrane, where they form functional ACh-receptor-channel complexes (Barnard *et al.*, 1982). More recently, Miledi and Sumikawa (1982) have shown that poly(A)$^+$ mRNA extracted from denervated or innervated skeletal muscles of the cat is also translated by *Xenopus* oocytes, and again the various protein subunits which make up the nicotinic ACh-receptor-channel complex are correctly assembled and inserted into the oocyte membrane. The present paper describes some of the properties of the ACh-receptor-channels encoded by mRNA from denervated muscle. A comparison with receptors translated from mRNA derived from innervated muscle will be presented at a subsequent date.

Results

Nicotinic and muscarinic ACh responses

When ACh was applied to the surface of oocytes injected with mRNA from denervated cat muscle (Cden-mRNA) a response was elicited in practically all the oocytes examined >2 days after injection. As already mentioned, many oocytes already have ACh-receptors (Kusano *et al.*, 1977, 1982), but the functional characteristics of the native receptors are so different from those of the new receptors, encoded by the injected Cden-mRNA, that both types of receptors can be identified unambiguously. For example, a very striking difference is the latency and time course of the membrane currents induced by ionophoretic application of ACh (Figure 1). In control oocytes (not injected, or injected with water or mRNA from fibroblasts) the response begins after a long delay and frequently has a characteristic 'oscillatory' nature, which is particularly evident in the oocyte used to obtain Figure 1B. On the other hand, oocytes injected with Cden-mRNA show an almost immediate response to ACh and the membrane current (Figure 1A) has the simple rise and fall, which is usually seen when ACh is applied in this way to the endplate region of skeletal muscle fibres of vertebrates.

The two types of ACh-receptors can also be easily distinguished by pharmacological means: the native ACh-receptors of the oocytes are of the muscarinic type and are blocked by atropine (Kusano *et al.*, 1977, 1982) while the receptors encoded by the Cden-mRNA have nicotinic properties: i.e., they are blocked by curare and α-bungarotoxin, but not by atropine (Miledi and Sumikawa, 1982). In many of the experiments reported here, the native muscarinic receptors were blocked by the addition of atropine (5×10^{-7} M) to the bathing solutions. This simplified the analysis, since otherwise injected oocytes could give both types of responses to ACh application (e.g., a small muscarinic response can be seen ~30 s after the application of ACh in Figure 1A). However, some control experiments (described later) were also made on oocytes not treated with atropine, to ascertain that

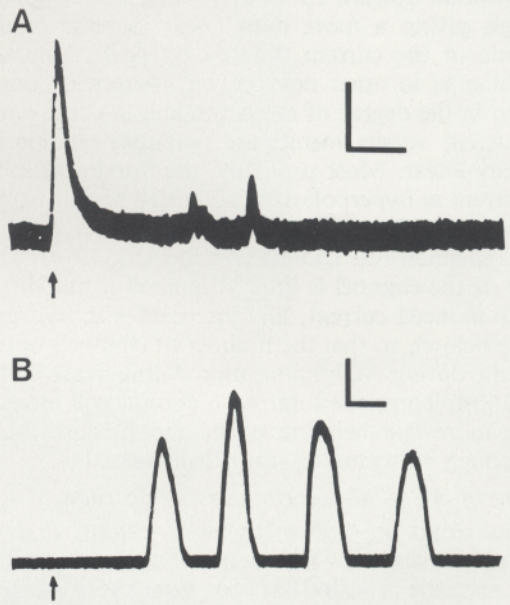

Fig. 1. Nicotinic and muscarinic responses to ACh applied ionophoretically to *X. laevis* oocytes. Each record shows the change in clamp current elicited by an ionophoretic pulse of ACh of 1 s duration, given at the time indicated by the arrows. **(A)** Predominantly nicotinic response to ACh in an oocyte (membrane potential −100 mV) after injection of Cden-mRNA. Note the short latency and rapid time course of the main response. A small muscarinic component is also present at 20–30 s after the onset of the ACh pulse. **(B)** Muscarinic response to ACh in a control oocyte (membrane potential −60 mV). Note the long delay and oscillatory nature of the response. In this and following figures, inward current is denoted by an upward deflection. Calibration bars are 10 s and 50 nA in each record.

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the drug was not significantly affecting the properties of the membrane channels opened by activation of the nicotinic ACh-receptors. For those experiments, the ACh was applied ionophoretically at a spot on the oocyte where almost pure nicotinic responses were elicited. Selection of such spots was not difficult because the receptors induced by Cden-mRNA, like those produced by *Torpedo* mRNA, were preferentially inserted into the vegetal hemisphere of the oocyte (Barnard *et al.*, 1982; Miledi and Sumikawa, 1982), whilst the native muscarinic receptors are preponderantly located in the animal hemisphere (Kusano *et al.*, 1982).

Current/voltage relation of ACh-induced channels

The amplitude of the current induced by activation of nicotinic ACh-receptors in oocytes injected with mRNA from cat muscle varies with membrane potential (Miledi and Sumikawa, 1982), as would be expected if ACh opens ionic channels in the membrane. This relationship was examined by recording the currents induced by a constant ionophoretic pulse of ACh applied while the membrane was clamped at different potentials. Atropine (5×10^{-7} M) was usually present in the bathing solution to block any muscarinic response.

Typical records are illustrated in Figure 2. The ACh-induced membrane current decreased as the membrane potential was reduced, became zero at about -10 mV, and inverted when the potential was made more positive. A mean value of -9.6 ± 0.6 (s.e.) mV was obtained for the equilibrium potential in eight oocytes injected with Cden-mRNA; a value which confirms that found in a different batch of oocytes (Miledi and Sumikawa, 1982). The current/voltage relation obtained from an injected oocyte is shown in Figure 3 (open symbols). In this oocyte the relationship shows an upward curvature, with increasingly negative potentials giving a more than linear increase in the peak amplitude of the current induced by ACh. Similar results were obtained in other oocytes, but there was considerable variation in the degree of curvature and in some oocytes the ACh current *versus* membrane potential relationship was practically linear. Most probably, the non-linear increase in ACh current at hyperpolarized potentials results from a voltage-dependent change in the mean lifetime of the ACh-induced channels (cf. Dionne and Stevens, 1974). The mean lifetime of the channel is brief compared to the duration of the ACh-induced current, and increases with hyperpolarization (see below), so that the number of channels open at any given time during ACh application will increase with hyperpolarization. Thus, the total ACh current will increase both because more channels are open, and because the current flow through individual channels is increased.

Blocking of ACh-induced channels by procaine

Several drugs are known to block current flow through endplate channels in the muscle membrane. The action of the local anaesthetic procaine has been extensively studied (Furukawa, 1957; Kordas, 1970; Katz and Miledi, 1975, 1980), and it was therefore interesting to examine the effect of procaine on the ACh-receptor-channel complexes which are incorporated into the oocyte membrane after injection of cat muscle mRNA. The filled circles in Figure 3 show the currents induced by ACh in the presence of 0.1 mM procaine. Two main features can be noted: firstly, the amplitudes of the currents are greatly reduced as compared to currents obtained in normal Ringer; and secondly the voltage dependence of the currents is changed so that hyperpolarization beyond about -80 mV does not cause any further increase in their amplitude. Experiments were also performed in the presence of 0.3 mM procaine, but with this concentration the responses were very small. For instance, at a potential of -100 mV the ACh current was reduced to ~5% of the control value. These findings are similar to those obtained at vertebrate endplates, where a voltage-dependent block of ACh-induced channels by procaine has been observed (Kordaš, 1970; Katz and Miledi, 1980).

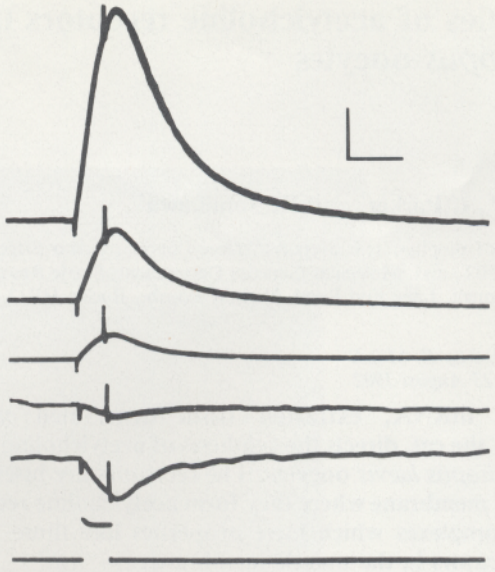

Fig. 2. Nicotinic responses to an ionophoretic pulse of ACh recorded at different membrane potentials from an oocyte injected with Cden-mRNA. The oocyte membrane was voltage clamped at (from top to bottom) -100, -40, -20, 0, and $+20$ mV. The lowest trace shows the ionophoretic pulse duration. The clamp current was recorded through a 30 Hz low pass filter. Bathing solution contained 5×10^{-7} M atropine. Calibration bars indicate 1 s and 20 nA for clamp current. Ionophoretic pulse was 60 nA.

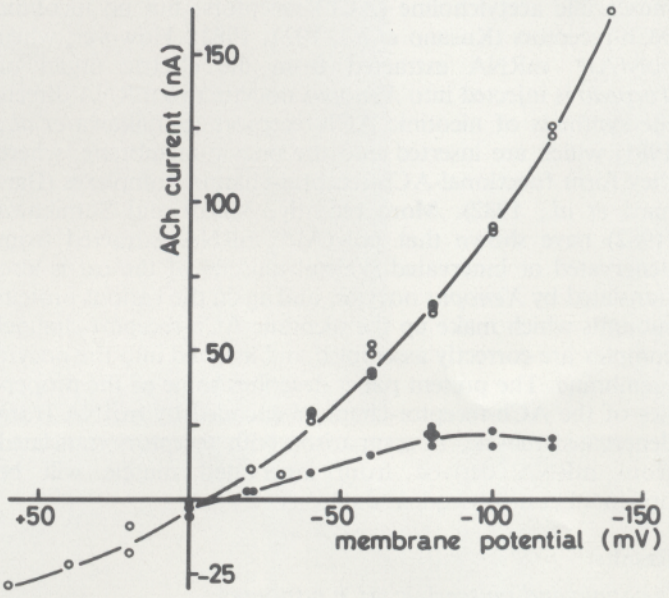

Fig. 3. Membrane voltage dependence of the ACh current induced by an ionophoretic pulse (0.5 s, 33 nA) in an oocyte injected with Cden-mRNA. Bathing solution included 5×10^{-7} M atropine. Open symbols, in normal Ringer. Closed symbols, ACh applied to the same spot in the oocyte after adding procaine (100 μM). The curves were drawn by eye.

ACh-induced membrane current noise

Statistical analysis of the membrane current fluctuations, during application of an agonist drug, can be used to derive the characteristics of the elementary events which underly the drug response (Katz and Miledi, 1971, 1972; Anderson and Stevens, 1973). We used this technique to obtain estimates of the mean lifetime (τ) and conductance (γ) of the cat muscle ACh-induced channels which appear in the oocyte membrane after injection of Cden-mRNA.

One problem with these experiments was that the background noise in the voltage clamp current was very high, partly because of the large electrical capacitance of the oocyte. The noise was minimized by using recording and current injecting microelectrodes of low resistance; and in this way a clear increase in membrane current noise was seen accompanying the current induced by ACh (Figure 4). Analysis of the noise was made by forming average power spectra. The lowest symbols in Figure 5 show the spectrum of background membrane and instrumental current noise, in the absence of ACh. The power of this noise increases at both high and low frequencies, as found in skeletal muscle fibres (Anderson and Stevens, 1973), but over the approximate range of 1 – 100 Hz it is sufficiently small to allow a clear resolution of the extra noise induced by ACh. Spectra of the noise induced by ACh when the oocyte membrane was held at −80 and −120 mV are illustrated by the upper traces in Figure 5. Both these spectra appear to comprise two components. At frequencies above ~10 Hz, all the data analysed so far (11 oocytes, 54 runs) could be fitted fairly well by a single Lorentzian component: $S(f) = S(0)/(1 + (f/f_c)^2)$; where $S(f)$ and $S(0)$ are the spectral densities at frequencies f and 0 Hz respectively, and f_c is the cut-off frequency at which the spectrum has decreased to half the power of the zero frequency asymptote. The cut-off frequency shifted to lower values at more negative potentials, as indicated by the arrows in Figure 5. At frequencies below 10 Hz there was usually a gradual increase in power with decreasing frequency, but this component varied considerably in different oocytes, and sometimes seemed to be absent.

The Lorentzian component in the spectra most probably arises from activation, by ACh, of ionic channels whose open lifetimes are distributed exponentially. ACh-induced channels in muscle behave in this manner (Katz and Miledi, 1972; Neher and Sakmann, 1976) and under normal conditions the spectrum of the membrane noise induced by ACh follows a single Lorentzian curve (Katz and Miledi, 1972; Anderson and Stevens, 1973). At present, the origin of the additional low frequency component seen in the oocyte is not clear. We analysed data obtained during either ionophoretic or bath application of ACh and composite spectra were seen with both methods of drug application.

Single channel lifetime (τ)

Estimates of the mean lifetime of the ACh-induced membrane channels were derived from the half-power frequency of the Lorentzian component in the noise spectra (cf. Materials and methods). Data obtained at various membrane potentials are illustrated in Figure 6. ACh-induced channels in muscle are known to increase their lifetime exponentially as the membrane is hyperpolarized (Anderson and Stevens, 1973; Dionne and Stevens, 1974) and the cat muscle channels incorporated into the *Xenopus* oocyte membrane appear to behave in a similar manner. At present, the results analysed are too few to be certain about the form of the voltage dependence, but it is clear from Figure 6 that membrane hyperpolarization results in an increase in lifetime. The line fitted to the data corresponds to an e-fold change in lifetime for a potential change of 60 mV.

Fig. 4. Current noise evoked by ionophoretic application of ACh to an oocyte injected with Cden-mRNA. The upper trace shows the clamp current recorded (DC) at low gain; the middle trace is a high gain (AC) recording of the same current; and the lowest trace shows the current flowing through the ionophoretic pipette. Note that during ACh application there is an increase in noise which is proportional to the total ACh current. The AC trace was bandpass filtered at 0.1 – 200 Hz. Maximum voltage error in the clamp was <2 mV. Ringer solution contained 5×10^{-7} M atropine. Calibration bars are 15 s, 200 nA for the DC current trace and 50 nA for the ionophoretic current monitor.

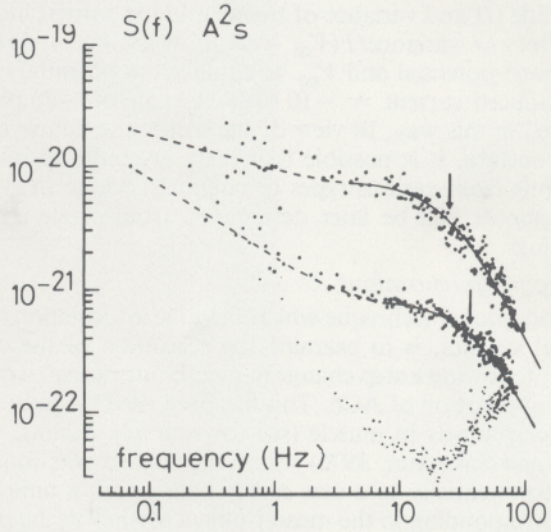

Fig. 5. Power spectra of ACh current noise in an oocyte injected with Cden-mRNA. Lowest curve (small dots) is the control background noise spectrum at −80 mV membrane potential. Middle and upper curves are the extra noise evoked by bath application of ACh (1 μM) at membrane potentials of −80 and −120 mV, respectively. For these two traces control noise spectra taken immediately before ACh application were subtracted from the noise during ACh action to remove contributions from non-receptor membrane and electronics. Solid curves follow a Lorentzian component and were fitted by eye to the data. Dashed curves were drawn by eye. Arrows mark the half-power frequencies (cf. Materials and methods) and are at 35 Hz and 23 Hz for the data at −80 and −120 mV, respectively.

Single channel conductance (γ)

The estimation of the mean conductance of the membrane channels opened by ACh in the oocytes was complicated by the composite nature of the power spectra (cf. Figure 5). The relative magnitudes of the two components was rather variable among different oocytes, making it difficult to analyse them separately. Therefore, a preliminary estimate of the single channel conductance (γ) was obtained from the mean

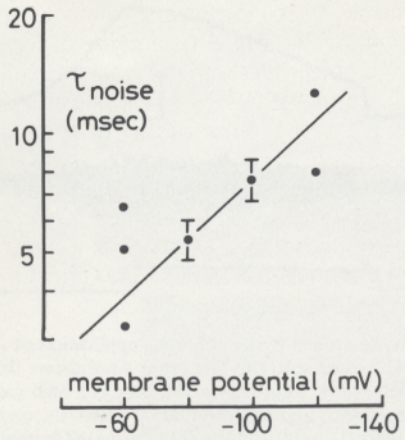

Fig. 6. Voltage dependence of the lifetime (τ) of single channels induced by bath applied ACh in oocytes injected with Cden-mRNA. Estimates of τ were derived from noise spectra similar to those in Figure 5. Error bars indicate ± 1 s.e.; points without bars are single observations.

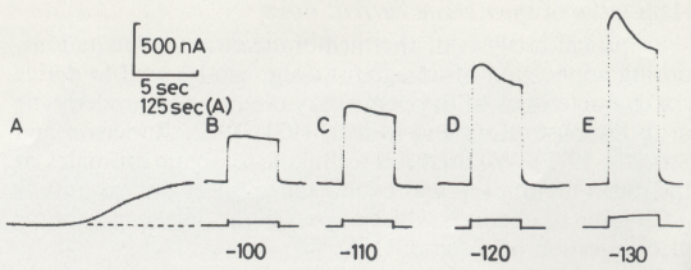

Fig. 7. Current relaxations elicited by voltage jumps during continuous bath application of ACh (2×10^{-6} M) to an oocyte injected with Cden-mRNA. **Frame A** shows the increase in membrane current due to application of ACh, at a holding potential of −80 mV. Time calibration is 125 s for this frame. **B**–**F** show currents elicited, during the same run of ACh application, by voltage steps to the potentials indicated (in mV). In each frame the lower traces give current in the absence of ACh, and the upper traces are during ACh application.

amplitude ($\bar{I}$) and variance of the membrane current induced by ACh (γ = variance/$\bar{I}$ ($V_m - V_{eq}$); where V_m = clamped membrane potential and V_{eq} = equilibrium potential of the ACh-induced current = −10 mV). A value of ~20 pS was obtained in this way. In view of the composite nature of the power spectra, it is possible that these are built up by contributions from various types of channels, whose individual conductances will be later determined from single channel recordings.

Voltage jump relaxations

An additional technique which can give information about channel kinetics, is to examine the relaxation of the clamp current following a step change in membrane potential during steady application of ACh. This has been used to study ACh-induced channels in muscle (see for example Adams, 1977; Neher and Sakmann, 1975) where the current was found to relax exponentially to a new steady value, with a time constant corresponding to the mean channel lifetime at the potential following the step.

Records of voltage jump responses obtained from an oocyte injected with denervated cat muscle mRNA are shown in Figure 7. When voltage pulses are applied in the absence of ACh, the ensuing membrane currents are small and approximately linearly related to the pulse amplitude. In contrast, the same voltage steps applied during the action of ACh gave much larger currents, which show a non-linear relation and comprise at least two different components. During a hyperpolarizing pulse, these components are seen as an initial increase in current, with a time constant of several hundred milliseconds, followed by a slower decline in current. Both components become more obvious at strongly hyperpolarized potentials (Figure 7D,E). The cause of these interesting changes in membrane current remains to be investigated. Unfortunately, a large capacitative surge frequently masked the current records during the first hundred milliseconds following a voltage step, and this has prevented us from resolving clearly any rapid relaxation corresponding to the channel lifetime derived from noise analysis.

Number of cat ACh channels incorporated into the oocyte membrane

An approximate estimate of the total number of ACh-activated channels which are incorporated into the oocyte plasma membrane, can be obtained by measuring the total current flow elicited by a high concentration of bath applied ACh, and dividing this by the single channel current estimated from noise analysis.

Measurements were made on five oocytes, which had been injected with Cden-mRNA 4–13 days previously. Over this time scale, and in the oocytes analysed so far, there did not appear to be any large change in ACh sensitivity. ACh concentrations of 2×10^{-5} M or 10^{-4} M were used to activate the receptors, both concentrations being sufficient to elicit a near maximal response. In these experiments atropine (5×10^{-7} M) was used to block any native muscarinic ACh-receptors. The oocytes were clamped at −20 or −30 mV so that the large currents evoked did not cause clamp failure. The peak currents elicited by bath application of ACh to the whole membrane of the ococyte were measured; and because desensitization is very slight for the ACh-induced channels translated from Cden-mRNA (e.g., Figure 4) it is unlikely that this factor introduces a large source of error in estimating the maximum current flow. The mean conductance change (peak ACh current/($V_m - V_{eq}$)) during maximal activation by ACh was 105 ± 64 μS (± 1 s.d.). Taking the value derived above for the single channel conductance, γ = 20 pS, this would indicate that the number of channels incorporated was at least $\sim 5 \times 10^6$.

A few oocytes from the same batch were used to estimate the number of ACh-receptors in the surface membrane of the oocytes, using ^{125}I-labelled α-bungarotoxin. The toxin is probably incapable of crossing the membrane and would therefore not have access to ACh-receptors in the cell's interior. Eight control and two mRNA-injected oocytes, all derived from the same donor, were incubated in α-toxin for 1 h. After extensive washing, the mRNA-injected oocytes had bound toxin, in excess of a small amount of toxin bound unspecifically to control oocytes. Assuming two toxin sites per ACh-receptor (Changeux, 1981) the excess toxin bound by the mRNA-injected oocytes corresponded to $\sim 10^8$ ACh-receptors per oocyte. This is somewhat larger than the number of ACh-activated channels estimated electrophysiologically, but further work is needed to assess the significance of this preliminary observation.

Discussion

A variety of neurotransmitter receptors are known to be present in the plasma membrane of *Xenopus* oocytes. For in-

stance, the oocytes can have ACh-receptors of the muscarinic type and receptors to (−)-epinephrine, dopamine, and serotonin (Kusano *et al.*, 1977, 1982). Moreover, we have recently found that some oocytes have receptors to enkephalin, substance P, adenosine, and some amino acids (Miledi and Sumikawa, 1982, and upublished data). It should be noted that a particular oocyte does not contain all these receptors at the same time, but several may co-exist in the oocyte membrane. We do not known why the oocyte should exhibit these receptors; but their presence may be a consequence of a largely derepressed genome. Sea urchin oocytes contain a complexity of mRNA such that ~20 000 different genes are represented (Galau *et al.*, 1976). It seems likely that a similar complexity of transcripts exists in *Xenopus* oocytes; in which case, it would not be surprising that some of these transcripts correspond to, and are translated into, neurotransmitter receptors. What is surprising, is that nicotinic ACh-receptors have not been detected in the large number of oocytes examined so far.

It could be thought that nicotinic ACh-receptors are absent because the oocyte is incapable of translating the corresponding mRNA, or is incapable of processing correctly the products of its translation. However, the present experiments, and those reported earlier (Barnard *et al.*, 1982; Miledi and Sumikawa, 1982) show conclusively that *Xenopus* oocytes are capable of translating the mRNAs that encode the various subunits which make up nicotinic receptors in other cells; and show also that the products of translation are fully processed and incorporated into the oocyte membrane. Since only a few transcripts would need to be active to produce an amount of ACh-receptors detectable by electrophysiological methods (cf. Miledi and Sumikawa, 1982), it appears that normal oocytes do not contain the mRNA that codes for nicotinic ACh-receptors. Incidentally this shows that although the genome of the oocytes is derepressed, the messages are not simply transcribed randomly or, if they are, some trancripts are then selectively 'masked'.

The question arises as to whether the cat muscle ACh-receptors embedded in the *Xenopus* oocyte membrane are the same as those in the original muscle membrane. We have previously found that translation of mRNA extracted from the electric organ of *Torpedo* yielded a product with a subunit structure very similar to that of the native *Torpedo* ACh-receptor (Sumikawa *et al.*, 1981). We have now found that the oocytes injected with mRNA extracted from denervated cat muscle synthesized ACh-receptors whose subunit structure was similar to that of the native receptor in cat muscle (unpublished). Moreover, a comparison of the peptide maps of the putative α-subunit synthesized in oocytes injected with *Torpedo* mRNA, and the α-subunit of native *Torpedo* receptors, showed that the two are very similar proteins (Sumikawa *et al.*, 1982). In view of all this, it would seem likely that translation of cat muscle mRNA in *Xenopus* oocytes yields ACh-receptor proteins which are very similar, if not identical, to those produced when the mRNA is translated by the muscle.

Therefore, the cat ACh-receptors in the oocyte membrane might be expected to have the same properties as the receptors in the muscle. This is true, at least qualitatively, for some basic properties such as their reaction to various agonists (such as carbachol and suberyldicholine) and antagonists; the nature of the ions that flow through the ACh-induced channels, etc. However, there may be some quantitative differences. For instance, at room temperature, activation of the cat ACh-receptors in the oocytes injected with mRNA from denervated muscle, opens membrane channels whose lifetime is ~5 ms (at −80 mV); while the lifetime of the channels induced at innervated endplates in the cat tenuissimus muscle is only ~1 ms (Wray, 1980). This difference could merely be a reflection of the longer lifetime of the channels in denervated muscle (cf. Katz and Miledi, 1972); but the analysis carried out so far suggests that the ACh-receptor-channels induced in the oocyte after injection of mRNA from innervated muscle are also slower than the normal endplate channels. Although this difference could result from a slight difference in the proteins which make up the native receptors and those encoded by the cat mRNA in the oocyte, a more likely interpretation is that the different characteristics of the ACh-receptor-channel complexes reflect differences in the membranes in which they are embedded, or in the cytoskeleton of the oocyte.

ACh-receptors in the cat muscle membrane are known to desensitize slowly (Zaimis and Head, 1976; Wray, 1981) and a similar slow rate of desensitization was observed in the cat receptors incorporated into the oocyte membrane (cf. Figure 7). In contrast, nicotinic ACh-receptors incorporated into the membrane in oocytes injected with mRNA extracted from the electric organ of *Torpedo*, desensitize more rapidly (unpublished). The different rates of desensitization of *Torpedo* and cat ACh-receptors cannot be attributed to differences in their 'environment' since in our experiments both types of receptors are made to reside in the same membrane. It seems likely, therefore, that the different rates of desensitization are a consequence of differences in the respective ACh-receptor-channel complexes.

The *Xenopus* oocyte system (Gurdon, 1974) is an efficient translator of the mRNA which codes for nicotinic ACh-receptors. In ~2 days the number of cat ACh-receptors synthesized by an oocyte is more than the total number of ACh receptors in a mammalian muscle fibre (Miledi and Sumikawa, 1982). Our preliminary experiments appear to indicate that the number of ACh-receptors incorporated into the oocyte membrane reaches a more or less stable level a couple of days after injection of the mRNA. Yet, if the ACh-receptors are blocked by α-bungarotoxin, within a short time, new ones appear on the oocyte membrane (Miledi and Sumikawa, 1982). It is as if some process regulates the incorporation of receptors and prevents them from occupying the entire surface membrane.

Using α-bungarotoxin, we are able to measure the amount of nicotinic ACh-receptors in the oocyte and, using electrophysiological methods, we can also estimate the number of ACh-receptors which can open membrane channels. In the few oocytes where this was done, the number of ACh-receptors in the surface membrane was greater than the number of channels which were activated by large doses of ACh. Some of this difference may be a consequence of the presumed two-step reaction between ACh and receptors (cf. Katz and Miledi, 1972); but it is possible that there are ACh-receptors which bind toxin but cannot open membrane channels. More experiments are needed to ascertain whether 'silent' receptors do exist in the membrane.

Materials and methods

Preparation of mRNA and injection into oocytes

Poly(A)$^+$ mRNA was isolated from denervated muscles of adult cats after tissue homogenization, phenol extraction, and oligo(dT)-cellulose chromatography. *Xenopus* oocytes were injected with ~25 ng of poly(A)$^+$ mRNA in water or Hepes buffer (5 mM, pH 7.5). For further details see Miledi and Sumikawa (1982).

Electrophysiological recording and noise analysis

After injection, the oocytes were kept at room temperature in Barth's fluid with gentamicin (0.1 mg/ml) and nystatin (50 U/ml). At various times after injection, oocytes were placed in a bath of normal frog's Ringer (in mM; NaCl 115, KCl, $CaCl_2$ 1.8, Hepes 5 at pH 7.2), and examined using methods described previously (Kusano *et al.*, 1977, 1982; Miledi, 1982). Oocytes were voltage clamped with two microelectrodes with their tips broken to reduce clamp current noise. The voltage electrode was filled with 3 M KCl and the current electrode was usually filled with 2–4 M K-acetate. ACh was applied either to a small membrane area by ionophoresis, or to the entire oocyte membrane by bath application, using a perfusion system to exchange the bathing fluid.

The membrane current fluctuations produced during steady application of ACh (cf. Katz and Miledi, 1971, 1972), were low-pass filtered to 500 Hz and recorded on FM tape for subsequent analysis by computer. After appropriate filtering to avoid aliasing, blocks of 512 points were digitized usually at a sampling interval of 5 ms and, after editing out any blocks containing artefacts, power spectra were computed using a fast Fourier transform algorithm. In some cases the same data were sampled twice, at 5 and 25 ms, and the resulting spectra were combined so as to cover an extended frequency range of 0.007–100 Hz. An average spectra was formed for each run, and averaged control noise spectra obtained before each application of ACh were subtracted from the spectra obtained during ACh action.

Spectra were analyzed as previously described (Katz and Miledi, 1972; Anderson and Stevens, 1973). The mean channel life time (τ) was estimated by measuring the half-power frequency of the spectra (f_c), and calculating $\tau = 1/2\pi f_c$).

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