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COMPARATIVE DEVELOPMENT OF END-PLATE CURRENTS IN TWO MUSCLES OF *XENOPUS LAEVIS*

By R. KULLBERG and J. L. OWENS*
From the Department of Biological Sciences, University of Alaska-Anchorage, Anchorage, Alaska 99508

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SUMMARY

1. The development of miniature end-plate currents (m.e.p.c.s) was studied in the superior oblique and interhyoideus muscles of *Xenopus laevis*. An analysis of m.e.p.c. decays shows that each muscle possesses its own characteristic programme of end-plate current development.

2. In the superior oblique, the exponential decay constants of m.e.p.c.s were initially about 3 ms; they declined within half a day to 1 ms and remained at that value for six weeks. They then gradually became longer, reaching a mean value of 1·7 ms at late metamorphosis.

3. In the interhyoideus, m.e.p.c. decay constants were initially about 6 ms. They declined in less than one day to a mean value of 2·6 ms and remained there for the following seven weeks. Upon completion of metamorphosis, the decay constants underwent a further decrease to about 1 ms.

4. In both muscles, the changes in m.e.p.c. decays were correlated with developmental changes in muscle contraction speeds, as measured by maximum twitch frequencies.

5. The above changes in end-plate currents in the superior oblique and interhyoideus muscles are discussed in terms of the development of acetylcholine receptor channel gating and acetylcholinesterase activity.

INTRODUCTION

In adult amphibian skeletal muscle, the gating kinetics of acetylcholine receptors (AChRs) differ according to muscle fibre type (Miledi & Uchitel, 1981; Fedorov, Magazanik, Snetkov & Zefirov, 1982). AChR channel open times are 3-fold longer in slow-muscle fibres than in fast-twitch fibres, and this difference gives rise to end-plate currents of comparatively long duration in slow muscle. Intermediate channel open times and end-plate currents of corresponding duration are present in some fibres, such as the multiply innervated twitch fibres of submaxillaris (Miledi & Uchitel, 1981) and the singly innervated fibres in tonic bundles of ileofibularis and cruralis (Fedorov *et al.* 1982). Levels of acetylcholinesterase (AChE) activity are also reported to differ

* To whom reprint requests should be sent

according to fibre type (Lannergren & Smith, 1966), with comparatively low levels being present in slow muscle.

The existence of such differences at adult end-plates implies that variations in the development of function should also be found at immature end-plates of different muscles. Much information has been gained about the development of end-plate function in amphibian myotomal muscle (reviewed by Cohen, 1980; Dennis, 1981), but little is known about other developing amphibian muscles. An examination of other muscles should reveal at least as much variability in synaptogenesis as there is in the function of adult end-plates. In amphibia, it is of particular interest to examine synapse development in muscles which are present in both larval and adult animals. The functions of such muscles may change during metamorphosis and there may be corresponding changes in end-plate properties.

We have done a comparative study of the development of end-plate currents in two muscles of *Xenopus laevis* which persist through metamorphosis: the superior oblique and interhyoideus. The former is an extraocular muscle which is responsible for rapid, saccadic movements of the tadpole eye. The interhyoideus is a broad, flat muscle of the hyoid arch, which elevates the floor of the mouth (Sedra & Milad, 1957; Gradwell, 1968). Its rhythmic activity in the tadpole contributes to irrigation of the buccal cavity. Both muscles become active at about the same time in *Xenopus* tadpoles, allowing a convenient comparison of their developmental schedules. As a means of studying the development of AChR gating kinetics and AChE activity, we have described the time courses of end-plate currents and their response to anticholinesterase treatment. We have found that each muscle has a distinctive programme of end-plate current development which contrasts with that of the other muscle.

METHODS

Animals

Experiments were done on *Xenopus laevis* ranging in age from 3 days to more than 2 years. Younger tadpoles were produced by gonadotropin-induced mating of adult animals. Older tadpoles (stages 58–65) and adults were obtained commercially (Nasco, U.S.A.). Tadpoles were reared in dechlorinated tap water and fed a commercial dried food (Nasco frog brittle or Carolina Biological Supply Co. tadpole food) 3–5 times weekly. Mating pairs of adult animals were kept in 5-gallon aquaria containing dechlorinated tap water and were fed frog brittle twice weekly. The staging of tadpoles was done according to the criteria of Nieuwkoop & Faber (1967).

Muscles

The superior oblique is an extraocular muscle which is homologous to that found in other vertebrates. It originates rostrally and medially to the eye and inserts on the dorsal sclera. The position of the muscle remains constant throughout development. It is innervated solely by the trochlear nerve. The paired interhyoideus muscles originate at a mid-line tendinous junction and insert laterally on the ceratohylia (Sedra & Milad, 1957). They are the dominant muscles of the floor of the mouth in the premetamorphic tadpole. During metamorphosis, they move caudally and acquire new insertions on the palatoquadrate cartilages. They become small in relation to the paired intermandibularis muscles, which in turn become the dominant muscles of the floor of the mouth. The shape and position of these muscles in the tadpole and frog are illustrated in Fig. 1. The interhyoideus muscles are innervated by branches of the hyomandibularis nerves (Nieuwkoop & Faber, 1967). Both the interhyoideus and the superior oblique muscles are easily accessible by removing the overlying skin and connective tissue.

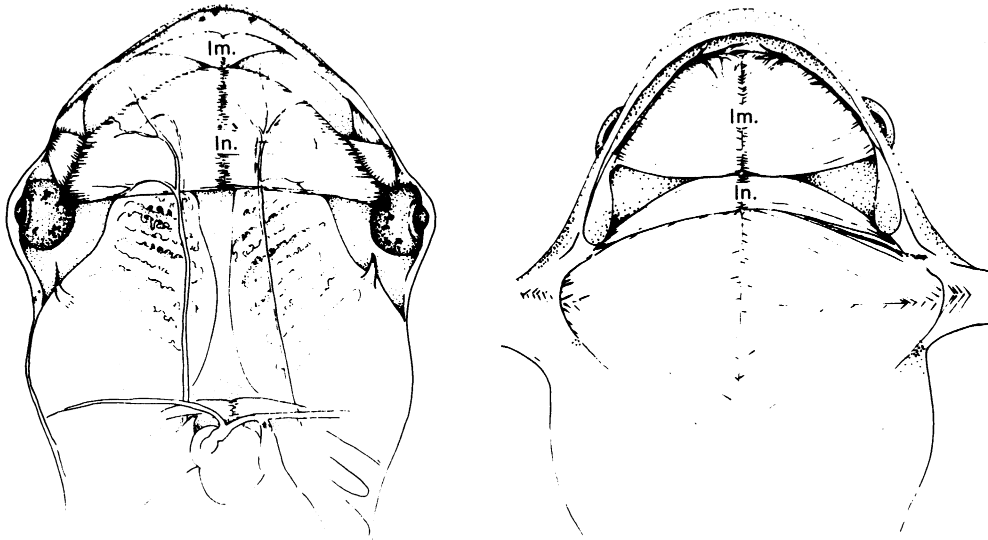

Fig. 1. Ventral view of stage 47 tadpole (left) and newly metamorphosed frog (right) illustrating the interhyoideus (ih.) and intermandibularis (im.) muscles.

Recording techniques

Muscles were bathed in Ringer solution containing 110 mM-NaCl, 3 mM-KCl, 1·8 mM-$CaCl_2$, and 8 mM-HEPES buffer, pH 7·4, plus 1 μg TTX/ml. Experiments were done at room temperature (22–24 °C). M.e.p.c.s were recorded with extracellular, fire-polished electrodes having inner tip diameters of 3–35 μm. Larger-tipped electrodes were found to give better recordings from the interhyoideus because they tended to rest on the muscle surface, rather than breaking through the tissue. Recording electrodes were filled with Ringer solution. Although the term 'miniature end-plate current' (m.e.p.c.) is used to describe the synaptic events, the recorded signal was in fact an extracellular voltage which was proportional to the synaptic current (del Castillo & Katz, 1956; Katz & Miledi, 1973). The extracellular m.e.p.c.s were amplified by a low noise differential amplifier (WPI DAM-5), bandpass filtered at 0·2–5 kHz and recorded on FM tape (Racal Store 4DS).

Data analysis

Analysis of m.e.p.c.s was done off-line by computer (DEC PDP 11/34) on records which were digitized at sample intervals of 0·05–0·30 ms. Up to eighty-five m.e.p.c.s were analysed from each recording site, with typical sample sizes being twenty to thirty. The rise time and decay constant(s) of each m.e.p.c. were measured. The rise time was defined as the time interval between the onset of the synaptic current and the peak amplitude. The time of onset was defined as the first point to fall within twice the standard deviation of the base-line noise, when the m.e.p.c. was searched backward from the peak. The decay phases of individual m.e.p.c.s were fitted by single or double exponential curves, as described previously (Kullberg, Owens & Vickers, 1985). The mean rise time and decay constant(s) were calculated for the sample of m.e.p.c.s from each recording site. Those values were in turn used to calculate the grand mean rise time and decay constant(s) at each developmental stage.

Muscle stimulation

Muscle twitches were evoked by direct electrical stimulation of the muscles near their origins or insertions and by stimulation of the trochlear or hyomandibularis nerves. Stimulating currents were passed between a Ringer solution-filled pipette (30 μm i.d.), positioned next to the muscle or nerve, and a ground electrode in the bath. Square-wave stimulus pulses of 1 ms duration were used.

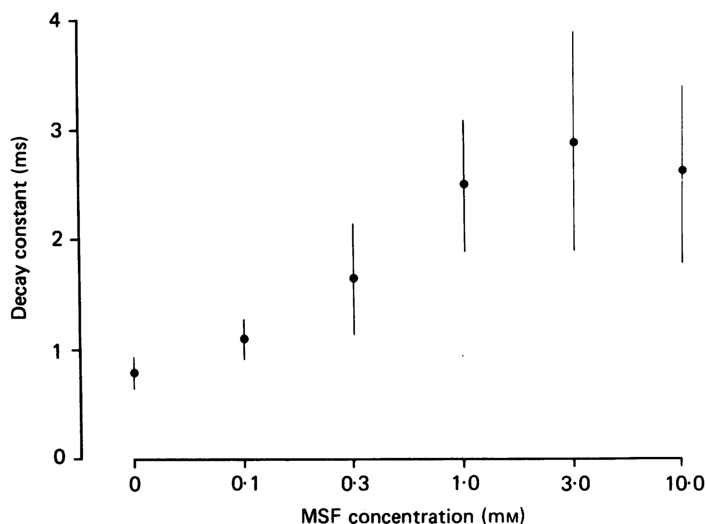

Fig. 2. Dose–response data showing the effect of methanesulphonyl fluoride (MSF) at different concentrations on m.e.p.c. decay constants. Each point represents the mean decay constant (±S.D.) of several recording sites. The numbers of recording sites at different concentrations, from left to right, were 38, 12, 9, 15, 24, 15.

Anticholinesterase experiments

AChE activity was blocked by an irreversible anticholinesterase, methanesulphonyl fluoride (MSF) (Kordas, Brzin & Majcen, 1975). AChE is more resistant to block by anticholinesterases in *Xenopus* than it is on other species (Kullberg, Mickelberg & Cohen, 1980). In order to determine the dose of MSF required to produce maximal inhibition of AChE, we measured its effect on the decay constants of m.e.p.c.s recorded at mature myotomal synapses (stages 47–52), where AChE is abundant (Kullberg *et al.* 1980). The muscle was soaked in different concentrations of MSF for 45 min, followed by 30 min wash-out. The dose–response data are plotted in Fig. 2. Application of 3 mM-MSF produced a maximal prolongation of m.e.p.c.s and also completely eliminated staining of AChE by the method of Karnovsky & Roots (1964). Similar application of MSF to interhyoideus and superior oblique muscles eliminated any AChE detectable by Karnovsky–Roots stain. In all MSF experiments reported here, the drug was used at 3 mM concentration with the above exposure and wash-out times.

RESULTS

Onset of synaptic activity

The earliest stage at which we were able to detect spontaneous synaptic activity or movement in either the superior oblique or interhyoideus was stage 41 or approximately 3 days after fertilization of the egg. Both muscles first became anatomically distinct about stage 39, which was 20 h before the earliest detected synaptic activity. Examples of m.e.p.c.s recorded at stage 41 and at later stages in each muscle are shown in Fig. 3.

M.e.p.c. development in superior oblique

The earliest m.e.p.c.s recorded in superior oblique had predominantly single-exponential decay phases with a mean time constant of 3·3 ms (Fig. 4, Table 1). There

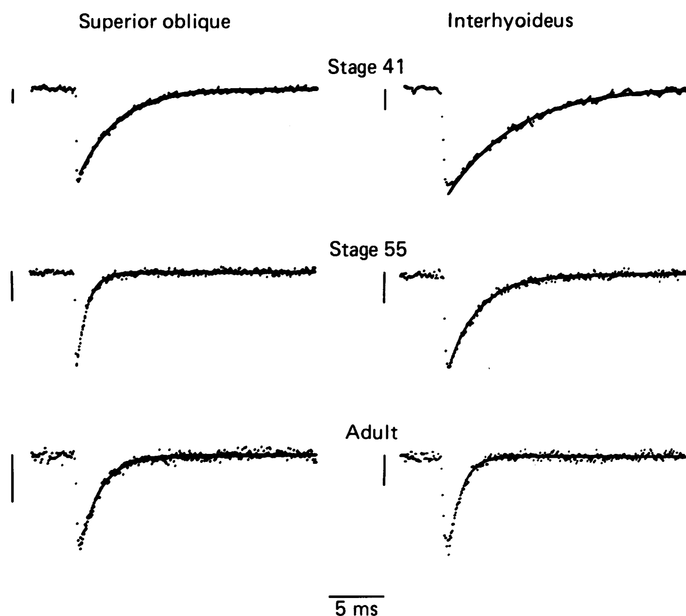

Fig. 3. Examples of extracellular m.e.p.c.s recorded at different developmental stages. The best-fitting single-exponential functions overlap each trace. The decay constants of m.e.p.c.s recorded from the superior oblique were 3·4 ms (stage 41), 1·0 ms (stage 55), and 1·8 ms (adult). The decay constants of interhyoideus m.e.p.c.s were 6·2 ms (stage 41), 2·7 ms (stage 55) and 1·1 ms (adult). Vertical bars indicate 0·1 mV.

was a broad distribution of time constants, ranging from 1 to 9 ms (Fig. 5). A small number of m.e.p.c. decays clearly deviated from a single-exponential fit (Fig. 6). These could be satisfactorily fitted by the sum of two exponential curves having average time constants of 1·2 and 4·2 ms (Table 1). Such decays, which may have resulted from two classes of AChRs with different gating times, were apparent in about 5% of the m.e.p.c.s analysed at stage 41.

We found no evidence of AChE activity in stage 41 superior oblique. Karnovsky–Roots stain did not reveal any localized accumulations of AChE, and application of MSF, under conditions which should entirely block AChE (see Methods), did not lengthen the time course of m.e.p.c.s (Table 1).

We did not directly estimate the AChR channel gating times at the newly formed end-plates in superior oblique. However, the m.e.p.c.s recorded at this stage had decay constants which were comparable to those observed in mature myotomal muscle, after block of AChE (Fig. 2), and there the majority of AChR channels have gating times of less than 1 ms (Kullberg & Kasprzak, 1985). This suggests that channels present at stage 41 in the superior oblique were predominantly fast.

M.e.p.c. durations became briefer during the first several hours of synapse development. Within 11 h, the decay constants of single-exponential m.e.p.c.s decreased to an average value of 1·0 ms and remained there during the following 6 weeks of development (Fig. 4, Table 1). The distribution of decay constants throughout this intermediate period of development had less variability than at earlier or later stages (Fig. 5).

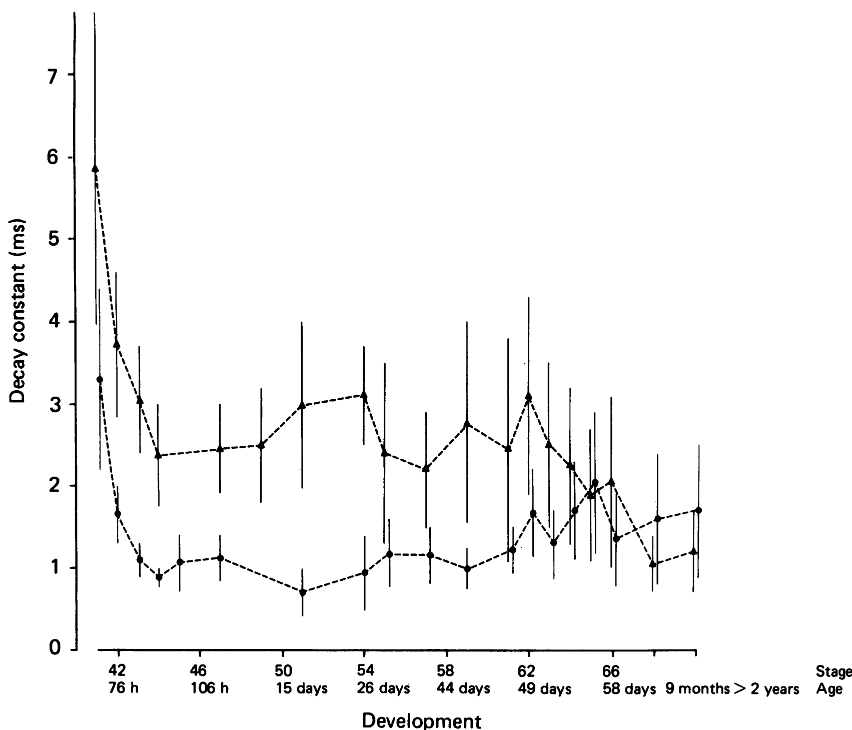

Fig. 4. Mean decay constants of single-exponential m.e.p.c.s recorded at different developmental stages. Bars indicate standard deviations. Each symbol represents the mean decay constant obtained from all recording sites at a single stage of development. The average number of recording sites at each stage was thirteen (range: 2–38). ●, superior oblique; ▲, interhyoideus.

The early decline in m.e.p.c. duration was probably due in part to the development of AChE. Whereas we detected no AChE in stage 41 muscle, localized accumulations of the enzyme were obvious in Karnovsky–Roots stained muscle at stage 44. Also, anticholinesterase treatment became effective in prolonging the duration of synaptic currents. M.e.p.c. decays at stages 43–59 were approximately doubled by blocking AChE activity with MSF (Table 1). However, even after MSF treatment, the decays at intermediate stages were significantly faster than those recorded at stage 41, which implies that the development of AChE is not the only explanation for the change in decay constant during the early period of synapse development.

Part of the early decline in m.e.p.c. duration could be due to a change in the junctional AChR channels, such as a decreased gating time or an increased relative number of fast channels. Evidence regarding these possibilities was obtained from m.e.p.c.s with double exponential decays (Fig. 6). Such decays, which may have been due to two kinetic classes of receptors, were observed in about 15 % of the m.e.p.c.s recorded during the 6-week period following stage 43. The mean fast and slow time constants were 0·6 and 2·8 ms (Table 1). Because AChE was abundant during this time, we take the decay constants as upper-limit estimates of the channel open times of two kinetic classes of receptors. Following block of AChE, the fast and slow decay

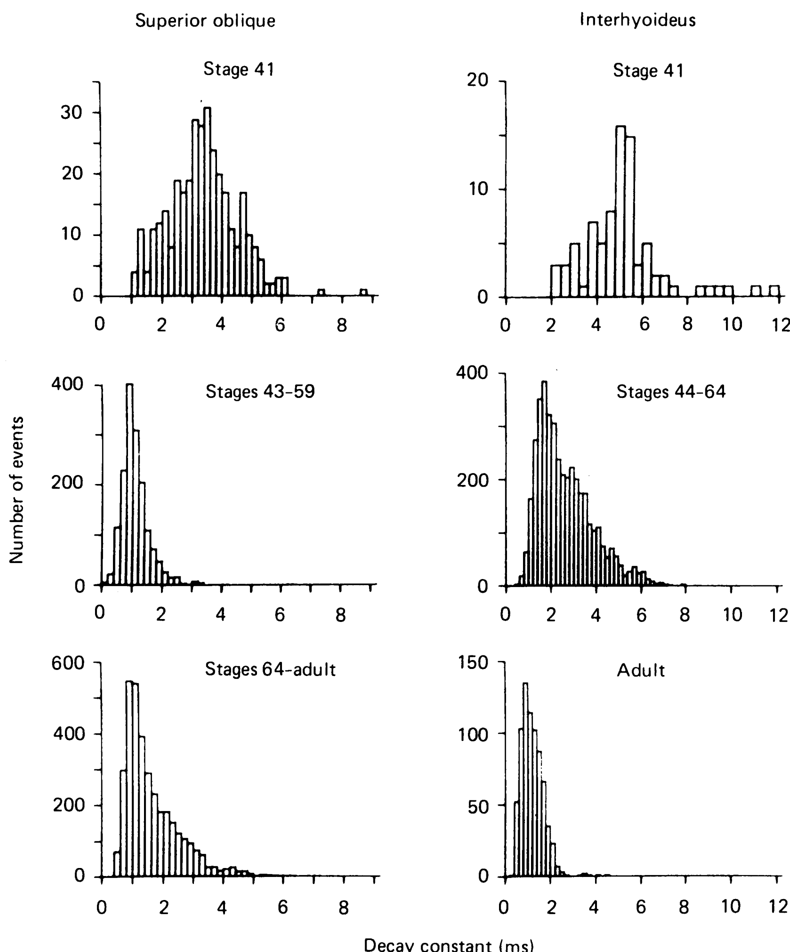

Fig. 5. Histograms of m.e.p.c. decay constants at different developmental stages. Each histogram is composed of all the m.e.p.c. decay constants measured at the indicated stages. A value of F, defined as the ratio of the variance of decay constants *between* sites to the variance *within* sites was calculated for each histogram. The F ratio, the m.e.p.c. sample size (n), and the number of recording sites (k) for each histogram are as follows. Superior oblique: stage 41, $F = 8{\cdot}55$, $n = 340$, $k = 18$; stages 43–59, $F = 16{\cdot}0$, $n = 1591$, $k = 82$; stages 64–adult, $F = 64{\cdot}2$, $n = 3515$, $k = 119$. Interhyoideus: stage 41, $F = 8{\cdot}6$, $n = 83$, $k = 6$; stages 44–64, $F = 55{\cdot}0$, $n = 4127$, $k = 151$; adult, $F = 33{\cdot}4$, $n = 735$, $k = 32$. For all histograms, the F ratios are significant beyond the 1 % level, indicating that differences between recording sites contributed to the distribution of decay constants.

constants were comparable to those recorded at stage 41 (Table 1). This result suggests that the gating times of the two classes of channels were unchanged during the early phase of development and indirectly supports the alternative possibility that the fast channels became more abundant.

Following the 6-week plateau (stages 43–59), there began a gradual increase in the decay constants of single exponential m.e.p.c.s (Fig. 4) which continued through late metamorphosis (stage 64). The values recorded in 2-year-old adult superior oblique were not significantly different from those recorded from tadpoles in late

TABLE 1. M.e.p.c. time course in superior oblique

	Stage 41	Stages 43–59	Stages 64–adult
Control			
τ (single)	$3{\cdot}3 \pm 1{\cdot}0$, $n = 18$	$1{\cdot}0 \pm 0{\cdot}3$, $n = 81$	$1{\cdot}7 \pm 0{\cdot}7$, $n = 118$
τ (fast)	$1{\cdot}2 \pm 0{\cdot}4$, $n = 5$	$0{\cdot}6 \pm 0{\cdot}2$, $n = 27$	$0{\cdot}7 \pm 0{\cdot}2$, $n = 39$
τ (slow)	$4{\cdot}2 \pm 1{\cdot}3$, $n = 5$	$2{\cdot}8 \pm 0{\cdot}8$, $n = 27$	$3{\cdot}8 \pm 1{\cdot}2$, $n = 39$
Rise time	$1{\cdot}0 \pm 0{\cdot}4$, $n = 18$	$0{\cdot}6 \pm 0{\cdot}1$, $n = 81$	$0{\cdot}7 \pm 0{\cdot}1$, $n = 118$
MSF treated			
τ (single)	$3{\cdot}3 \pm 1{\cdot}0$, $n = 10$	$2{\cdot}2 \pm 1{\cdot}0$, $n = 78$	$4{\cdot}8 \pm 2{\cdot}3$, $n = 63$
τ (fast)	—	$1{\cdot}0 \pm 0{\cdot}4$, $n = 17$	$1{\cdot}0 \pm 0{\cdot}4$, $n = 3$
τ (slow)	—	$3{\cdot}9 \pm 1{\cdot}0$, $n = 17$	$7{\cdot}1 \pm 0{\cdot}4$, $n = 3$
Rise time	$1{\cdot}1 \pm 0{\cdot}3$, $n = 10$	$0{\cdot}8 \pm 0{\cdot}2$, $n = 78$	$1{\cdot}2 \pm 0{\cdot}3$, $n = 63$

All values are given as mean ± S.D. (in ms). Sample sizes (n) refer to number of recording sites. τ (single) refers to decay constants of single-exponential m.e.p.c.s. τ (fast) and τ (slow) refer to decay constants of double-exponential m.e.p.c.s.

metamorphosis. The mean decay constant at stages 64–adult was 1·7 ms (Table 1) and the distribution of values was skewed (Fig. 5). This gradual increase in decay constant was probably not due to a decline in the activity of junctional AChE, since application of MSF at stages 64–adult lengthened the decay constants of m.e.p.c.s to 4·8 ms (Table 1). If the increase in m.e.p.c. duration following stage 59 had been due to a decline in esterase, then MSF treatment at late stages should have had little or no effect on the decay constants of m.e.p.c.s. At most, they should have been lengthened to slightly more than 2 ms which would have been comparable to the values recorded in stage 43–59 muscle after block of AChE (Table 1).

Double exponential decays were apparent in about 12 % of the m.e.p.c.s recorded at stage 64–adulthood (Fig. 6). The analysis of these m.e.p.c.s suggests that the lengthening of m.e.p.c. duration may have been due in part to a longer gating time of the slow class of AChR channels. The slow decay constants increased by about 40 % from stage 59 through metamorphosis, while there was no significant change in the fast component (Table 1). A similar, selective change in the gating time of slow channels has also been seen in developing *Xenopus* myotomal muscle *in vitro*, although there the slow channels became faster with age (Leonard, Nakajima, Nakajima & Takahashi, 1984).

At any given stage in the development of the superior oblique, the distribution of decay constants (Fig. 5) reflected a substantial variation *between* recording sites in addition to the variability *within* single sites. The decay constants were narrowly distributed at individual recording sites relative to the variation from site to site, and an analysis of variance indicates that the differences between recording sites were significant (Fig. 5, legend). We did not detect any clustering of values, which would have implied the existence of a few discrete types of synapses; instead, there seemed to be a continuum of values ranging from slow to fast at different recording sites.

M.e.p.c. development in interhyoideus muscle

During larval development, m.e.p.c.s recorded in interhyoideus muscle were typically longer than those recorded in superior oblique (Fig. 4). The earliest m.e.p.c.s recorded in interhyoideus muscle at stage 41 had a mean decay constant of 5·9 ms,

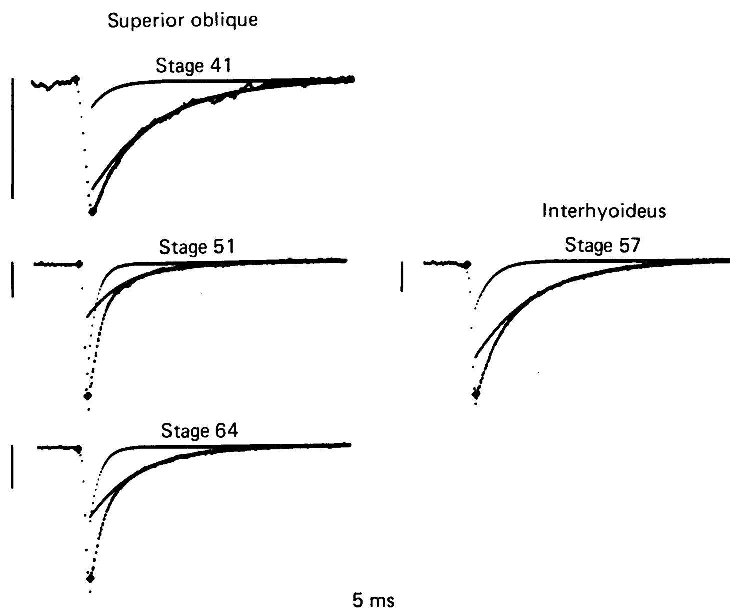

Fig. 6. Double-exponential m.e.p.c.s recorded at end-plates of superior oblique and interhyoideus muscles at different developmental stages. The fast and slow exponential components are shown as well as their sums, which in all cases closely overlap the data points. In the sample m.e.p.c.s from superior oblique end-plates, the fast and slow decay constants were 1·4 and 4·0 ms (stage 41), 0·7 and 2·9 ms (stage 51) and 0·7 and 3·6 ms (stage 64). The fast and slow decay constants in the sample m.e.p.c. from stage 57 interhyoideus were 1·2 and 4·5 ms. Vertical bars indicate 0·1 mV.

TABLE 2. M.e.p.c. time course in interhyoideus

	Stage 41	Stages 44–64	Adult
Control			
τ (single)	5·9 ± 2·0, n = 6	2·6 ± 1·0, n = 151	1·1 ± 0·4, n = 32
τ (fast)	—	1·0 ± 0·4, n = 56	—
τ (slow)	—	4·0 ± 1·3, n = 56	—
Rise time	2·0 ± 1·0, n = 6	0·9 ± 0·2, n = 56	0·7 ± 0·2, n = 32
MSF treated			
τ (single)	9·5 ± 3·1, n = 16	6·2 ± 2·8, n = 113	3·9 ± 2·3, n = 43
τ (fast)	—	1·8 ± 0·8, n = 5	—
τ (slow)	—	7·5 ± 1·5, n = 5	—
Rise time	3·4 ± 0·8, n = 16	1·7 ± 0·7, n = 113	1·3 ± 0·5, n = 43

See legend to Table 1.

almost twice that of the superior oblique (Tables 1 and 2). We observed no double-exponential decays, which suggests that most or all of the channels had similar gating times. Unlike the superior oblique, there appeared to be active AChE at the newly formed interhyoideus end-plates. Application of MSF lengthened the m.e.p.c. durations by about 50 % (Table 2). Despite that fact, we did not detect any focal accumulations of AChE after Karnovsky–Roots staining, although a diffuse background stain was evident. A similar observation has been reported at the newly

formed end-plates of rat diaphragm (Ziskind-Conhaim, Inestrosa & Hall, 1984). Because the end-plate currents were comparatively slow in the interhyoideus, in spite of the junctional AChE activity, it seems likely that the AChR channels initially present in this muscle had longer gating times than those in the superior oblique at a similar stage.

Although m.e.p.c.s in interhyoideus were longer than those in superior oblique, the early schedule of change in the two muscles occurred in parallel (Fig. 4). Within about 16 h after the first recorded spontaneous synaptic activity, m.e.p.c. durations had stabilized at a briefer time course and remained more or less unchanged during the following 7 weeks (stages 44–64). During this intermediate period of development the mean decay constant was 2·6 ms (Table 2).

Part of the early decrease in m.e.p.c. duration may have been due to a further increase in AChE activity. By stage 44, localized accumulations of esterase were revealed by Karnovsky–Roots stain, and MSF treatment had a greater effect on the time course of m.e.p.c.s (Table 2). However, the decay constants were less than those recorded at stage 41, after identical treatment with MSF. We therefore conclude that AChE development does not account for all of the change in m.e.p.c. duration during the developmental period from stage 41 to stage 44.

M.e.p.c.s with double-exponential decays were recorded at stages 44–64 (Fig. 6), whereas they were not present at stage 41. The emergence of a second population of AChR channels with faster gating kinetics could explain the occurrence of double-exponential decays and could also account for part of the decline in m.e.p.c. duration between stages 41 and 44. Double-exponential decays were evident in 9% of the m.e.p.c.s recorded at intermediate stages and their mean fast and slow components were 1·1 and 4·0 ms (Table 2). Because AChE was abundant, we use these values as upper-limit estimates of the open times of two classes of AChR channels.

The mean fast and slow decay constants of double-exponential m.e.p.c.s in interhyoideus were 1·7- and 1·4-fold longer, respectively, than those recorded in superior oblique at intermediate developmental stages (Tables 1 and 2). This difference cannot be attributed to relatively lower AChE activity in interhyoideus, because it persisted after MSF treatment. Rather, it raises a possibility that both classes of channels in interhyoideus had longer gating times than those in the superior oblique.

The single-exponential decay constants of m.e.p.c.s recorded in the interhyoideus were 2·6-fold longer on the average than those recorded in the superior oblique at intermediate developmental stages. The differences in channel gating time suggested above are not great enough to account for the difference in single-exponential m.e.p.c. decays. Differing levels of AChE activity are not a likely explanation either, since MSF treatment had nearly identical effects on the decay times in the two muscles: a 2·4-fold increase in interhyoideus and a 2·2-fold increase in superior oblique (Tables 1 and 2). A likely alternative is that the longer m.e.p.c. durations in interhyoideus resulted from a relatively greater proportion of slow AChR channels.

Beginning in late metamorphosis (about stage 64), the interhyoideus end-plates entered another phase of development during which the durations of m.e.p.c.s declined markedly (Fig. 4). In adult interhyoideus muscle, m.e.p.c.s decayed as single exponentials with a mean time constant of 1·1 ms (Table 2). This value is comparable

to the open time of the fast class of AChR channels, as estimated from double-exponential decays in stages 44–64. After metamorphosis, no double-exponential decays were detected, which suggests that the m.e.p.c.s arose from a more or less homogeneous class of fast AChR channels. In comparing the histograms of decay constants at different developmental stages in interhyoideus, it appears that the slower m.e.p.c.s dropped out, leaving behind a single population of decay constants centred about 1 ms (Fig. 5). This change in m.e.p.c. duration was probably not due to additional cholinesterase development, since complete block of AChE in adult interhyoideus did not produce m.e.p.c.s as long as those recorded in stages 44–64 with AChE blocked (Table 2).

As in the superior oblique, the distribution of decay constants in the interhyoideus resulted from significant differences between recording sites as well as variability within single sites (Fig. 5, legend). No clustering of values, indicative of a small number of types of synapses, was evident.

Muscle contraction speed

In stage 50 tadpoles, direct stimulation of the superior oblique or the interhyoideus by extracellular electrodes evoked twitches as well as slower contractions. In order to compare the speed of contraction in the two muscles, we estimated by eye the maximum frequency at which repetitive twitches could be evoked. The maximum twitch frequency in the superior oblique was 28–30 Hz. At higher frequencies, the twitches fused into a sustained tetanic contraction. Single twitches were visibly slower in the interhyoideus than in the extraocular muscle and the maximum twitch frequency ranged from 10 to 16 Hz. Twitches in both muscles, particularly the interhyoideus, were superimposed on slower contractions. With increasing stimulus frequency, it appeared that some fibres reached tetany well before the fastest fibres did. Identical behaviour was observed when the muscles were indirectly stimulated via the trochlear or hyomandibularis nerves. Application of TTX (2 μg/ml) abolished propagated twitches, however local contractions could still be produced at the site of contact of the stimulating electrode with the muscle. In the adult interhyoideus, the maximum twitch frequency was increased to 20–24 Hz, and the slower contractions observed in the tadpole were usually absent. In the adult superior oblique, the maximum twitch frequency ranged from 18 to 24 Hz, which was somewhat slower than in the tadpole. It therefore appears that the contraction speeds of both muscles change during the development from larval stages to adulthood.

DISCUSSION

The comparison of end-plate currents in developing interhyoideus and superior oblique muscles illustrates that end-plates of different muscles may vary in their programmes of physiological development and that more than one distinct phase of development can occur within a single muscle. In other studies of developing end-plates in vertebrate skeletal muscle, changes in synaptic currents have been shown to be due primarily to the development of AChR channel kinetics and AChE activity (Sakmann & Brenner, 1978; Fischbach & Schuetze, 1980; Michler & Sakmann, 1980; Kullberg *et al.* 1980; Kullberg & Kasprzak, 1985; Vicini & Schuetze, 1985). It is likely

that these two factors can account for most or all of the developmental changes in synaptic current duration which we have seen in the superior oblique and interhyoideus muscles of *Xenopus*. Our results suggest a specific programme, which we summarize below, for the development of end-plate function in each muscle.

Superior oblique

At newly formed end-plates in superior oblique there is negligible AChE activity. The enzyme accumulates rapidly and, within half a day after the first detectable synaptic activity, the decay times of end-plate currents decrease 3-fold. The AChR channels present at the onset of synaptic activity have predominantly brief open times (less than 1 ms). A second, less numerous class of channels with slow gating times is also present. During the following half day of development, there is an increased relative amount of fast-channel activity, with no change in the gating time of either class of channels. The estimated mean open times of the two classes of channels are 0·6 and 2·8 ms. After the initial decline in m.e.p.c. duration, there follows a 6-week period during which no change occurs in the properties of AChR channels or AChE activity. At about the time of metamorphosis, end-plate currents gradually become slower, possibly due to a lengthened gating time of the slow class of receptors. The adult state of the end-plates is reached shortly before the completion of metamorphosis, and there is no further change in AChE activity or channel properties in the subsequent 2 years of adulthood.

Interhyoideus

At the time of first detectable synaptic activity, the interhyoideus differs from the superior oblique in two respects: AChE activity is already evident, and the gating times of AChR channels are predominantly or exclusively slow. During the following day, there is further development of AChE activity and an emergence of fast AChR channel activity. After the initial decline in m.e.p.c. duration, there is a 7-week period during which no further change occurs in channel properties or AChE activity. M.e.p.c. decays are 2·6-fold longer on the average than those in superior oblique, due to a predominance of slow AChR channels. Both fast and slow channels are active and their estimated gating times are 1·0 and 4·0 ms, somewhat longer than those in superior oblique at the same stages. Following metamorphosis, the durations of end-plate currents decline, due to a loss of slow-channel activity. At adult interhyoideus end-plates, AChE is abundant, the predominant channels have an open time of about 1 ms, and there is little or no slow-channel activity.

The schemes proposed above are based on the analysis of changes in m.e.p.c. decays. In order to study the development of AChE, we have used an irreversible anticholinesterase, MSF, under conditions which should entirely block the enzyme activity. Any developmental changes in end-plate current decay which persisted after MSF treatment we have tentatively attributed to changes in the properties of AChRs, either in their gating times or in the relative numbers of slow and fast receptors. We have not directly measured AChR channel gating times, but have assumed that the end-plate-current decay constant sets an upper-limit estimate of the mean channel open time at end-plates which have abundant AChE (Anderson & Stevens, 1973). The true open time will tend to be over-estimated by this procedure, due to the bursting behaviour of channels (Colquhoun & Sakmann, 1981; Dionne & Leibowitz, 1982).

Also, even when AChE is fully active, the decay of end-plate currents at adult-frog neuromuscular junctions (at 21 °C) is reported to be slower than the channel open time, due to the rebinding of ACh to receptors (Feltz, Large & Trautmann, 1977). Our conclusions about the development of channel gating therefore remain to be confirmed by direct recordings of channel open times.

The likelihood that each muscle expresses two discrete classes of AChRs is argued by the presence of double-exponential decays in both muscles. Similar double-exponential m.e.p.c.s have been recorded in rat skeletal muscle (Sakmann & Brenner, 1978; Fischbach & Schuetze, 1980; Michler & Sakmann, 1980; Vicini & Schuetze, 1985) and *Xenopus* myotomal muscle (Kullberg & Kasprzak, 1985), and in those cases the fast and slow decay constants have been shown to resemble the mean open times of two classes of AChR channels. In this study, we have used the fast and slow components of double-exponential decays as estimates of the gating times of two classes of AChR channels, at those stages where AChE is abundant. The fact that most m.e.p.c. decays were apparently well fitted by single-exponential curves probably reflects our inability to resolve two components by eye when one component largely outweighs the other. In general, the single-exponential decays fell within the limits of the fast and slow channel open times previously described by spectral analysis of ACh noise and by single channel recordings from myotomal muscle (Brehm, Kullberg & Moody-Corbett, 1984; Brehm, Kidokoro & Moody-Corbett, 1984; Kullberg & Kasprzak, 1985).

The duration of end-plate currents was found to be correlated with muscle contraction speed, as measured by maximum twitch frequencies. The slowest end-plate currents and contraction speeds were observed in the larval interhyoideus, whereas the fastest of each were present in the larval superior oblique. During development, the end-plate current decays and contraction speeds both became faster in interhyoideus and slower in the superior oblique. If our explanation of the change in m.e.p.c. decays according to channel properties is correct, these results suggest that the ratio of channel types and/or their gating properties are regulated mutually with the contraction speed of the twitch muscle fibres. It would follow that the distribution of m.e.p.c. durations which we have observed within each muscle may correspond to a variety of functionally different fibre types.

In both the superior oblique and interhyoideus, there are some obvious changes in muscle function which are correlated with the development of synaptic currents and with changes in contraction speed. The rapid saccadic movements of the tadpole eye cease during metamorphosis and the only movement of the adult eye is a protective retraction. The phasic contractions of the interhyoideus, which are essential to feeding and branchial ventilation in the tadpole, also cease during metamorphosis. In *Rana catesbeiana*, the phasic contractions of the interhyoideus are produced by fibres which have some of the properties of adult slow muscle, such as resistance to fatigue when stimulated directly, sustained contraction in the presence of ACh, and simple end-plate morphology (Gradwell & Walcott, 1971). However, these fibres also generate twitches and therefore may be similar to the intermediate type fibres described by Lannergren (1979). The fibres responsible for slow, phasic contractions disappear after metamorphosis in *Rana catesbeiana*, leaving only fibres which have the properties of fast-twitch muscle (Gradwell & Walcott, 1971).

We have not determined whether the metamorphic changes of end-plate currents occur within individual fibres or whether they represent the appearance of new types of fibres. In *Rana temporaria*, larval interhyoideus fibres are histolysed and replaced by new, adult fibres during metamorphosis (De Jongh, 1968). If a similar process occurs in *Xenopus*, then the change in time course of m.e.p.c.s at metamorphosis may be due to the establishment of new end-plates on adult muscle fibres, rather than to the further maturation of old end-plates.

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