Comparison of Excitatory Currents Activated by Different Transmitters on Crustacean Muscle

II. Glutamate-activated Currents and Comparison with Acetylcholine Currents Present on the Same Muscle

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ABSTRACT The properties of glutamate-activated excitatory currents on the gm6 muscle from the foregut of the spiny lobsters *Panulirus argus* and *interruptus* and the crab *Cancer borealis* were examined using either noise analysis, analysis of synaptic current decays, or slow iontophoretic currents. The properties of acetylcholine currents activated in nonjunctional regions of the gm6 muscle were also examined. At 12°C and −80 mV, the predominant time constant of power spectra from glutamate-activated current noise was ~7 ms and the elementary conductance was ~34 pS. At 12°C and −80 mV, the predominant time constant of acetylcholine-activated channels was ~11 ms with a conductance of ~12 pS. Focally recorded glutamatergic extracellular synaptic currents on the gm6 muscle decayed with time constants of ~7–8 ms at 12°C and −80 mV. The decay time constant was prolonged e-fold about every 225-mV hyperpolarization in membrane potential. The Q_{10} of the time constant of the synaptic current decay was ~2.6. The voltage dependence of the steady-state conductance increase activated by iontophoretic application of glutamate has the opposite direction of the steady-state conductance activated by cholinergic agonists when compared on the gm6 muscles. The glutamate-activated conductance increase is diminished with hyperpolarization. The properties of the marine crustacean glutamate channels are discussed in relation to glutamate channels in other organisms and to the acetylcholine channels found on the gm6 muscle and the gm1 muscle of the decapod foregut (Lingle and Auerbach, 1983).

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INTRODUCTION

In the preceding paper (Lingle and Auerbach, 1983), the properties of acetylcholine (ACh)-activated excitatory channels on lobster and crab gml neuromuscular preparations were described. Here, we have tried to take advantage of the presence of both ACh- and glutamate-activated excitatory currents on particular decapod crustacean foregut muscles to make a direct comparison of the two types of excitatory currents.

The study of transmitter-gated excitatory ionic channels has been limited largely to two types of channels: ACh-activated channels at the vertebrate neuromuscular junction (Katz and Miledi, 1972; Anderson and Stevens, 1973; Magleby and Stevens, 1972; Neher and Sakmann, 1975) and *Aplysia* neurons (Ascher et al., 1978) and glutamate-activated channels at arthropod neuromuscular junctions (Anderson et al., 1978; Dudel, 1974; Onodera and Takeuchi, 1975, 1978; Crawford and McBurney, 1976; Cull-Candy and Parker, 1982; Cull-Candy et al., 1981). Although these two different types of channels share some common features, for example, somewhat similar ionic selectivity (Dwyer et al., 1980; Anwyl, 1977*a*, *b*), several aspects of the kinetic properties and ionic conductance differ. Specifically, the apparent mean open time of ACh-activated excitatory channels tends to be prolonged by hyperpolarization (Anderson and Stevens, 1973; Magleby and Stevens, 1972; Ascher et al., 1978; Lingle and Auerbach, 1983), whereas the apparent mean open time of glutamate-activated channels of the locust and crayfish show a weak tendency to be shortened (Anderson et al., 1978; Dudel, 1974; Onodera and Takeuchi, 1975, 1978) or unchanged (Anwyl and Usherwood, 1974) with hyperpolarization. In addition, the mean conductance of the locust glutamate channel is substantially greater than the conductance of the ACh channel of either the vertebrate neuromuscular junction (review by Steinbach, 1980), the mammalian sympathetic ganglion (Rang, 1981), or *Aplysia* neurons (Ascher et al., 1978).

The factors that determine voltage dependencies in channel kinetics and the properties that determine channel conductance remain incompletely understood. However, both the kinetic properties and the conductance of ionic channels are to some extent sensitive to extracellular ionic manipulation (Marchais and Marty, 1979; Gage and Van Helden, 1979; Cull-Candy and Miledi, 1980) and possibly to changes in membrane fluidity (Gage et al., 1978; review by Steinbach, 1980). The voltage dependence of the apparent mean open time of the locust glutamate channel is in fact reversed in the presence of an isotonic calcium medium (Cull-Candy and Miledi, 1980). It remains possible that some of the observed differences in the properties of ionic channels may not reflect differences in molecular structures but rather differences in the membrane or extra- or intracellular environment between cells of different organisms. The present paper addresses this issue by comparing some of the aspects of the kinetic and conductance properties of glutamate and ACh currents found on different and, in some cases, the same neuromuscular preparations of lobster and crab foregut. Within the experimental limitations imposed by these crustacean muscle fibers, the results indicate that these ACh-

and glutamate-activated excitatory currents are different in a manner similar to their differences when found in different phyla.

MATERIALS AND METHODS

The materials and methods were basically identical in all respects to those presented in the previous paper (Lingle and Auerbach, 1983). When glutamate iontophoretic electrodes were used, they were filled with 1 M L-glutamate and adjusted to pH 8.0. The gm6 muscle, which receives a glutamatergic type of innervation but, in *P. interruptus* displays nonjunctional ACh receptors (Lingle, 1980), was used in this study. Muscles from either the lobsters *Panulirus argus* and *interruptus* or the crab *Cancer borealis* were studied.

Noise Analysis

The details of the noise analysis procedure were presented in the previous paper (Lingle and Auerbach, 1983). With respect to glutamate-activated currents, some additional discussion of the problems in obtaining meaningful spectra of glutamate-activated noise on these foregut neuromuscular preparations is warranted. These problems have been extensively analyzed for crayfish neuromuscular preparations (Finger and Stettmeier, 1980). For crayfish muscle fibers >100 μm in diameter, an effective space clamp at frequencies up to 250–500 Hz can be obtained only within 100 μm of the clamping electrodes. Assuming that for lobster muscle fibers of 50–150 μm diam the effective space clamp is similar to that of medium to large crayfish fibers, the increase in glutamate-activated noise might fail to reflect adequately the frequency components of the glutamate-activated channels for several reasons. First, glutamate may activate channels in relatively unclamped regions and the resulting currents, when conducted down the fiber, would then reflect the filtering characteristics of the muscle fiber; higher-frequency signals would be less likely to be seen by the clamping electrodes. Second, the glutamate-activated conductance might alter the area of the membrane that is adequately space-clamped, thereby changing the characteristics of the background noise in the presence and absence of glutamate.

The above problems are relatively more severe for glutamate-activated currents than for the ACh-activated currents presented in the previous paper (Lingle and Auerbach, 1983). This results primarily from the fact that glutamate-activated current noise apparently contains higher-frequency components than ACh-activated noise. To adequately record glutamate currents, a larger region of the fiber must be effectively clamped in order to avoid filtering by the muscle fiber. In addition, because of the localized nature and sparse distribution of glutamate-sensitive sites, the location of the glutamate-sensitive receptors relative to the clamped region is more uncertain. A third problem is that glutamate currents appear to undergo much more rapid and substantial desensitization than the cholinergic currents. Thus, DC recordings of glutamate currents frequently show a rapid peak followed by a rapid diminution to a minimal stable level. As a result, since stable mean current increases are generally only ~10–30 nA, the variance in the high-gain current noise is also undesirably small. The severity of the above problems was underscored in the present experiments by the observation that occasionally spectra of glutamate current noise obtained at 22°C rolled off at lower frequencies than spectra obtained at 12°C, which clearly indicates that at 22°C the properties of the increase in current noise probably reflect the filtering properties of the muscle fibers and an inadequate clamp of the glutamate currents.

Several procedures were used to try to minimize problems associated with the

limited space clamp. These included the addition of 20 mM manganese chloride or 5 mM cesium chloride (Dudel et al., 1977) to the saline to increase the muscle fiber input resistance and to perform the experiments at ~12°C. Combining such treatments, muscle fiber resistances upwards of 0.5 MΩ could be obtained. Under such conditions, it is likely that the cutoff frequency of glutamate-activated current noise is within the range of clamped frequencies. Such conditions yielded spectra similar to that shown in Fig. 6. Although no direct test for the low concentration limit was made, the mean currents activated by glutamate in these experiments are a small portion of the maximal current that can be activated at a single glutamate-sensitive site. Glutamate spectra were not clearly single Lorentzian functions but, for the comparative purposes of this study, spectra were approximated with single Lorentzians.

RESULTS

Comparison of Glutamate and ACh Responses on the gm6 Muscle

The gm6 muscle is one of several in the foregut of some decapod species that exhibit excitatory responses to either application of ACh or glutamate (Fig. 1A; Lingle, 1980). Some description of the similarities and differences between those responses will be useful here. Inward currents in response to the application of ACh can be elicited at virtually any position on the *P. interruptus* gm6 muscle, whereas glutamate-sensitive sites can only be found at restricted locations sparsely distributed on the muscle fibers. Excitatory synaptic potentials on the gm6 muscle are not blocked by cholinergic agents that do block ACh-evoked potentials (Figs. 1B and C). This includes hexamethonium, decamethonium, curare, dihydro-β-erythroidine, trimethapan, pempidine, and mecamylamine. Similar tests with glutamatergic agents are limited by the paucity of definitive blockers (Fig. 1D). However, chlorisondamine, a sympathetic ganglionic nicotinic blocker and blocker of these crustacean ACh responses (50% inhibition of iontophoretic responses $\simeq 2 \times 10^{-6}$ M at −80 mV) is also effective in reducing both synaptic and glutamate-evoked currents (50% inhibition $\simeq 2 \times 10^{-4}$ M at −80 mV) on the gm6 muscle (Lingle et al., 1981; Lingle, 1981, 1983). Chlorisondamine is similarly effective in reducing synaptic responses on the presumed glutamatergic lobster opener muscle.

Divalent cation sensitivity also distinguishes between the glutamate and cholinergic responses. In low-Ca^+ saline, glutamate potentials are severely attenuated (Lingle, 1980; Thieffry and Bruner, 1978), while ACh potentials are unchanged. The addition of 20 mM manganese to zero-calcium saline restores the glutamate-activated potentials, which indicates that the glutamate-activated response does not occur via a presynaptic effect on the nerve terminal.

Finally, after washout of a prolonged application of glutamate, synaptic responses on the gm6 muscle are greatly attenuated even after restoration of the normal cell input resistance. After washout of cholinergic agonists, no desensitization of the synaptic response is observed. Thus, the glutamate and ACh responses on the gm6 muscle are unrelated and the gm6 synaptic responses can be considered glutamatergic-like.

Synaptic Currents at gm6 Glutamatergic Excitatory Junctions

Synaptic currents (excitatory junctional currents: ejc's) on the gm6 muscles of *P. interruptus* and *C. borealis* were recorded by measuring the potential difference between an extracellular focal pipette located directly over a synaptic region and a second reference pipette (Lingle and Auerbach, 1983).

In contrast to the relatively simple exponential decays observed on the cholinergic neuromuscular junctions of the gm1 muscle of *P. interruptus* and *C.*

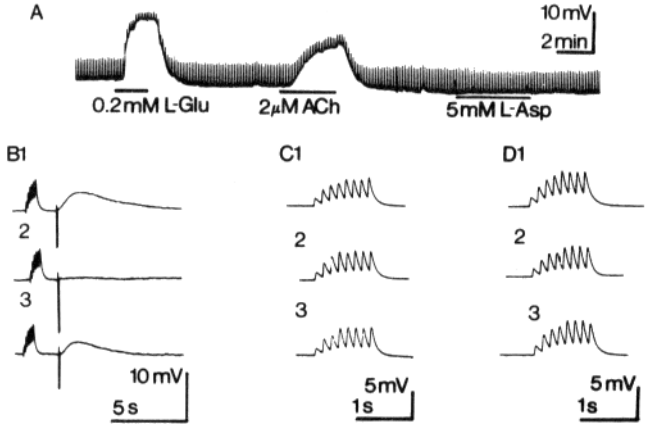

FIGURE 1. Synaptic and agonist-induced potential changes on the gm6 muscle of *P. interruptus*. (A) Effects of bath-applied agonists on membrane potential. The nerve to the muscle was stimulated with a 500-ms train of pulses. Each deflection corresponds to a train of excitatory junction potentials (EJPs). Drugs (0.2 mM L-glutamate, 2 mM ACh with 10^{-5} g/ml tensilon, 5 mM L-aspartate) were introduced into the perfusion system during the periods indicated by the bars. At this temperature (~14°C) and perfusion rate, no desensitization of synaptic responses by glutamate was apparent. (B) Effect of 5×10^{-5} M *d*-tubocurare on gm6 EJPs and ACh iontophoretic potentials. Each trace shows a train of EJPs followed by the response to an iontophoretic pulse of ACh. B1 and B3 correspond to the control and wash, respectively. B2 is in the presence of 5×10^{-5} M curare. (C) Effect of 5×10^{-4} hexamethonium on gm6 EJPs. Top and bottom correspond to control and wash C2 is in the presence of hexamethonium. Temperature: 12°C. (D) Effect of 2 mM L-glutamate-γ-methylester on gm6 EJPs. Top and bottom correspond to control and wash junctional potentials. In D2 a slight reduction in amplitude of the gm6 EJPs occurs. Temperature: 12°C.

borealis, on the gm6 muscle a rather complex synaptic current decay is observed (Fig. 2). In general, the ejc decay begins rather slowly and does not reach an exponential time course until close to 50% of peak. In some cases the decay never clearly follows a simple exponential time course. To provide a means of comparison of gm6 ejc decay rates with those from cholinergic (gm1) synapses, a time constant was determined over the portion of the decay that approached exponentiality. The time constant of decay of gm6 synaptic currents near 12°C and −80 mV was 8.5 ± 0.2 ms (SEM of 10 determinations) for *P. interruptus* and 6.2 ± 0.2 ms (SEM of 26 determinations) for *C. borealis*.

Glutamatergic synaptic current decays on other preparations also tend to exhibit a slow approach to apparent exponentiality (Onodera and Takeuchi, 1975; Clark et al., 1980).

The effect of membrane potential on the apparent time constant of the decay of the ejc's was examined. The time constant of ejc decay was slightly

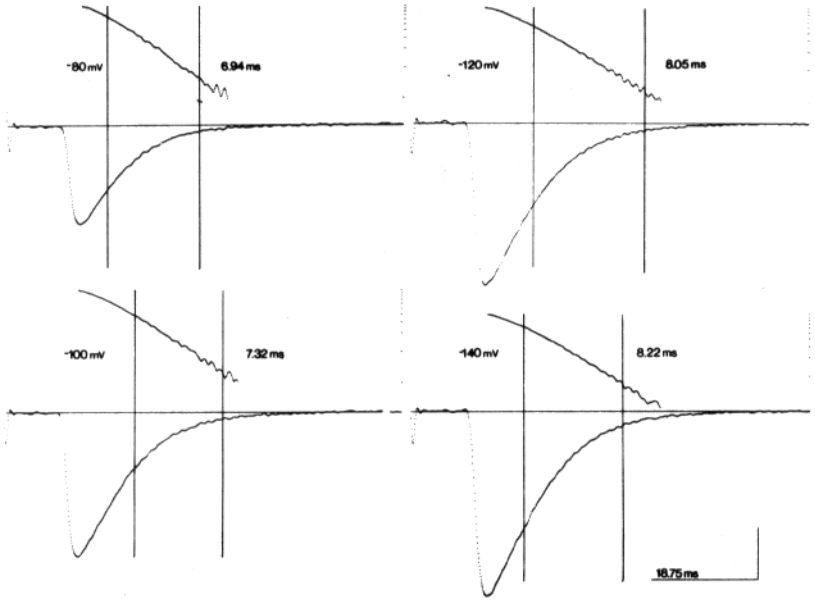

FIGURE 2. Voltage dependence of decay of extracellular ejc's recorded from a gm6 muscle of *C. borealis*. A saline-filled pipette of 10–20 μm diam was positioned along a muscle fiber until a potential was found corresponding to the synaptic current flow in response to stimulation of the excitatory motor nerve to the muscle. Two intracellular electrodes were then placed in the muscle fiber adjacent to the focal pipette to allow control of the membrane potential. The nerve to the muscle was stimulated continuously at ~5 Hz and the evoked currents were stored on tape. At least 40 ejc's were then averaged (triggering on the stimulus pulse) with a MINC-11 computer. The recorded top portion of each trace shows the semilogarithmic plot of the averaged currents shown below. A time constant was calculated by a least-squares linear regression over the range indicated by the vertical lines. The decays only slowly approached a simple exponential decay, although this was somewhat variable among sites. On the left: for the top trace, −80 mV and 6.94 ms; for the bottom trace, −100 mV and 7.32 ms. On the right: for the top trace, −120 mV and 8.05 ms; for the bottom trace, −140 mV and 8.22 ms. Horizontal calibration was 18.75 ms and the vertical calibration is in arbitrary units, the same for all traces. Temperature: 11.5°C.

prolonged by hyperpolarization (Fig. 2). This is illustrated for one gm6 synaptic site from *P. interruptus* for which a voltage dependence of 213 mV/e-fold change was observed (Fig. 3A). Similar values were obtained at gm6 synapses of *C. borealis*, as seen in Fig. 3B. This trend towards prolongation with hyperpolarization was observed for every site examined, even in cases

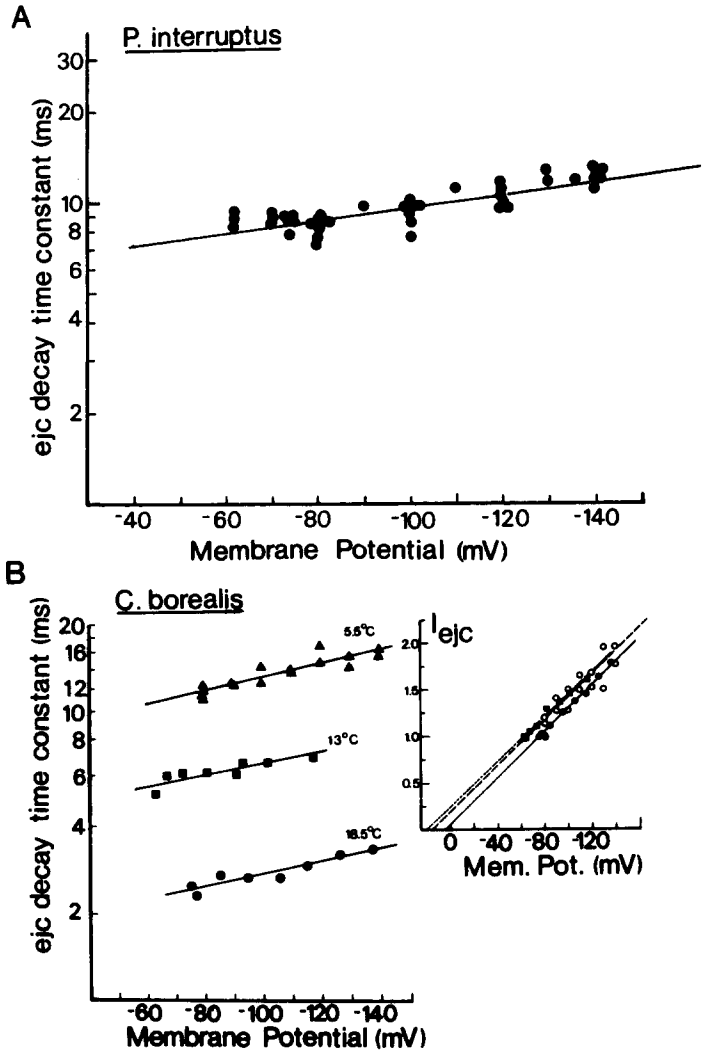

FIGURE 3. (A) Voltage dependence of ejc decay time constants on the gm6b muscle of *P. interruptus*. Records similar to those shown in Fig. 2 were used to generate ejc decay time constants at various membrane potentials. The ordinate corresponds to the value for the ejc time constant and the abscissa corresponds to the membrane potential at which a given set of ejc's was generated. Each point corresponds to the average of at least 40 single evoked currents. The temperature was 13°C and an e-fold change in the ejc decay time constant occurred every 213 mV as determined by the linear regression to the points. (B) Voltage dependence of ejc decay time constants on the gm6 muscle of *C. borealis*. The values for the ejc decay time constants for three muscle fibers shown in Fig. 1 are plotted as a function of membrane potential. From top to bottom, at 5.5°C (open circles) an e-fold change in the τ_{ejc} was produced about every 230 mV, at 13°C an e-fold change in τ_{ejc} occurred about every 220 mV, and at 18.5°C (filled circles) an e-fold change in τ_{ejc} occurred about every 200 mV. In the inset the amplitudes of the ejc's as a function of membrane potential is plotted for these three fibers. The amplitudes are normalized to the amplitude of the response obtained at the most depolarized potential in each case. The symbols in the inset correspond to the symbols used for the plot of ejc decay time constants, except the open circles of the inset, which correspond to the triangles. The extrapolated reversal potentials determined from linear regressions to all points at a particular site are: at 5.5°C, +16 mV; at 13°C, +23 mV; and at 18.5°C, +4.3 mV.

with decay time constants <3 ms. For 30 determinations of the voltage dependence of τ_{ejc} from 10 fibers, τ was prolonged threefold for every 245.4 ± 34.2 mV (SEM) of hyperpolarization.

This prolongation with hyperpolarization, although slight, is in general at variance with studies of the glutamatergic synapses of the locust and crayfish (Dudel, 1974; Onodera and Takeuchi, 1978). However, Onodera and Takeuchi (1975, 1978) reported a prolongation of intracellular synaptic currents obtained with crayfish muscle fibers at membrane potentials in the range of −50 to −140 mV. They attributed this result to a presynaptic effect of the currents set up by clamping to hyperpolarized potentials. This produced asynchronous vesicular release when observed at single synaptic sites with an extracellular focal electrode. Such a possibility was addressed by the experiment shown in Fig. 4A. Single sweeps of focal synaptic potentials were captured at −74 and −140 mV. At the more hyperpolarized membrane potential, a number of the evoked currents exhibited decays of abnormal appearance. These decays might be indicative of some effect on the presynaptic terminal that results in a prolonged release of neurotransmitter. If these abnormalities were to account for the apparent prolongation of the averaged ejc decay, one might expect that single events not manifesting deviant decay might not show prolongation with hyperpolarization. However, a histogram of the time constant of decays determined from single evoked synaptic events at both −74 and −140 mV fails to indicate a substantial occurrence of synaptic decays at −140 mV that are faster than those at −74 mV (Fig. 4B). The distribution of events at −140 mV again is suggestive of a trend towards a slight prolongation of synaptic currents with hyperpolarization. The occurrence of visibly abnormal decays cannot simply account for the prolongation. Similarly, in results not shown, at sites at which spontaneous miniature quantal events could be observed, hyperpolarization also produced a prolongation of such currents.

Katz and Miledi (1973) observed that the presence of an extracellular focal electrode at a synaptic site on the frog neuromuscular endplate can prolong the time course of spontaneous miniature synaptic currents beneath the focal electrode presumably by a physical alteration of pathways for diffusion of ACh from the cleft. If this "compression artifact" were occurring here, the time course of the presence of transmitter in the junctional region might control the time course of ejc's. One might thus expect that variation in the amount of transmitter released from the terminal might affect the rate of ejc decay. Since the gm6 neuromuscular synapses undergo substantial frequency-dependent facilitation, in several fibers ejc's were examined over a range of frequencies of nerve stimulation to ascertain whether the amount of transmitter released presynaptically might affect the time course of synaptic currents (Fig. 4C). Increases in stimulation frequency resulted in a large facilitation in the ejc amplitude without any clear effect on the ejc decay time constant. The absence of any clear prolongation suggests that the amount of transmitter in the junctional area may not directly influence the synaptic current decays, although other interpretations of this result are possible.

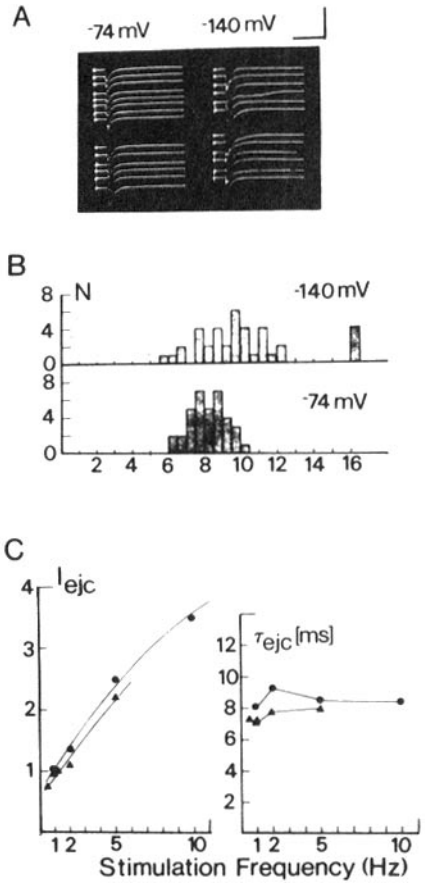

FIGURE 4. Assessment of factors contributing to the ejc decay process. (A) Single sweeps of evoked excitatory synaptic currents at a gm6 muscle of *Panulirus interruptus* are shown. The left column shows single ejc's at −74 mV and the right column shows ejc's at −140 mV. At −140 mV, several ejc's decay with nonexponential or abnormally prolonged time course. The vertical calibration is 1 mV and the horizontal calibration is 20 ms. (B) Histograms of the decay time constant of single evoked ejc's as shown in A. For abnormal decays the time constant was calculated over the fastest portion of the decay. The distribution of ejc decay time constants is much broader at −140 mV. Note that the fastest decaying events apparently occur at −140 mV. The largest values at −140 mV correspond to any value above 16 ms. (C) The effect of ejc amplitude on the ejc decay time constant of the gm6 excitatory currents is plotted. The frequency of stimulation of the excitatory motor nerve was varied between 0.5 and 10 Hz. The ejc amplitude increases with stimulation frequency because of presynaptic facilitation. On the left, the vertical axis displays the normalized ejc amplitude vs. the stimulation frequency for two separate fibers, both near −60 mV and at ~12°C. On the right the vertical axis shows the ejc decay time constant plotted as a function of stimulation frequency. Each point is the average of at least 60 single evoked currents.

The effect of temperature on the synaptic current decay rate was also examined. Similar results were obtained from synaptic currents of the gm6 muscles of either *C. borealis* or *P. interruptus*. Increasing the temperature produced a marked increase in the decay rate of synaptic currents (Fig. 5). The time constant of synaptic current decay was prolonged ~2.6-fold for every 10°C decrease in temperature over the range of 5–21°C. This temperature dependence is similar to that of other transmitter-gated currents and somewhat greater than might be expected if the synaptic decay were controlled simply by diffusion of transmitter out of the cleft.

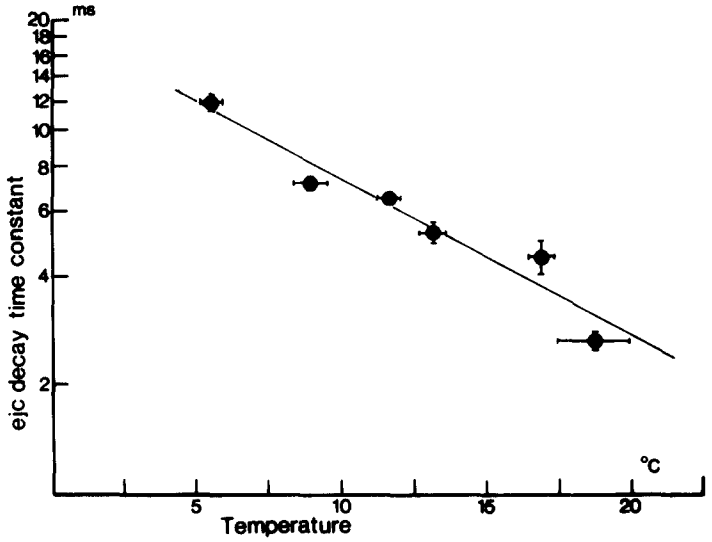

FIGURE 5. Temperature dependence of the ejc decay time constant of the gm6 glutamatergic synapse of *C. borealis*. The vertical axis corresponds to the ejc decay time constant of the averaged excitatory synaptic currents and the horizontal axis corresponds to the temperature. Time constants were determined as shown in previous figures. Each point is the mean of at least five determinations within a particular range of temperatures. The vertical error bars indicate the SD of the ejc decay time constants and the horizontal error bars indicate the SD of the temperatures. Values were obtained at membrane potentials between −80 and −100 mV. The line is the linear regression to the means and yields a Q_{10} of ~2.6.

Synaptic current amplitude increased with hyperpolarization in a manner consistent with an increased electrical gradient on the ions passing through the opened channels (Fig. 2). Over the range of voltages examined, the peak ejc amplitude varied in a close-to-linear fashion with membrane potential. For 31 determinations from over 10 fibers, the extrapolated reversal potential was +12.8 ± 3.9 mV (SEM).

Glutamate- and ACh-activated Current Noise on the gm6 Muscle

Glutamate-sensitive locations on the gm6 muscles of *P. argus* and *P. interruptus*

are sparsely distributed. After locating such a site, intracellular electrodes were positioned within 50 μm of the iontophoretic glutamate electrode. The region was then voltage-clamped and application of glutamate resulted in an inward current flow. Power spectral analyses of the frequency components of the glutamate-activated noise after subtraction of the background noise, in the best cases, yielded spectra similar to that shown in Fig. 6A. Although such spectra were not clearly simple Lorentzian functions, for purposes of compar-

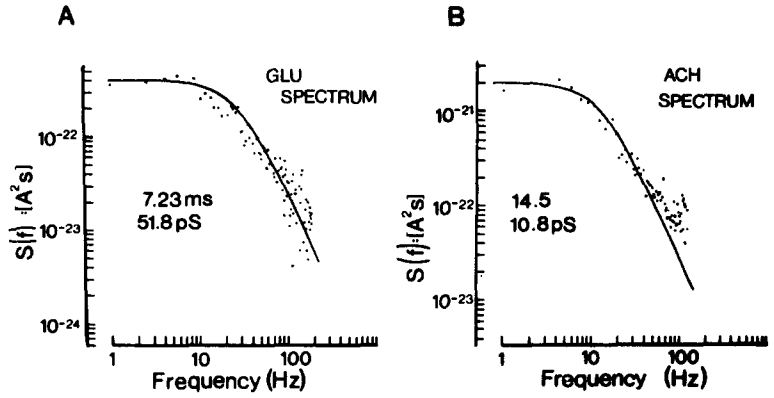

FIGURE 6. Power spectra of glutamate- and ACh-activated current noise at the same voltage-clamped region of a gm6 muscle of *P. interruptus*. A glutamate-sensitive region was initially voltage-clamped, and high-gain records of glutamate-activated currents were obtained by application of glutamate from a 1-M glutamate-containing micropipette. The glutamate electrode was then withdrawn and a 1-M ACh-containing micropipette was positioned between the clamping electrodes. High-gain records of ACh-activated current noise were then obtained. FFT analysis of the current noise yielded the spectra shown. Points were digitized at 500 Hz and low-pass-filtered at 250 Hz. The lines indicate single Lorentzian functions aligned by eye to the points. A three-point smoothing function was used to generate the plots. Estimates of τ_n and γ were similar whether the smoothing function was applied or not. Points above ~150 Hz have been omitted for clarity. Details of the noise analysis procedure are presented in Lingle and Auerbach (1983). For the spectrum of glutamate current noise (A), the apparent mean open time was 7.23 ms with a single-channel conductance of 51.8 pS. For the spectrum of ACh-activated current noise (B) the apparent mean open time was 14.5 ms and the mean single-channel conductance was 10.8 pS. Temperature was 12°C and the membrane potential was −80 mV.

ison spectra were fit with a single Lorentzian. However, it was difficult to obtain such spectra reliably (see Materials and Methods) and, therefore, information that could be gained concerning the glutamate-activated channels using the noise methodology was limited. From glutamate current noise spectra, an estimate for a predominant time constant of the glutamate-activated currents, τ_n, was obtained from the cutoff frequency of the single

Lorentzian. Again assuming a single Lorentzian function, an estimate of the mean single-channel conductance, γ, was determined from the zero-frequency asymptote and the half-power frequency of the fitted Lorentzian (Lingle and Auerbach, 1983). For five determinations, τ_n = 7.3 ± 0.6 ms (SEM), while γ = 34.8 ± 5.9 ps (SEM) at 12°C. These values include determinations at membrane potentials from −60 to −120 mV. The limited number of values precluded an estimate of the voltage dependence of these parameters.

The gm6 muscle of *P. interruptus* allows a comparison of current noise resulting from either ACh or glutamate application on the same cell. The diffuse distribution of cholinergic receptors on the gm6 muscle extends at least close to the synaptic regions, since double-barreled iontophoretic electrodes filled with ACh and glutamate can at appropriate sites elicit responses to either agonist. Taking advantage of this arrangement of receptors, Fig. 6 displays the power spectra generated from a gm6 muscle in which glutamate- and ACh-activated currents were elicited at the same voltage-clamped region. The values for predominant time constant and conductance for the glutamate-activated currents correlate well with those values obtained from other sites on the gm6 muscle. For four spectra of ACh-activated current noise on the gm6 muscle at −80 mV and 12°C, τ_n = 11.1 ± 1.3 ms (SEM) and γ = 12.0 ± 2.5 pS (SEM). These values for τ_n and γ for the ACh-activated currents on the gm6 muscle are within the range of values observed for the gm1 ACh channels at 12°C (Lingle and Auerbach, 1983). Thus, the amplitude and frequency characteristics of the elementary units underlying the currents generated by ACh appear to differ from the properties of the elementary units activated by glutamate when examined on the same gm6 muscle fibers.

Voltage Dependence of Steady-State Agonist-induced Currents

On the gm1 muscle, the voltage dependence of the amplitude of the iontophoretically activated cholinergic currents was of similar direction to the voltage dependence of the apparent mean open time, which suggests that iontophoretic currents may reflect a pseudo–steady-state condition of the processes underlying the cholinergic conductance (Lingle and Auerbach, 1983). Since the analysis of synaptic current decays of the gm6 muscle indicated that hyperpolarization may also favor a slight prolongation of mean channel open time on this apparently glutamatergic muscle, it was therefore of interest to examine the voltage dependence of the amplitude of the glutamate iontophoretic currents.

Such an experiment is illustrated in Fig. 7 for both glutamate- and carbachol-induced currents on the gm6 muscle. As with the cholinergic currents on the gm1 muscle, the amplitude of glutamate-activated currents showed a nonlinear dependence on voltage. However, the direction of that voltage dependence was the opposite of that observed for carbachol, with the glutamate-activated conductance being less favored by hyperpolarization. This result was observed in all fibers examined from both *Panulirus* and *Cancer* either with or without 20 mM manganese chloride added to the saline. The removal of calcium from the saline with 20 mM Mn^{++} present did not alter the nature of the curvature.

DISCUSSION

The main goal of this study was to characterize the properties of both glutamatergic and cholinergic currents when found on similar and the same crustacean muscles. The results show that both the glutamatergic synaptic current decays and noise spectra are suggestive of complex kinetic processes that contrast with the properties of ACh current noise on the gm6 muscle (this paper) and to the properties of the cholinergic synaptic current decays

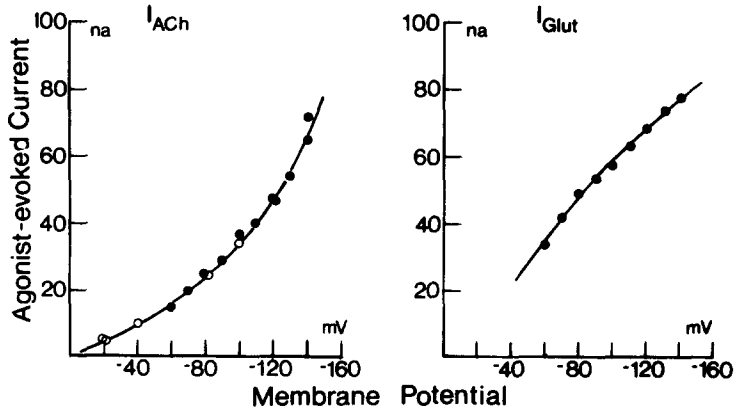

FIGURE 7. The effect of membrane potential on the amplitude of iontophoretically activated carbachol and glutamate currents on the gm6 muscle of *P. interruptus*. The two panels correspond to two separate experiments. In both cases, a region of the muscle fibers was voltage-clamped with two intracellular electrodes and the appropriate agonist applied iontophoretically. The amplitude of the agonist-induced currents is plotted as a function of the clamped membrane potential. For the carbachol-activated currents the open circles correspond to points obtained in saline with no calcium and 20 mM Mn^{++} to enable movement of the membrane potential to more depolarized levels. Each point corresponds to a single iontophoretic response. For the glutamate-activated currents each point corresponds to a single iontophoretic response. For the glutamate-activated currents each point is the mean of at least six iontophoretic responses with SEMs smaller than the size of the points. The lines are drawn by eye to indicate the trends of the points.

and current noise on the gm1 muscle (Lingle and Auerbach, 1983). In addition, the pseudo-steady-state currents activated by glutamate on the gm6 muscle show a different voltage dependence than ACh-activated currents on the same muscle. Before elaborating on the implications of these differences between the glutamatergic and cholinergic currents, a comparison of the gm6 glutamate currents with other arthropod glutamatergic systems is warranted.

Properties of Glutamate Currents on the gm6 Muscle

Within the limitations of the present analysis, the magnitude of the time constant obtained at 12°C from the synaptic current decays from the gm6 muscles of *P. interruptus* (8.5 ms at −80 mV) corresponded reasonably well to

that derived from the cutoff frequency of the Lorentzian fit to glutamate current noise spectra from *P. interruptus* and *P. argus* (7.3 ms at −80 mV). This suggests that both types of measurements are probably similar approximations of a predominant time constant of some aspect of the glutamate-activated channels. However, the interpretation of the physical meaning of these time constants rests on assumptions about the kinetic scheme underlying the opening and closing of ion channels which the present experiments are insufficient to address. As a result, a simple correspondence of the constants derived from the results with a definable physical process is precluded.

Irrespective of the physical basis of the measured kinetic constants, the predominant time constant of the glutamate currents of the gm6 muscle is fairly similar to the apparent mean open time of glutamate channels analyzed on other arthropod preparations. When differences in experimental temperatures are taken into account, glutamate-activated channels appear to stay open on the average ~1.5–3 ms at ~20°C (Crawford and McBurney, 1976; Onodera and Takeuchi, 1975, 1978; Anderson et al., 1978; Stettmeier et al., 1978, 1979). The values we have obtained from marine decapods are on the upper end of this range (see Fig. 5).

In contrast to the similarities in apparent mean open time among glutamate channels, the conductance of the lobster glutamate channel (~34 pS at 12°C) appears to be less than that of locust muscle (~70 pS near 12°C; Anderson et al., 1978), but somewhat more than that reported for the crayfish (20 pS at ~20°C; Stettmeier et al., 1978). The estimate of single-channel conductance depends on an accurate estimate of the reversal potential of the glutamate-activated currents, which for simplicity was assumed here to be 0 mV. The value of E_r obtained from extrapolations of the ejc amplitudes was +12.8 mV, a value which is in the range of other reported values for the glutamate reversal potential on crustacean preparations (Taraskevitch, 1971; Dudel, 1974; Onodera and Takeuchi, 1975, 1978). However, if 0 mV is a substantial underestimate of the true reversal potential value, our estimates of γ would be too large. On the other hand, the possible loss of high-frequency components in glutamate noise spectra due either to imperfect space clamp or the limited frequency response of the system would tend to underestimate γ. Based on such considerations, it is difficult to form conclusions concerning the extent of similarities or dissimilarities among values of single-channel conductance from different arthropod preparations.

In contrast to previous investigations of glutamatergic synaptic currents in Crustacea (Dudel, 1974; Onodera and Takeuchi, 1978), a slight prolongation of glutamatergic synaptic current decays with hyperpolarization was observed in the present experiments. Although the magnitude of the voltage dependence of the apparent mean open time of glutamate-activated channels from different preparations is in all cases slight, a different direction of voltage dependence would be of some interest. Several possible artifactual sources of this result were mentioned in the Results. Other possible explanations follow.

One simple answer might be that the synaptic transmitter at the gm6 muscle is simply not L-glutamate. However, in other respects the gm6 excit-

atory innervation is similar to other crustacean glutamatergic excitatory neuromuscular systems (Lingle, 1980) and this synapse can at least be considered glutamate-like.

Second, the nonexponential aspect of the glutamatergic ejc decays and the voltage dependence might result from contamination by potentials generated from nonfocal, nonsynaptic currents. However, using identical methods, the decays of cholinergic ejcs on the gm1 muscle are fairly good simple exponentials (Lingle and Auerbach, 1983). Thus, although this possibility cannot be definitely excluded, if such currents were a contaminating feature of glutamate ejc's, it would be surprising if a similar effect were not occurring with cholinergic ejc's.

Finally, the apparent voltage dependence of the kinetics of glutamate current relaxations in these marine decapods may in fact be different from that of the freshwater crayfish and the terrestrial locust. In the locust, isotonic calcium saline shortens the glutamate channel apparent mean open time and reverses the direction of the voltage dependence of the open time (Cull-Candy and Miledi, 1980). Since the origins of voltage dependence remain obscure (review by Steinbach, 1980) particularly in light of the complicated nature of the kinetic processes that may underly glutamate channel activation (Patlak et al., 1979; Cull-Candy and Parker, 1982), the meaning of reversals in voltage dependence is certainly not clear.

Comparison of Glutamate-activated Currents with ACh-activated Currents

The ACh currents of the gm1 and gm6 muscle differ from the glutamate currents of the gm6 muscle in the following ways.

(*a*) The time constants estimated from spectral analyses of ACh-activated noise are greater than for glutamate-activated noise. At 12°C and −30 mV, τ_n (ACh) is ~13 ms and τ_n (Glu) is ~7 ms.

(*b*) The estimated single-channel conductance for glutamate-activated channels (~35 pS at 12°C) is greater than that of ACh-activated channels (~18 pS at 12°C).

(*c*) ACh synaptic currents decay as simple exponentials, whereas glutamate synaptic currents do not. At −80 mV and 12°C, the decay of ACh synaptic currents (14–15 ms) is slower than for glutamate synaptic currents (7–8 ms).

(*d*) The magnitude of the voltage dependence of cholinergic ejc decay time constant (85–100 mV/e-fold change) is larger than that for glutamate ejc's (>200 mV/e-fold).

(*e*) The direction of the voltage dependence of the amplitude of pseudo-steady-state currents activated by the two transmitters differs.

Taken as a whole, these results suggest that the properties of the molecules underlying the openings and closings of the channels, and possibly the rate of ion movement through the channels, are fundamentally different for ACh and glutamate channels when found in the same or similar cells.

In many invertebrate preparations, a striking similarity in the pharmacological sensitivity of responses activated by different transmitters has been observed. As one example, picrotoxin, commonly considered a blocker of γ-

aminobutyric acid (GABA)-activated chloride conductances, is also known to block an ACh-activated excitatory conductance (Marder and Paupardin-Tritsch, 1980), a glutamate-activated chloride conductance increase (Lingle and Marder, 1981), and a glutamate-activated potassium conductance increase (Marder and Paupardin-Tritsch, 1978). In most of such cases, the mechanism of pharmacological blockade is unknown. However, one viewpoint is that in some cases the ionic channel or link between the channel and receptor may be similar among different transmitter-activated responses. Different protein subunits containing transmitter recognition sites may hook up with common ionic channel subunits (Swann and Carpenter, 1975; Carpenter et al., 1977; Ono and Salvaterra, 1981).

The types of information obtained in the present investigation partially address the above issue. If channels gated by different transmitters were in fact structurally different, it would be expected that the structural differences would be manifested in functional differences. Based on both the physiological results presented here and pharmacological data (Lingle, 1981; Lingle et al., 1981; Lingle, 1983), there is no compelling reason to think that the crustacean glutamate and ACh channels, when found in the same cells, are in any way identical molecular entities or share molecular components that can be interchanged. Rather, the present results support the observation that ACh-activated excitatory channels among different phyla appear to share features that differ clearly from the properties of glutamate-activated channels. Such differences would suggest that the distinguishing features of ionic channels activated by different transmitters arose fairly early through evolutionary time and that to a large extent those features have been conserved. Any apparent similarities in particular transmitter-gated ionic channels may simply reflect common structural features that large cation-conducting membrane holes might be expected to share.

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REFERENCES

Anderson, C. R., S. G. Cull-Candy, and R. Miledi. 1978. Glutamate current noise: post-synaptic kinetics investigated under voltage clamp. *J. Physiol. (Lond.)*. 282:219–242.

Anderson, C. R., and C. F. Stevens. 1973. Voltage-clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *J. Physiol. (Lond.)*. 235:655–691.

Anwyl, R. 1977*a*. Permeability of the post-synaptic membrane of an excitatory glutamate synapse to sodium and potassium. *J. Physiol. (Lond.)*. 273:367–388.

Anwyl, R. 1977*b*. The effect of foreign cations, pH and pharmacological agents. *J. Physiol. (Lond.)*. 273:389–404.

Anwyl, R., and P. N. R. Usherwood. 1976. Factors affecting the time course of decay of excitatory postsynaptic currents at a glutamate synapse. *J. Physiol. (Lond.)*. 254:46–47P.

Ascher, P., A. Marty, and R. O. Neild. 1978. Life time and elementary conductance of the channels mediating the excitatory effects of acetylcholine in *Aplysia* neurones. *J. Physiol. (Lond.)*. 278:177–206.

Carpenter, D. O., J. W. Swann, and P. J. Yarowsky. 1977. Effect of curare on responses to different putative neurotransmitters in *Aplysia* neurons. *J. Neurobiol.* 8:119–132.

Clark, R. B., K. A. F. Gration, and P. N. R. Usherwood. 1980. Influence of glutamate and aspartate on time course of decay of excitatory synaptic currents at locust neuromuscular junctions. *Brain Res.* 192:205–216.

Crawford, A. C., and R. M. McBurney. 1976. On the elementary conductance event produced by L-glutamate and quanta of the natural transmitter at the neuromuscular junctions of *Maia squinado*. *J. Physiol. (Lond.)*. 258:205–225.

Cull-Candy, S. G., and I. Parker. 1982. Rapid kinetics of single glutamate-receptor channels. *Nature (Lond.)*. 295:410–412.

Cull-Candy, S. G., and R. Miledi. 1980. Factors affecting the channel kinetics of glutamate receptors in locust muscle fibers. *In* Receptors for Neurotransmitters, Hormones and Pheromones in Insects. D. B. Satelle, L. M. Hall, and J. G. Hildebrand, editors. Elsevier/North-Holland Biomedical Press, New York. 161–173.

Cull-Candy, S. G., R. Miledi, and I. Parker. 1981. Single glutamate-activated channels recorded from locust muscle fibres with perfused patch-clamp electrodes. *J. Physiol. (Lond.)*. 321:195–210.

Dudel, J. 1974. Nonlinear voltage dependence of excitatory synaptic current in crayfish muscle. *Pflügers Arch. Eur. J. Physiol.* 352:227–241.

Dudel, J., W. Finger, and H. Stettmeier. 1977. GABA-induced membrane current noise and the time course of the inhibitory synaptic current in crayfish muscle. *Neurosci. Lett.* 6:203–208.

Dwyer, T. M., D. J. Adams, and B. Hille. 1980. The permeability of the endplate channel to organic cations in frog muscle. *J. Gen. Physiol.* 75:469–492.

Finger, W., and H. Stettmeier. 1980. Efficacy of the two-microelectrode voltage clamp technique in crayfish muscle. *Pflügers Arch. Eur. J. Physiol.* 387:133–141.

Gage, P. W., R. M. McBurney, and D. Van Helden. 1978. Octanol reduces end-plate channel lifetime. *J. Physiol. (Lond.)*. 274:279–298.

Gage, P. W., and D. Van Helden. 1979. Effects of permeant monovalent cations on end-plate channels. *J. Physiol. (Lond.)*. 288:509–528.

Katz, B., and R. Miledi. 1972. The statistical nature of the acetylcholine potential and its molecular components. *J. Physiol. (Lond.)*. 224:665–700.

Katz, B., and R. Miledi. 1973. The binding of acetylcholine to receptors and its removal from the synaptic cleft. *J. Physiol. (Lond.)*. 231:549–567.

Lingle, C. 1980. The sensitivity of decapod foregut muscles to acetylcholine and glutamate. *J. Comp. Physiol.* 138:187–199.

Lingle, C. 1981. Open acetylcholine (ACh)-gated channels blocked by chlorisondamine undergo a transition to a blocked-closed state. *Neurosci. Abstracts.* 7:701.

Lingle, C. 1983. Blockade of cholinergic channels by chlorisondamine on a crustacean muscle. *J. Physiol. (Lond.)*. In press.

Lingle, C., and A. Auerbach. 1983. Comparison of excitatory currents activated by different transmitters on crustacean muscle. I. Acetylcholine-activated channels. *J. Gen. Physiol.* 81:547–569.

Lingle, C., J. Eisen, and E. Marder. 1981. Block of glutamatergic excitatory synaptic channels by chlorisondamine. *Mol. Pharmacol.* 19:349–353.

Lingle, C., and E. Marder. 1981. A glutamate-activated choride conductance on a crustacean muscle. *Brain Res.* 212:481–488.

Magleby, K. L., and C. F. Stevens. 1972. The effect of voltage on the time course of end-plate currents. *J. Physiol. (Lond.).* 223:151–171.

Marder, E., and D. Paupardin-Tritsch. 1978. The pharmacological properties of some crustacean neuronal acetylcholine, gamma-aminobutyric acid, and L-glutamate responses. *J. Physiol. (Lond.).* 280:213–236.

Marder, E., and D. Paupardin-Tritsch. 1980. Picrotoxin block of a depolarizing ACh response. *Brain Res.* 181:223–227.

Marchais, D., and A. Marty. 1979. Interaction of permeant ions with channels activated by acetylcholine in *Aplysia* neurones. *J. Physiol. (Lond.).* 297:9–45.

Neher, E., and B. Sakmann. 1975. Voltage-dependence of drug-induced conductance in frog neuromuscular junction. *Proc. Natl. Acad. Sci. USA.* 72:2140–2144.

Ono, J. K., and P. M. Salvaterra. 1981. Snake α-toxin effects on cholinergic and noncholinergic responses of *Aplysia californica* neurons. *J. Neurosci.* 1:259–270.

Onodera, K., and A. Takeuchi. 1975. Ionic mechanism of the excitatory synaptic membrane of the crayfish neuromuscular junction. *J. Physiol. (Lond.).* 242:295–318.

Onodera, K., and A. Takeuchi. 1978. Effects of membrane potential and temperature on the excitatory post-synaptic current in the crayfish muscle. *J. Physiol. (Lond.).* 276:183–192.

Patlak, J. B., K. A. F. Gration, and P. N. R. Usherwood. 1979. Single glutamate-activated channels in locust muscle. *Nature (Lond.).* 278:643–645.

Rang, H. P. 1981. The characteristics of synaptic currents and responses to acetylcholine of rat submandibular ganglion cells. *J. Physiol. (Lond.).* 311:23–55.

Steinbach, J. H. 1980. Activation of nicotinic acetylcholine receptors. *In* The Cell Surface and Neuronal Function. C. W. Cotman, G. Poste, and G. L. Nicolson, editors. Elsevier/North-Holland Biomedical Press, New York. 119–156.

Stettmeier, H., W. Finger, and J. Dudel. 1978. Synaptic current noise induced by glutamate in crayfish muscle. *Pflügers Arch. Eur. J. Physiol.* 377:R44/174.

Stettmeier, H., J. Dudel, and W. Finger. 1979. Activation of synaptic channels by glutamate in crayfish muscle. *Pflügers Arch. Eur. J. Physiol.* 381:R34/134.

Swann, J. W., and D. O. Carpenter. 1975. The organization of receptors for neurotransmitters on *Aplysia* neurons. *Nature (Lond.).* 258:751–754.

Taraskevitch, P. S. 1971. Reversal potentials of L-glutamate and the excitatory transmitter at the neuromuscular junction of the crayfish. *Biochim. Biophys. Acta.* 241:700–703.

Thieffry, M., and J. Bruner. 1978. Sensibilité des fibres musculaires d'écrevisse au glutamate et au médiateur naturel dans des solutions pauvres en calcium. *C.R. Acad. Sci. (Paris).* 286:1813–1816.