

# Dual Effect of L-Glutamate on Excitatory Postjunctional Membranes of Crayfish Muscle

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**ABSTRACT** Ionophoretically applied glutamate produces different excitatory postjunctional permeability changes on separate muscle fibers in a single crayfish muscle. At junctions on some fibers glutamate appears to increase the conductance to both sodium and potassium whereas at others its effect is primarily on the sodium conductance. These results were obtained by studying the reversal potential for the extracellularly recorded glutamate potential under conditions of varied extracellular sodium and potassium concentrations.

## INTRODUCTION

At crustacean neuromuscular junctions it has been suggested that L-glutamate is the excitatory transmitter (Robbins, 1958, 1959; van Harreveld, 1959; van Harreveld and Mendelson, 1959; Grundfest and Reuben, 1961; Takeuchi and Takeuchi, 1964; Ozeki et al., 1966; Kravitz et al., 1970). A further test of this suggestion is to determine the postjunctional conductance change produced by L-glutamate and to compare it to that produced by the excitatory transmitter. The ionic mechanisms underlying the action of a neurotransmitter can be revealed by studying the reversal potential (Ginsborg, 1967). The reversal potential is that postjunctional membrane potential where the synaptic transmitter produces no net ionic current. Thus, at a given junction, substances producing the same postjunctional conductance change have the same reversal potential. It has been shown that the reversal potentials for L-glutamate and the excitatory transmitter are the same at the crayfish neuromuscular junction (Taraskevich, 1971; Takeuchi and Onodera, 1973). Takeuchi and Onodera (1973) demonstrated that the reversal potential was positive with respect to the bath solution, and since only changes in external sodium but not potassium or chloride concentration were effective in shifting the reversal potential, they concluded that the excitatory junctional current and glutamate current were carried mainly by sodium. Taraskevich (1971), on the other hand, reported positive and negative reversal potentials on different fibers of the same

muscle. The present study was undertaken to determine the ionic basis of the positive and negative glutamate reversal potentials.

#### METHODS

The experiments were done on the abductor of the dactylopodite of the first and occasionally the second walking leg of the crayfish *Orconectes virilis* (Nasco-Steinhilber, Wisc.). The dissection and chamber were essentially the same as described by Dudel and Kuffler (1961). The opener muscle consists of obliquely arranged fibers which originate on the exoskeleton and insert on a central tendon. In the preparations used, the fiber diameters ranged from 70 up to a few hundred microns and were 0.8–2.0 mm in length. The most proximal and most distal fibers are smaller in diameter than those located intermediately. The proximal thin fibers tend to be more numerous on one side of the segment than the other. The first 5–15 of these fibers also have different physiological properties than other fibers also in the segment (see Results). The nerve innervating the opener muscle consists of a single excitatory and single inhibitory axon. These axons branch extensively and form numerous synaptic contacts on each muscle fiber (van Harreveld, 1939; Fatt and Katz, 1953; Dudel and Kuffler, 1961).

For intracellular recording and current passing, microelectrodes were filled with 3 M KCl and 2 M K-citrate, respectively, and had resistances of 5–10 M $\Omega$ . Extracellular recording of glutamate potentials was done with low resistance (3–10 M $\Omega$ ) pipettes filled with 1 M Tris Cl-Tris base (pH 7.0). The bath electrode was a coiled Ag-AgCl wire. To compensate for changes in junctional potentials at the indifferent electrode after solution changes, the intracellular electrode was withdrawn from the cell, the potential nulled with a bucking unit and the electrode reinserted. Tip potentials of the intracellular recording electrode were minimized by carefully breaking the tip of the KCl electrode (Adrian, 1956) on hairs extending from the cuticle. This produced electrode tip potentials of < 5 mV (determined at the end of the experiment by breaking the electrode further on the bottom of the chamber [Agin, 1969]). L-glutamate was applied iontophoretically from microelectrodes filled with either 1 M sodium glutamate (pH 8.0) or 1 M Tris-glutamate (pH 8.0–8.2) having resistances of 80–200 M $\Omega$ . Diffusion of glutamate from the pipettes was prevented by applying a positive breaking voltage to the inside of the glutamate electrodes. In order to reduce the interaction between the current and recording electrodes, the stems of the glutamate and intracellular current electrode were wrapped in aluminum foil which was grounded.

Conventional high input impedance negative capacitance amplifiers were used for potential recording. Current was measured by using a ground clamp (Gage and Eisenberg, 1969). The current necessary to maintain the bath potential at virtual ground was monitored by the potential drop across a 1-M $\Omega$  resistor. The potentials were displayed on a Tektronix R564B memory oscilloscope (Tektronix, Inc., Beaverton, Ore.)

The method used to determine glutamate reversal potentials is illustrated in Fig. 1 A. A muscle fiber was impaled with two electrodes one to pass current to set, the other to record the membrane potential. The distance between these intracellular electrodes was always greater than 500  $\mu$ m. Glutamate potentials were recorded extracellularly with a microelectrode from a glutamate-sensitive region located within

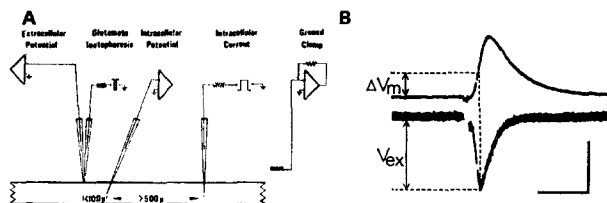


FIGURE 1. (A) Diagram depicting typical electrode positioning during experiment. Glutamate iontophoresed at site of extracellular recording. Intracellular potential recorded within  $100\ \mu\text{m}$  of extracellular recording region. Intracellular current electrode inserted at least  $500\ \mu\text{m}$  from site of intracellular recording. (Note that  $\mu$  in figure stands for  $\mu\text{m}$ .) (B) Diagram demonstrating method used to measure potentials.  $V_{\text{ex}}$ : amplitude of extracellular glutamate potential measured from base line to peak potential.  $\Delta V_m$ : difference between membrane potential and internal polarization at peak external potential. Vertical calibration:  $10\ \text{mV}$  for intracellular trace,  $400\text{-}\mu\text{V}$  for extracellular trace. Horizontal calibration:  $400\ \text{ms}$ .

$100\ \mu\text{m}$  of the intracellular recording electrode. The close proximity of the sites of extracellular and intracellular recording ensured accurate measurement of membrane potential at the glutamate spot under investigation. Localization of glutamate-sensitive regions was accomplished by using one of two techniques. The first was to stimulate the excitatory axon at a frequency of  $5\text{--}10\ \text{s}^{-1}$  and find a place on the muscle fiber where the extracellularly recorded nerve spike was followed by a negative synaptic potential (Dudel and Kuffler, 1961). The glutamate pipette was then lowered to the same spot and positioned to give a relatively fast-rising extracellular as well as intracellular glutamate potential. The second method was to record intracellularly and search along the surface of a muscle fiber with a glutamate pipette for a spot where iontophoretic ejection of glutamate produced an intracellular depolarization (Takeuchi and Takeuchi, 1964). The region of the fiber at the site of iontophoresis was visualized with the aid of a Wild dissecting microscope ( $\times 50$ ) and the extracellular recording electrode was lowered to this region. It was usually necessary to then reposition the glutamate pipette and adjust the amplitude and duration of the iontophoretic current until a relatively fast extracellular potential (total duration  $500\ \text{ms}$  or less) of approximately  $400\text{-}\mu\text{V}$  amplitude was obtained. The iontophoretic dose of glutamate necessary to produce such a potential often varied from fiber to fiber and depended on the placement of the glutamate and external recording electrodes. There was however no consistent difference in the dose of glutamate used on fibers in different regions of the opener muscle. Since the second procedure did not require nerve stimulation for extracellular localization of glutamate-sensitive regions, it was not necessary to maintain either nerve conduction or transmitter release from nerve terminals. Takeuchi and Takeuchi (1964) have shown that the glutamate-sensitive areas of crayfish muscle fibers are localized at the synaptic regions and in some cases in the present experiments, spontaneous extracellular junctional potentials were clearly visible at glutamate spots localized externally without the aid of nerve stimulation. Glutamate was pulsed once every  $8\text{--}20\ \text{s}$  and the extracellular and intracellular glutamate potentials were photographed. The muscle fiber potential was changed by a current pulse of  $0.6\text{--}1.2\text{-s}$

duration. Each change in membrane potential was preceded and followed by a control at the resting potential.

The amplitude of the extracellular glutamate potential was measured from the base line preceding the glutamate pulse to the peak of the external response (Fig. 1 B). In the present experiments, the amplitude of the intracellular glutamate potentials ranged between 2 and about 20 mV. To take into account the potential change produced by glutamate application, the level of intracellular potential was measured at the peak of the extracellular glutamate potential (Fig. 1 B). This method thus gave an accurate measurement of membrane polarization at peak external potential. The amplitude of the extracellular glutamate responses at various levels of membrane potential were plotted as the fraction of control amplitude at the resting potential. The reversal potential was determined by extrapolation of the line fitted to the points, by linear regression, to that membrane potential at which the fraction of control amplitude was zero. Normalizing the responses was useful for two reasons. First, since the amplitude of the responses at altered levels of membrane potential was compared to the amplitude of controls immediately preceding and following the change in potential, variation in the response amplitude due to gradual changes in the absolute magnitude of the control response during the experiment was taken into account. Second, data from different experiments could be compared directly on the same graph by choosing a membrane potential which was common to the experiments to be included in the comparison, and then assigning that potential a value of 1.0 for the fraction control amplitude, and shifting the rest of the points relative to it. This procedure changes the slope of the data (the slope, however, has no meaning in terms of conductance as it does in voltage clamp), but the X-intercept remains the same. Only experiments in which the correlation coefficient of the linear regression line was  $>0.93$  were used.

Intracellular  $K^+$  activity was measured by ion-specific liquid ion exchanger microelectrodes (Walker, 1971). The method of fabrication and calibration of the  $K^+$ -specific electrodes as well as the procedure used to measure intracellular  $K^+$  activity were essentially the same as described by Brown et al. (1970).

The normal bathing solution had the following ionic composition (in mM): NaCl, 195; KCl, 5.4;  $CaCl_2$ , 13.5;  $MgCl_2$ , 2.6; NaOH, 10; Tris-maleate to make pH 7.0–7.5. Sodium-deficient solutions were prepared by replacing NaCl with either equiosmotic amounts of sucrose or equimolar concentrations of choline-Cl. The potassium concentration was altered by either adding or deleting KCl. No attempt was made to maintain the product of potassium and chloride concentrations constant.  $MnCl_2$  was used as a substitute for  $CaCl_2$  in many of the bathing solutions. The use of Mn aided in determination of the reversal potential by inhibiting muscle contraction (Orkand, 1962) and thus permitted the membrane potential to be depolarized over a wider range without producing movement. In solutions containing normal Na concentration, there was no observable difference between the reversal potential determined in Ca or Mn solutions. Mn was not used as a Ca substitute in low Na solutions because it appeared to increase the difficulty in maintaining glutamate potentials of constant amplitude and time-course.

The bath consisted of two interconnected chambers and had a total volume of

about 10 ml. The solution level was kept constant by a U-tube connected with a vacuum source. When solutions were changed, both chambers of the bath were first drained from the bottom and the new solution was flowed in from the back chamber. The bath was voided and refilled twice and then superfused at constant level with at least five bath volumes of the solution. A minimum of 15 min was allowed for equilibration in each solution. All experiments were done at room temperature (18–20°C).

## RESULTS

### *Glutamate Reversal Potential*

The effect of membrane potential on the amplitude of the extracellular glutamate potential recorded on a fiber in the proximal portion of the opener muscle is shown in Fig. 2. There is a nearly linear relation between membrane potential and amplitude of the extracellular glutamate potential. In the experiment illustrated in Fig. 2, the reversal potential, estimated by extrapolation, was  $-10$  mV. The mean reversal potential obtained from fibers in the proximal region of the muscle was  $-0.2 \pm 2.0$  mV (SE of mean of 13 experiments).

Experiments on fibers located in the middle-to-distal portion of the opener muscle produced quantitatively different results than those mentioned above.

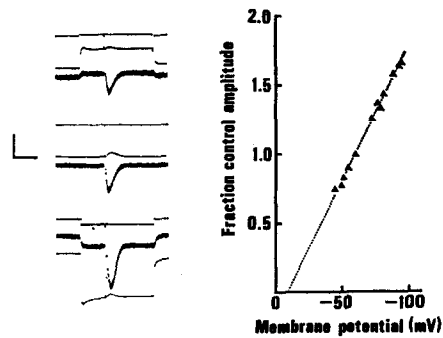


FIGURE 2

FIGURE 2. Relation between amplitude of extracellular glutamate potential (plotted as fraction control amplitude) and membrane potential from fiber in proximal portion of opener muscle. Traces: extracellular glutamate potential at various levels of membrane potential. Top traces monitor current for changing membrane potential and glutamate ejection. Intracellular potential, second trace in top and middle set of records, bottom trace in lower set. Extracellular potential, third trace in top and middle set, second trace in lower set. Vertical calibration:  $2 \times 10^{-7}$  A for current traces, 20 mV for intracellular traces, and 400  $\mu$ V for extracellular traces. Horizontal calibration: 400 ms. Resting potential  $-65$  mV ( $Mn^{++}$  solution). Extrapolated reversal potential,  $-10$  mV.

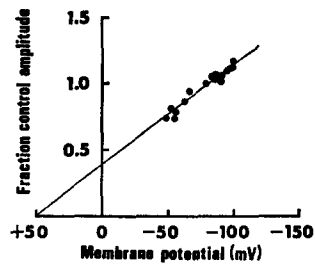


FIGURE 3

FIGURE 3. Relation between membrane potential and amplitude of extracellular glutamate potential (plotted as fraction control amplitude) from a fiber in the middle portion of opener muscle. Extrapolated reversal potential,  $+50$  mV. Resting potential,  $-79$  mV.

The relation between amplitude of the glutamate potential (plotted as fraction control amplitude) and membrane potential for one such fiber is shown in Fig. 3. The extrapolated reversal potential obtained from these fibers was  $50.6 \pm 3.3$  mV (mean  $\pm$  SE of mean of nine experiments). Fibers having a reversal potential of 0 mV were never found in the middle-to-distal portion of the muscle; however, slightly positive reversal potentials (20–30 mV) were on occasion encountered in the proximal portion. The fibers located between these two regions were not studied extensively, but the few experiments that were done on these fibers yielded intermediate values of reversal potential. Since the borders between these intermediate fibers and the proximal fibers on one side and the middle-to-distal fibers on the other side could not be determined visually with any certainty, the majority of experiments were performed on fibers that were either clearly proximal or well into the middle-to-distal portion of the muscle. This selection procedure yielded two distinct and fairly homogeneous populations of fibers based on the level of reversal potential and thus simplified data analysis. The existence of a gradient of reversal potentials from 0 to 50 mV in the proximal to middle-to-distal direction is at present problematical.

#### *Glutamate-Induced Permeability*

The different levels of glutamate reversal potential could arise from different ionic gradients across the muscle fiber membranes or from differences in the glutamate-induced permeability properties of the postjunctional membranes (Ginsborg, 1967). The first possibility was tested in part, by measuring intracellular  $K^+$  activity ( $a_K^i$ ) of the two fiber groups with  $K^+$ -specific liquid ion-exchanger microelectrodes (Brown et al., 1970; Walker, 1971). The results of these experiments are shown in Table I. There was no difference between  $a_K^i$  in proximal and middle-to-distal fibers ( $P \gg 0.05$ ). Since only intracellular  $K^+$  activity was measured, the different reversal potentials could result from differences in the distribution of some other ion or ions (e.g.,  $Na^+$ ) across the fiber membranes; however, as is shown below, asymmetric ion distributions

TABLE I  
INTRACELLULAR POTASSIUM ACTIVITY IN PROXIMAL AND MIDDLE-TO-DISTAL MUSCLE FIBERS

	$E_m$	$a_K^i$	$E_K$	$n$
	$-mV$	$mM$	$-mV$	
Proximal fibers	$78.1 \pm 1.3$	$118.1 \pm 9.4$	$85.5 \pm 1.7$	11
Middle-to-distal fibers	$77.0 \pm 0.9$	$121.7 \pm 5.0$	$86.1 \pm 0.9$	43

Mean  $\pm$  SEM  $n$  is number of fibers.

cannot fully account for the different reversal potentials. The second possibility, namely, that there are differences in glutamate-induced permeability properties of the postjunctional membranes was tested by determining the glutamate reversal potential in solutions of varied ionic composition. Since the reversal potentials of the natural excitatory transmitter and L-glutamate are the same (Taraskevich, 1971; Takeuchi and Onodera, 1973), presumably, the results obtained from studying glutamate action are applicable to the natural transmitter as well (see also Takeuchi and Onodera, 1973).

**Na IONS** The  $\text{Na}^+$  concentration of the normal bathing solution was varied between 205 and 20.5 mM by replacing NaCl with either sucrose or choline-chloride. In low sodium solutions it was often difficult to maintain glutamate potentials of constant amplitude and time-course. This appeared to result from an enhancement of the process of desensitization (Takeuchi and Takeuchi, 1964). Smaller doses of glutamate and longer intervals between doses had to be used in sodium-deficient solutions in order to avoid desensitization.

The effect of reducing the external  $\text{Na}^+$  concentration on the level of the positive reversal potential is shown in Fig. 4. Decreasing the external sodium concentration from 205 mM (filled circles) to 50 mM (open triangles) caused the reversal potential to shift about 35 mV. In contrast, the same change in sodium concentration resulted in about a 15-mV shift in the level of the zero reversal potential (Fig. 5). The value of the reversal potential at each altered sodium concentration appeared to be independent of the substitute used. In the sucrose-substituted sodium-deficient solutions the membrane potential under-

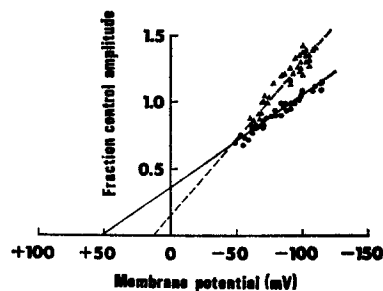


FIGURE 4

FIGURE 4. Effect of external sodium concentration on positive glutamate reversal potential. Filled circles: compiled data from three fibers in 205 mM  $\text{Na}^+$  solution. Open triangles: compiled data from three fibers after soaking in 50 mM  $\text{Na}^+$  solution.

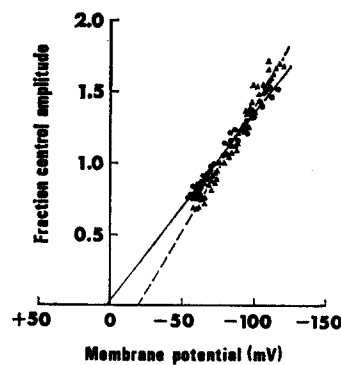


FIGURE 5

FIGURE 5. Effect of sodium concentration on zero glutamate reversal potential. Filled circles: compiled data of three fibers in 205 mM  $\text{Na}^+$  solution. Open triangles: compiled data of three fibers after soaking in 50 mM  $\text{Na}^+$  solution.

goes a transient depolarization due to the decrease in the chloride equilibrium potential (Zachar et al., 1964). The fibers were equilibrated long enough for the potential to return to the original resting level. Although the effect of changes in external chloride concentration on the reversal potential were not studied in these experiments, Takeuchi and Onodera (1973) have found that large changes in external chloride had no appreciable effect on the glutamate reversal potential.

The reversal potentials obtained in various external sodium concentrations are shown in Fig. 6. The positive reversal potential (filled squares) moved to

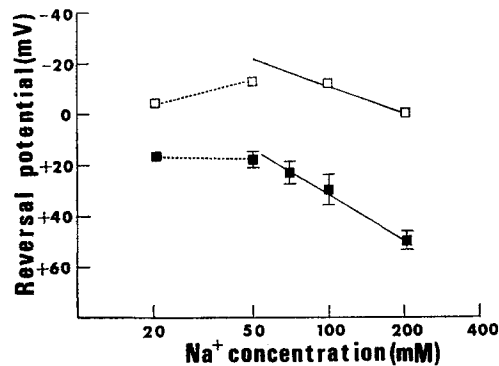


FIGURE 6. Glutamate reversal potential on proximal fibers (open squares) and middle-to-distal fibers (filled squares) plotted against external sodium concentration, semilogarithmic scale (mean  $\pm$  SE of the mean, where SE is greater than size of point). Solid line through open squares represents relation expected if  $\Delta g_{Na}/\Delta g_K$  is 1.70 (see Discussion). Solid line through filled squares represents relation expected if glutamate-induced conductance change involves only  $\Delta g_{Na}$  (slope 58 mV per 10-fold change in  $[Na^+]_o$ ). Dotted line used to indicate marked deviation of point in 20.5 mM  $Na^+$  from relation obtained in higher  $Na^+$  concentrations.

less positive values and the zero reversal potential (open squares) moved to negative values of membrane potential as the sodium concentration is decreased from 205 to 50 mM. The shift in the positive reversal potential over this range of  $Na^+$  concentrations followed closely the shift in the equilibrium potential for  $Na^+$  predicted by the Nernst equation. The shift in the zero reversal potential was less marked. In 20.5 mM  $Na^+$  the reversal potential of both fiber types was at a more positive level of membrane potential than expected by the relation obtained in higher sodium concentrations. This finding is considered in more detail in the Discussion.

**K IONS** Membrane potential and membrane resistance are affected by changes in external K concentration (Fatt and Ginsborg, 1958). When the  $K^+$  concentration was decreased below normal, membrane potential and resistance increased. Furthermore, in the reduced  $K^+$  solutions most fibers ex-



hibited a potential range of 20–40 mV over which it was not possible to obtain a stable level of depolarization during an applied pulse of constant current (Fig. 7). Within this range, membrane resistance, measured by the amplitude of hyperpolarizing potentials produced by passing a constant inward current through a second current-passing electrode, was much increased above what it was at the resting potential. In high  $K^+$  solutions membrane resistance was slightly decreased. The amplitude of the extracellular glutamate potential, however, was not affected by these changes in resistance and consequently varied linearly with membrane potential.



FIGURE 7. Four superimposed electrotonic potentials from muscle fiber soaked in low (1.0 mM)  $K^+$  solution. Resting potential,  $-115$  mV. Vertical calibration:  $4 \times 10^{-7}$  A for current injection (upper traces) and 20 mV for potential (lower traces). Horizontal calibration: 400 ms.

Fig. 8 shows an example of the reversal potentials determined from fibers in the middle region of the muscle in solutions containing 10.8 mM  $K^+$  (open circles) and 1.0 mM  $K^+$  (filled circles). The level of reversal is unaffected by the change in  $K^+$  concentration. In contrast, the level of the zero reversal is altered by changes in the external  $K^+$  concentration. Fig. 9 is an example of the reversal potential determined on fibers in the proximal portion of the muscle in 10.8 mM  $K$  (open circles) and 1.0 mM  $K$  (filled circles). In the high  $K$  solution, the reversal potential was  $4.3 \pm 2.8$  mV (mean  $\pm$  SE of mean of 10 experiments) and in the low  $K$  solution was  $-11.4 \pm 3.8$  mV (mean  $\pm$  SE of mean of 10 experiments). The relation between external  $K^+$  concentration and reversal potential on both proximal and middle-to-distal fibers is shown in Fig. 10. Altering the  $K$  concentration is without effect on the positive reversal potentials of the middle-to-distal fibers (filled circles). The zero reversal potentials of the proximal fibers, on the other hand, are shifted to more negative values of membrane potential as the  $K^+$  concentration is decreased from 10.8

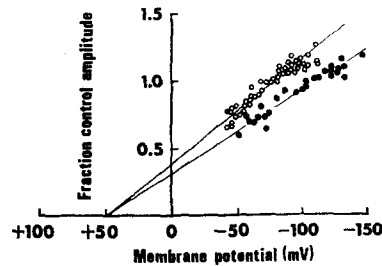


FIGURE 8. Effect of external potassium concentration on glutamate reversal potential of middle-to-distal fibers. Open circles: compiled data of three fibers obtained in 10.8 mM  $K^+$  solution. Filled circles: compiled data of three fibers obtained in 1.0 mM  $K^+$  solution.

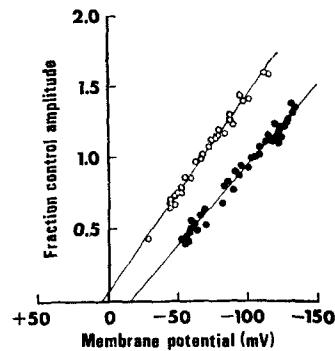


FIGURE 9

FIGURE 9. Effect of external potassium concentration on glutamate reversal potential in proximal fibers. Filled circles: compiled data from three fibers in 1 mM  $K^+$  solution. Open circles: compiled data from three fibers in 10.8 mM  $K^+$  solution.

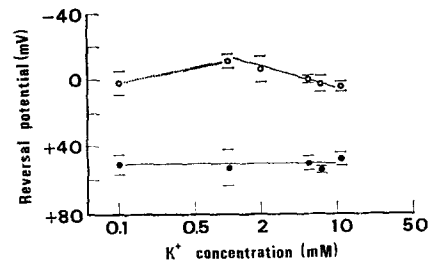


FIGURE 10

FIGURE 10. Glutamate reversal potential plotted against external potassium concentration semilogarithmic scale (mean  $\pm$  SEM). Open circles: proximal fibers, solid line represents expected relation if  $\Delta g_{Na}/\Delta g_K$  is 1.70 (see Discussion). Dotted line used to indicate deviation of point in 0.1 mM  $K^+$  from relation obtained in higher  $K^+$  concentrations. Filled circles: middle-to-distal fibers (mean  $\pm$  SE of mean).

to 1.0 mM. In 0.1 mM  $K^+$  the reversal potential on proximal fibers was about the same as in normal  $K^+$  solution (5.4 mM  $K$ ) perhaps indicating a concentration-dependent change in the  $\Delta g_{Na}/\Delta g_K$  ratio, but this was not pursued further in the present experiments.

#### DISCUSSION

There are several cases in which transmitters and suggested transmitter substances produce different conductance changes when applied to different cells. In vertebrates, for example, ACh increases sodium and potassium conductance in skeletal muscle (Takeuchi and Takeuchi, 1960) producing excitation,

but in cardiac muscle, it increases only potassium conductance (Burgen and Terroux, 1953), resulting in inhibition; however, these synaptic actions are mediated by different nerves. In *Aplysia* abdominal ganglion, it has been found that the transmitter released from different branches of a single neuron can produce an increase in sodium conductance leading to excitation, chloride conductance leading to inhibition, or both sodium and chloride conductance leading to dual excitation-inhibition in different follower cells, and these responses are mimicked by ACh application (Blankenship et al., 1971). Clearly, the mode of synaptic action is determined by the properties of the postjunctional membrane. Furthermore, the observations reported here suggest that in the crayfish opener muscle excitation mediated by a single axon can result from different ionic mechanisms.

On the majority of fibers in the opener muscle the glutamate reversal potential is positive with respect to the bath solution (see also Takeuchi and Onodera, 1973); however, on the first few proximal fibers it is about 0 mV. Since it was not possible to actually reverse the polarity of the glutamate potentials due to the large currents necessary to change membrane potential (Takeuchi and Takeuchi, 1964) and contraction at large depolarizations, the reversal potential was estimated by extrapolation of the linear relation between membrane potential and amplitude of external glutamate potential. Because of the long distance over which these relations were extrapolated, especially in the case of the positive reversal potentials, the absolute value of reversal may be in error. In other preparations, differences in actual reversal potential and that estimated by extrapolation from a limited range of membrane potentials have been observed at presumably isolated single synaptic regions (Miledi, 1969; Feltz and Mallart, 1971; Mallart and Trautman, 1973). The differences are produced by nonlinearities in the membrane potential-response amplitude relation at large membrane polarizations. Over the range of membrane potentials used in the present experiments (ranging from  $-30$  to  $-150$  mV) nonlinearities were not observed. Furthermore, the present analysis of glutamate-induced conductance is based primarily on measurement of changes in reversal potential produced by altering external ion concentrations rather than absolute values of reversal potential and differential changes could be measured by the method of extrapolation.

The shift in the positive reversal potential with changes in external sodium concentration from 205 to 50 mM followed closely the expected shift in the equilibrium potential for sodium from the Nernst equation. No appreciable change in the positive reversal potential occurred when external potassium concentration was altered. In 20.5 mM Na, the reversal potential was at a more positive level of potential than expected by the relation from 205 to 50 mM Na (Fig. 6, filled squares). This flattening out may be due to an increase in conductance of the junctional membrane to an ion (e.g.,  $\text{Ca}^{++}$ ) with a

positive equilibrium potential. A contribution of  $\text{Ca}^{++}$  to the glutamate current has been demonstrated by Takeuchi and Onodera (1973) in sodium-free solution. However, in normal bathing solution, the action of glutamate on the middle-to-distal fibers is to increase the permeability of the junctional membrane mainly to sodium and probably not to potassium ions, producing a positive reversal potential.

Changes in the external sodium or potassium concentration were effective in shifting the zero reversal potential, suggesting that glutamate increased the permeability of the junctional membranes of proximal fibers to both sodium and potassium ions. This permeability change is qualitatively similar to that produced by ACh at the frog neuromuscular junction (Takeuchi and Takeuchi, 1960). If it is assumed that  $\text{Na}^+$  and  $\text{K}^+$  are the only ions carrying glutamate current, then by applying the equivalent circuit analysis of Takeuchi and Takeuchi (1960) a  $\Delta g_{\text{Na}}/\Delta g_{\text{K}}$  ratio for the junction membranes of proximal fibers can be calculated from the following equation (from Maeno, 1966):

$$\Delta g_{\text{Na}}/\Delta g_{\text{K}} = (E_{\text{K}} - E_{\text{rev}})/(E_{\text{rev}} - E_{\text{Na}}), \quad (1)$$

where  $E_{\text{rev}}$  is the reversal potential and all other symbols have their usual meaning. Assuming that in normal bathing solution the level of positive reversal potential is indicative of the sodium equilibrium potential for all the fibers of the opener muscle (see preceding discussion, however)  $E_{\text{Na}}$  would be 50.6 mV,  $E_{\text{K}}$  is  $-86$  mV (Table I), and  $E_{\text{rev}}$  is  $-0.2$  mV, then  $\Delta g_{\text{Na}}/\Delta g_{\text{K}}$  is 1.70. The solid lines through the open symbols in Fig. 6 and Fig. 10 represent the expected relation between reversal potential and external  $\text{Na}^+$  and  $\text{K}^+$  concentration, respectively, calculated from the following equation using a conductance ratio of 1.70 (from Eq. 2 of Takeuchi and Takeuchi, 1960):

$$E_{\text{rev}} = (E_{\text{K}} + (\Delta g_{\text{Na}}/\Delta g_{\text{K}})E_{\text{Na}})(1 + \Delta g_{\text{Na}}/\Delta g_{\text{K}})^{-1}. \quad (2)$$

The observed change in the zero reversal potential with changes in external  $\text{K}^+$  follows fairly well the predicted change (Fig. 10); however, the change in the zero reversal potential with changes in external  $\text{Na}^+$  concentration does not (Fig. 6). When the external  $\text{Na}^+$  concentration was below 100 mM the observed reversal potential was at a less negative membrane potential than predicted by Eq. 2 (Fig. 6, open symbols). This deviation in low  $\text{Na}^+$  concentrations could result from either a concentration-dependent change in the  $\Delta g_{\text{Na}}/\Delta g_{\text{K}}$  ratio, or an increase in the conductance of another ion, for example  $\text{Ca}^{++}$ , in the low  $\text{Na}^+$  solutions (Takeuchi, 1963). Since there was no effect on the zero reversal potential when  $\text{Ca}^{++}$  was removed from a bathing solution containing normal  $\text{Na}^+$  and  $\text{K}^+$  concentration (Taraskevich, unpublished observation), it appears that in normal bathing solution the action of glutamate on the junctional membranes of proximal fibers is to increase the conductance primarily to  $\text{Na}^+$  and  $\text{K}^+$  resulting in a zero reversal potential.

Separate conductance channels for sodium and potassium have been proposed in the frog endplate (Maeno, 1966; Maeno et al., 1971; Deguchi and Narahashi, 1971; see, however, Kordaš, 1970; Mallart and Trautmann, 1973). In this context, the present observations could be explained by postulating that in the junctional membrane of middle-to-distal fibers the  $K^+$  conductance channel is absent. On the other hand, it is not necessary to propose separate channels; instead a single channel with anionic sites would suffice if the electric field strength of the sites is different in proximal as compared to middle-to-distal fibers. A channel with a low field strength would select cations in the order of their hydrated radii, while one with high field strength would select cations in the order of their ionic radii, the smallest being the most preferred in each case (see Diamond and Wright, 1969). Thus, if the field strength of the anionic sites in the conductance channels on proximal fibers was intermediate to low, whereas on middle-to-distal fibers it was high, the present results could be accommodated by a single-channel model.

In addition to differences in postjunctional permeability properties reported here, differences in release characteristics of the presynaptic axon terminals located on different fibers of the opener muscle have been reported (Bittner, 1968). Similar differences in facilitation properties for terminals of a single axon have also been reported on different fibers in a muscle of the lobster walking leg (Frank, 1973). Frank suggested that the differences in facilitation characteristics are regulated and maintained by a myotrophic influence. On the other hand, Atwood (1973) has proposed a neurotrophic influence to explain the observed diversity in properties of crustacean nerve and muscle. The present results appear to be best explained by assuming that the individual muscle fibers specify the permeability properties of their junctional membranes; however, the role of the axon in inducing, maintaining, or influencing the postjunctional membrane is as yet unknown.

I am indebted to Dr. R. K. Orkand for his guidance and encouragement throughout the course of these experiments, to Dr. S. Hagiwara and Dr. H. Bracho for their helpful comments on the manuscript, and to Dr. C. L. Ortiz for the many useful discussions. Special thanks to Dr. A. M. Brown for the generous use of his facilities making possible the ion-selective electrode studies. This paper is in part from a dissertation submitted to the University of California Los Angeles in partial satisfaction of the requirements for the degree Doctor of Philosophy in Biology. This work was supported by USPHS Research Grant 08346 to R. K. Orkand and Training Grant 05670.

*Received for publication 30 September 1974.*

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