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Differential Development of Two Classes of Acetylcholine Receptors in *Xenopus* Muscle in Culture

Abstract. *Physiological properties of acetylcholine receptors on muscle cells at very early stages of ontogeny were compared with those of cells at later stages. Two changes were observed that contributed to an overall shortening of the mean open time of single-channels. First, there was a shift in the relative proportions of two receptor types with different conductances and mean open times, such that the contribution of receptors with large conductance and short open time increased as development proceeded. Second, there was a sharp reduction in the mean open time of channels having small conductance, with no similar change in channels having large conductance.*

During development the mean open time of nicotinic acetylcholine receptor (AChR) channels gradually shortens (*1–3*). In rat muscle this shortening is associated with innervation (*4*), but in cultured *Xenopus* muscle it can occur in the absence of innervation (*3*). Not much is known, however, about the mechanism underlying these changes. It has been suggested that replacement of receptors of one class (small conductance, long open time) by receptors of another class (large conductance, short open time) is responsible for the developmental changes in mean open time (*1, 3, 5*). There is indirect evidence suggesting that this conversion is due to a modification of the receptor molecules (*1*).

In this study we examined the single-channel properties of AChR's that had just emerged in the surface membrane during ontogeny. We found that the open times of these AChR's were initially long and then shortened as development proceeded in vitro. A new mechanism, in addition to the redistribution of receptor types, must be involved in the maturation of early AChR's. The mechanism decreases the mean open time of channels belonging to a single conductance class of AChR's.

We cultured dissociated muscle cells isolated from myotomes of *Xenopus* embryos. General procedures were similar to those previously described (*6*); however, we dissected the myotomes at earlier stages of development so that the cells stuck to the bottom of the dish and thus became amenable to physiological experiments before first insertion of AChR's into the surface membrane. Myotomes were dissected from *Xenopus* embryos (*7*) at stages 14 to 16, dissociated in calcium-free EGTA Steinberg solution, and plated in culture dishes containing 60 percent L-15 (Gibco) and 1 percent fetal bovine serum, with or without penicillin and streptomycin. Cultures were maintained at 18° to 24°C. Single-channel currents activated by 500 n*M* ACh were recorded by using the conventional patch clamp method (*8*) in the cell-attached mode. Experiments were performed in standard frog Ringer solution containing tetrodotoxin (see legend to Fig. 1) at 11° to 14°C.

We shall express the developmental stage of cultured cells in terms of the corresponding stage of intact embryos maintained at the same temperature as the cultured dishes (*9*). We do not claim that cultured cells develop in the same way as in intact embryos, but we opt for this procedure because of its convenience (*10*).

Acetylcholine receptors first emerge on muscle surface membrane at stage 20 in *Xenopus* embryos (*11*). Recently, Bridgman *et al.* (*9*) showed that, even in culture, the cells start to acquire ACh sensitivity at stage 20. At this early stage overall ACh sensitivity is low, probably reflecting a low density of AChR's, but gradually increases, reaching almost the plateau level at stage 31 (16 hours later at 23°C).

We recorded the single-channel currents of AChR's as early as possible [stages 21 to 24, 1 to 5 hours after stage

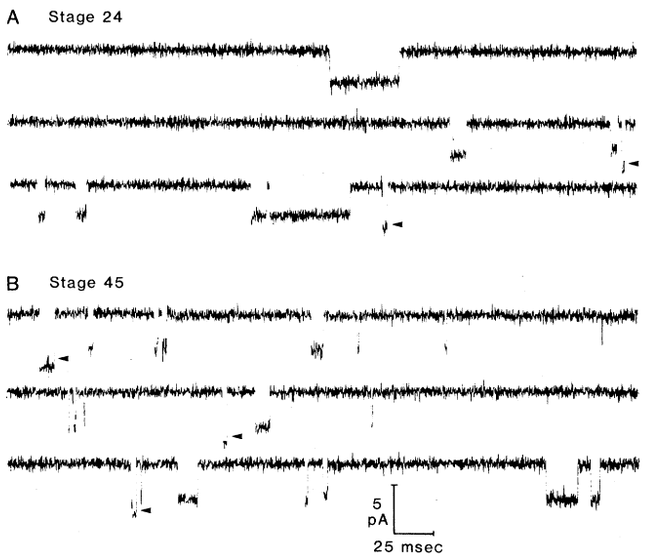


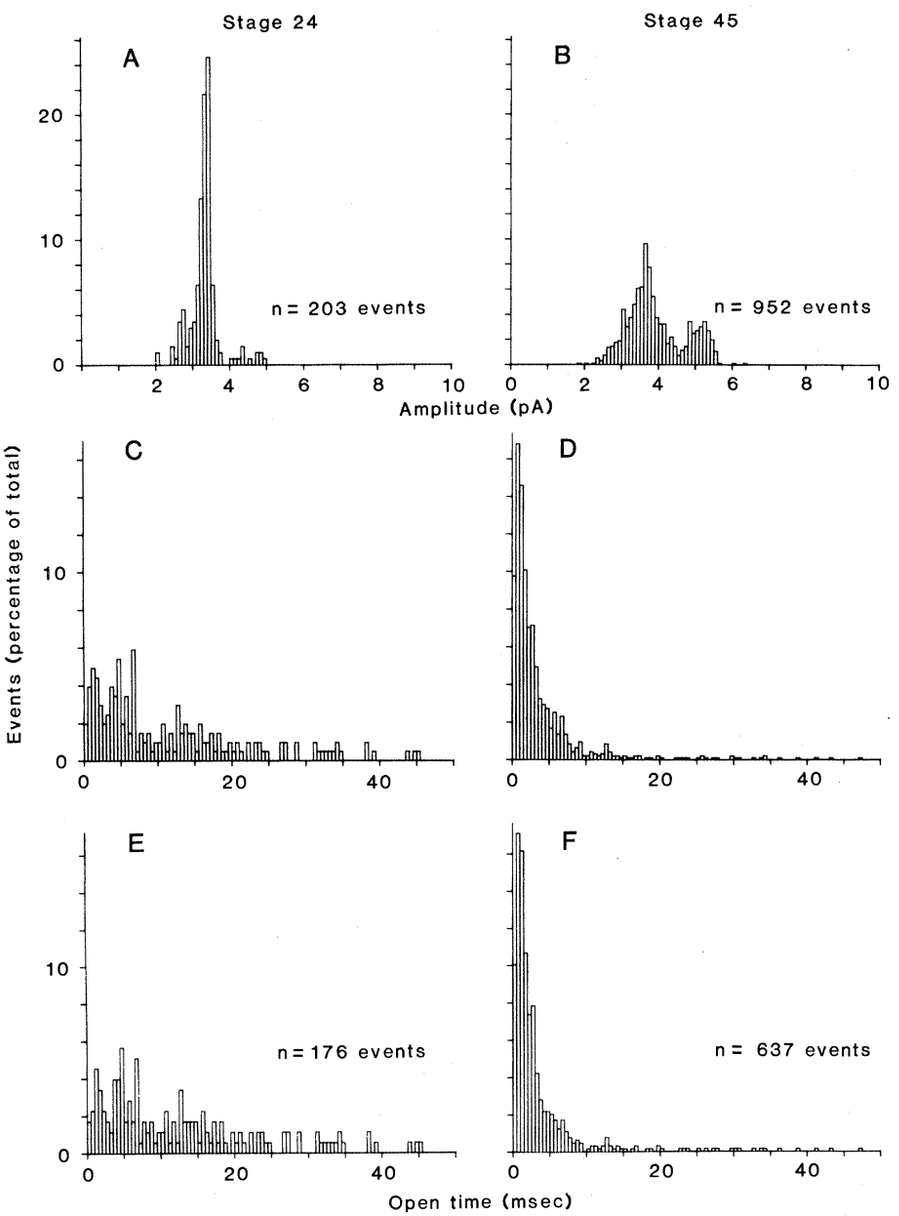
Fig. 1. Acetylcholine–induced single-channel currents recorded from two cells at stage 24 (A) and stage 45 (B). Channel openings produced inward currents, represented as downward deflections. Successive records are not continuous in time. Records at stage 24 were chosen to illustrate the existence of the two populations of conductance events. The frequency of large conductance openings (arrows) was very low at this early stage. Both cells were hyperpolarized from their resting potential by 50 mV. The bathing solution was composed of 115 m*M* NaCl, 2.5 m*M* KCl, 1.8 m*M* $CaCl_2$, 4 m*M* Hepes (*p*H 7.2), and tetrodotoxin (5×10^{-7} g/ml) (~13°C). The recording pipette contained 500 n*M* ACh dissolved in the bathing solution. Data were filtered to 2 kHz and digitized at 100-μsec intervals.

20, at 23°C (*7*)] and compared them with records for more mature muscle cells [stage 45, 3 days after stage 20, at 23°C (*7*)]. The records in Fig. 1B, taken from a "mature" cell at stage 45, reflect the two classes of channels: one with small conductance and long open time, the other with large conductance and shorter open time (*12*). The records in Fig. 1A are from a cell at stage 24. At this early stage the open times of channels are very long, and there are already two classes of receptors.

At stage 24 the amplitudes of single-channel events were almost exclusively between 2.7 and 3.7 pA (small conductance events), with only a small peak visible over 4 to 5 pA (large conductance events) (Fig. 2A). At this early stage the probability of occurrence of the large conductance events was very low, only 6 percent of the total number of events. Nevertheless, it appears that the small peak represents the large conductance class of receptors. Their amplitude and mean open time matched those of the well-defined large conductance channels measured later in development, and increased hyperpolarization led to clearer separation of the two peaks.

Recordings made at stages 21 to 25 usually revealed only small conductance events; only 4 of 12 cells displayed a few large conductance events, such as those shown in Fig. 2A. The mean probability of large conductance events for these four cells was 5 percent. As development proceeded, however, the probability of large conductance events increased. As shown in Fig. 2B, at stage 45 large conductance events constituted a substantial proportion of the total events (25 percent in this cell; an average of 53 percent for nine cells). These results agree with previous findings that showed a relative increase in the probability of large conductance events during development of noninnervated cells (*5*).

Histograms of the channel open times for all events are shown in Fig. 2, C and D. At stage 24 (Fig. 2C), open times were very long (mean, 14.1 msec), but at stage 45 (Fig. 2D) they were markedly shorter (4.8 msec). This result agrees with the noise analysis data of Kullberg *et al.* (*2*) on in situ myotome cells of *Xenopus* embryos. One might attribute this short-



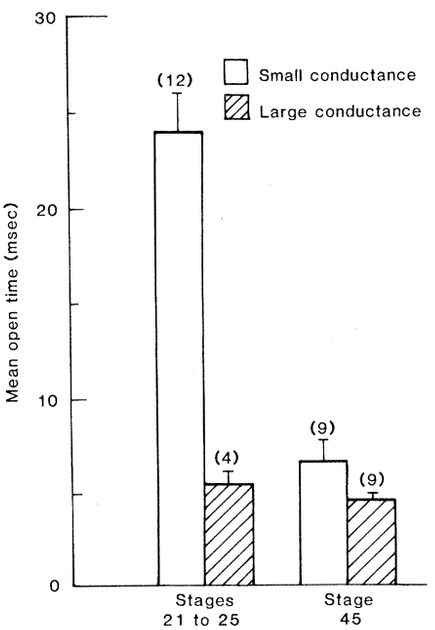


Fig. 2 (left). Analysis of the complete data on the two cells represented in Fig. 1. (A and B) Distribution of amplitudes of single-channel currents. Only events lasting 400 μsec or longer were included in the analysis, since openings of less than 400 μsec do not reach a plateau and measurements of their amplitudes are thus unreliable. Note that in stage 24 cells (A) almost all the events fall into a single amplitude class, whereas in stage 45 cells (B) amplitude distribution is bimodal. (C and D) Distribution of open times of all the events. (E and F) Distribution of open times of small conductance events. Only events that fell within amplitudes of 2.7 to 3.7 pA (E) and 3.0 to 4.5 pA (F) were regarded as small conductance events. Fig. 3 (above right). Effect of development on the open times of both classes of AChR's. Values are means ± standard errors for 21 cells. Mean open time is shown for each conductance class at stages 21 to 25 and stage 45. The numbers in parentheses indicate sample size. All cells were hyperpolarized from their resting potential by 50 mV. Temperature, 11°C to 14°C; small conductance, 25.5 ± 1.4 pS; and large conductance, 43.3 ± 2.4 pS. Conductances were determined from the differences in mean currents at different voltages.

ening of overall open times to the increase in the probability of large conductance events, which usually have short open times. However, there is another important factor contributing to the observed decrease in the mean open times. As shown in Fig. 2, E and F, the open times of the small conductance events alone are much longer at stage 24 (mean, 15.5 msec) than at stage 45 (mean, 5.3 msec).

The pooled results of several experiments (Fig. 3) indicate an approximately threefold reduction in the mean open time of the small conductance channels between stages 21 to 25 and stage 45. In contrast, the mean open time of the large conductance class does not appear to change during development. Other physiological characteristics, namely, single-channel conductance (determined from the differences of mean currents at different voltages, usually over the voltage range between resting potential and a 50-mV hyperpolarization), reversal potential, and voltage dependence of channel open time for both classes, do not appear to be influenced by development.

The observed shortening of open time is probably not the result of changes in resting membrane potential during development because (i) the reversal potential measured from the resting level did not change during development, (ii) the change in open time was in the opposite direction from what would be predicted by an increase in membrane potential, and (iii) the open time of only the small conductance events was affected.

In previous studies of AChR development in cultured *Xenopus* muscle a redistribution of receptor populations was reported (*3, 5*), but not a direct influence of development on the channel open times of either conductance class. One possible explanation for this is that most of the change in open time may have occurred before the time of the earliest recordings made by other investigators. Our evidence suggests that, after 1 day in culture, the mean open time of the small conducting channels (11.4 ± 1.9 msec at −50 mV; $n = 3$ cells) is already 50 percent shorter than that at stages 21 to 25.

Our results demonstrate a remarkable decrease in the open time of AChR channels during the earliest stage of development in the absence of innervation. This is caused partly by the redistribution of the receptor population from one class to the other (a mechanism observed previously), and partly as the result of a shortening of the open time of one class of receptor (a new mechanism). We do not know whether these changes are due to modification of existing receptor molecules or to replacement by new receptor molecules during development. Nor do we know whether there is more than one class of small conductance events. The observation that only one of the two classes is affected reduces the possibility of a nonspecific effect of membrane maturation.

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Antigens Encoded by the 3′-Terminal Region of Human T-Cell Leukemia Virus: Evidence for a Functional Gene

Abstract. *Antibodies in sera from patients with adult T-cell leukemia-lymphoma or from healthy carriers of type I human T-cell leukemia virus (HTLV) recognize an antigen of approximately 42 kilodaltons (p42) in cell lines infected with HTLV-I. Radiolabel sequence analysis of cyanogen bromide fragments of p42 led to the conclusion that this antigen is encoded in part by LOR, a conserved portion of the "X" region that is flanked by the envelope gene and the 3′ long terminal repeat of HTLV-I. It is possible that this novel product mediates the unique transformation properties of the HTLV family.*

The human T-cell leukemia viruses (HTLV) are a family of exogenous human retroviruses with three known types (*1, 2*). HTLV type I (HTLV-I) is etiologically associated with adult T-cell leukemia-lymphoma (ATLL) (*2, 3*). HTLV type II (HTLV-II) was isolated from a patient with a T-cell variant of hairy cell leukemia (*4*). HTLV type III (HTLV-III) refers to prototype virus isolated from patients with acquired immune deficiency syndrome (*5*).

HTLV-I and HTLV-II have several unusual features that distinguish them from the replication-competent retroviruses of mice and chickens. These include lack of chronic viremia in infected individuals, absence of common proviral integration sites in tumors (*6*), *trans*-activation of HTLV long terminal repeat (LTR)–directed transcription in infected cells (*7*), and ability to immortalize T cells in vitro (*8*). In addition to the *gag*, *pol*, and *env* genes of animal retroviruses, the HTLV genome contains a 1.5-kilobase region, initially described as the "X" region, and located between the *env* gene and the 3′ LTR (*9*). Sequence comparisons of this region between HTLV-I and HTLV-II demonstrate that it can be divided into a 5′ nonconserved region and a 3′ highly conserved region designated LOR (*10*).

In a previous study, serum samples from adult T-cell leukemia-lymphoma patients and from healthy carriers living in the HTLV-I endemic area of Japan were examined for the presence of antibodies to HTLV-associated membrane antigen (HTLV-MA). We reported that HTLV-specific antigens detected in an HTLV-I–infected tumor cell line, Hut 102, could be grouped into three categories (*11*). These included unglycosylated antigens encoded by the *gag* gene, glycosylated antigens encoded by the *env* gene, and an unglycosylated 42-kilodalton species (p42) whose coding origin