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Reversal potentials of L-glutamate and the excitatory transmitter at the neuromuscular junction of the crayfish

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SUMMARY

Extracellular recording was utilized to determine the reversal potentials for L-glutamate and the excitatory transmitter at the neuromuscular junction of the crayfish *Orconectes virilis*. The reversal potentials for both substances were the same. Thus, L-glutamate remains the best candidate as the naturally occurring transmitter at this synapse.

Low concentrations of L-glutamate applied to crustacean muscle fibers cause depolarization and contraction[1–4]. The glutamate-sensitive areas of crayfish muscle fibers are localized at the synaptic region[5]. These data suggest that L-glutamate is the excitatory transmitter. One necessary, though not sufficient, requirement to support this suggestion is that glutamate produces the same post-junctional permeability change as the naturally occurring transmitter. At the squid giant synapse, Miledi[6] has found that the permeability change produced by L-glutamate and the natural transmitter are not the same. This result tends not only to rule out glutamate as the transmitter at that synapse, but also infers that two excitatory chemicals acting at the same synaptic region can lead to different postsynaptic permeability changes. In this study the reversal potential for the change in ionic conductance produced by the excitatory transmitter and glutamate application was determined at the crayfish neuromuscular junction.

The abductor muscle of the dactylopodite of the first or second walking leg of the crayfish *Orconectes virilis* was dissected and mounted in the manner described by Dudel and Kuffler[7]. Two electrodes filled with 2 M potassium citrate were inserted into a muscle fiber; one to pass current to set and the other to record the membrane potential. Synaptic activity was recorded extracellularly with low resistance (1–6 MΩ) 3 M sodium propionate electrodes. L-Glutamate was applied iontophoretically from micropipettes filled with 2 M sodium glutamate (pH 8.0).

The reversal potential is that level of membrane potential where the change in

ionic conductance does not lead to a potential change. Substances which produce the same change in membrane permeability have identical reversal potentials[8]. The most direct measurement of the reversal potential is to find that level of membrane potential where the synaptic potential reverses polarity. For excitatory transmitters, this measurement is not always feasible, and few excitatory synaptic potentials have been reversed[8]. A less direct, but more usual, practice is to record the change in amplitude of the intracellular synaptic potential over a limited range of membrane potentials. The reversal potential is determined from the relation between amplitude of synaptic potential and membrane potential and extrapolating to zero synaptic potential.

Crayfish muscle fibers have two properties which make the usual analysis difficult. First, the membrane resistance of some of the muscle fibers is dependent on potential in an unusual manner. Hyperpolarization decreases membrane resistance, whereas depolarization initially increases and then decreases resistance. The effect of these changes in membrane resistance on intracellularly recorded glutamate potentials is shown in Fig. 1. Hyperpolarization cuases a decrease in the amplitude of the glutamate potential even though the driving force for the ionic currents produced by glutamate has been increased (see below). By contrast, small depolarizations (up to 15 mV) result in an increase in the glutamate potential. Increased depolarization eventually leads to a decrease in the amplitude of the glutamate potential but the relation between glutamate potential and membrane potential is non-linear. Similar results have been described in crayfish muscle fibers bathed in hyperosmotic solutions[9] and neurons of Aplysia[10]. Thus, determination of the reversal potential by extrapolation of the intracellular potential is invalid[6]

The second problem in using intracellular recording arises from the multiterminal innervation of these muscle fibers by the excitatory neuron[11]. When the potential of the fiber is altered by passing current at a single point, synapses at a distance from the intracellular electrode will be at a different level of membrane potential. This results in an estimate of the reversal potential which is too depolarized[12]. For these reasons the technique of extracellular recording[13,7] was employed in these experiments,

The extracellularly recorded synaptic potential is highly localized spatially to a region of 50 μm or less in diameter around the synapse[13]. Once a synaptic region was located extracellularly, the intracellular electrode was placed within 100 μm of the extracellular electrode. This allowed an accurate recording of the membrane potential at the

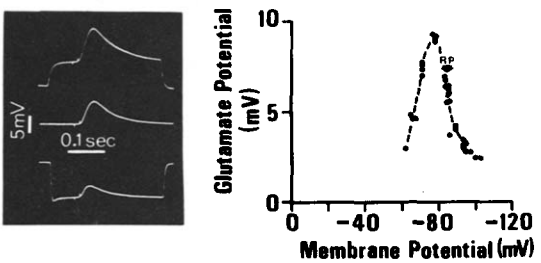

Fig. 1. Effect of membrane potential on amplitude of the intracellularly recorded glutamate potential. Glutamate potentials produced by iontophoresis of a constant dose of L-glutamate to a synaptic spot. Middle trace is glutamate induced depolarization at the resting potential (RP, −83 mV). Top trace shows the increase in the glutamate potential produced by a small depolarization of the muscle fiber. Note, the glutamate potential decreases in amplitude when the fiber is depolarized by more than about 15 mV (graph). Bottom trace shows the decrease in amplitude of the glutamate potential when the muscle fiber is hyperpolarized. Graph plots the amplitude of the glutamate potential at various levels of membrane potential.

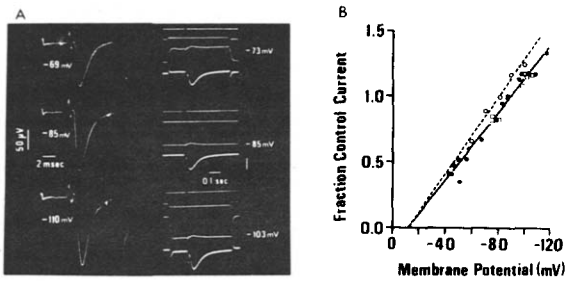

Fig. 2. A. Left column, average of 100 extracellularly recorded synaptic currents at three levels of membrane potential (resting potential −85 mV). Traces in column on right, single sweeps during glutamate application. Top traces, glutamate injection current. Second traces, monitored current passed to change membrane potential. Third traces, intracellular potential. Bottom traces, extracellularly recorded glutamate currents. Vertical bar equals (top to bottom) $5 \cdot 10^{-7}$ A, $1 \cdot 10^{-6}$ A, 10 mV, and 0.5 mV, respectively. B. Relation of extracellularly recorded currents (plotted as fraction of control) to membrane potentials (different experiment than A). ○, excitor currents; □, glutamate currents; ●, glutamate currents in Ringer in which all of the Ca^{2+} was replaced with Mn^{2+}. Extrapolated equilibrium potential is −12 mV. Resting potential −83 mV. Straight lines fitted to points by linear regression. Correlation coefficients for dashed and solid lines, 0.99 and 0.98, respectively.

synaptic region under investigation. Furthermore, the resistance of the ionic pathways in the synaptic membrane is much greater than the input resistance of the muscle fiber[7,13]; the amplitude of the extracellularly recorded currents is then independent of membrane resistance and should vary linearly with membrane potential.

Fig. 2B is a plot of the relation between the extracellularly recorded currents (plotted as fraction of control) and the membrane potential of the fiber. Because the quantal content of the excitor is low[7], the extracellular responses had to be averaged. For a control, 100 or 200 stimuli at 10/sec were averaged with a Northern Scientific NS-550 series digital memory oscilloscope and simultaneously photographed. The potential of the muscle fiber was then changed by a d.c. current through the current passing electrode about 1 mm from the potential recording electrode. At the new level of membrane potential the responses were again averaged and photographed. Fig. 2A, left column, is an example of the averaged extracellular currents at three different membrane potentials; each run was followed by a control. If the quantal contents before, during, and after the run were the same (as judged by the fraction of failures[7]) and the averaged controls on both sides of the run were the same, the averaged amplitude of the run was plotted as the fraction of the control current (Fig. 2B, open circles). After a variable number of runs were made with the excitor, a glutamate pipette was lowered to the same spot. The pipette was positioned to give a fast rising extracellular as well as intracellular glutamate potential which was approximately the same amplitude as the synaptic potentials. Glutamate was pulsed once every 8 sec and the amplitude of the extracellular glutamate currents was photographed and measured (Fig. 2A, right column). The membrane potential was changed by a current pulse of 0.6 to 1 sec duration. Each change in membrane potential was preceded and followed by a control at the resting potential. Glutamate currents were recorded in normal calcium and in a Ringer in which all of the Ca^{2+} was replaced with Mn^{2+}. Since Mn^{2+} inhibits contraction of crayfish muscle fibers[14], the muscle fiber could be depolarized over a wider range without producing contraction. However, in the Mn^{2+} Ringer, evoked transmitter release was abolished and consequently, only glutamate reversal potentials

could be determined. There was no difference in reversal potentials in Ca^{2+} and Mn^{2+} Ringer.

As seen in Fig. 2B, the extrapolated reversal potential for both the excitor and glutamate is the same; about −12 mV. The reversal potential for the excitor from 5 experiments was −4.4 ± 8.5 S.D. (range −12 to +12 mV), for glutamate from 6 experiments it was −2.5 ± 8.3 S.D. (range −12.7 to +9.1 mV). In a few other experiments, the extrapolated reversal potentials for the excitor and glutamate were at positive levels of membrane potential statistically outside of the normal population. In one such experiment, values for the excitor and glutamate were obtained from the same fiber; both were positive and outside the normal range. The average and standard deviation of these positive reversal potentials for the excitor were +34 ± 19 mV (2 experiments) and for glutamate +53 ± 14 mV (4 experiments). It is not clear at this time if the positive and negative reversal potentials result from variations in ionic gradients across two populations of fibers or from different ionic conductances caused by the transmitter on different fibers. The positive reversal potentials could be an artifact due to simultaneously recording extracellular currents from two adjacent fibers while altering the membrane potential of only one of them.

In insect muscle fibers the reversal potential for glutamate application and miniature end-plate potentials were found to be identical[15]. At the crayfish neuromuscular junction, Takeuchi and Takeuchi[5] suggested that the reversal potential for glutamate application was about zero. Grundfest and Reuben[16], using intracellular recording and a Ringer where Cs^+ replaced K^+, determined a reversal potential of −20 mV for the excitor in lobster muscle fibers. The results presented in this paper demonstrate that the reversal potential for glutamate and the excitor are the same. Thus, glutamate remains the best candidate as the naturally occurring transmitter at the crayfish neuromuscular junction.

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REFERENCES

1 J. Robbins, *Anat. Rec.*, 132 (1958) 492.
2 J. Robbins, *J. Physiol.*, 148 (1959) 39.
3 A. van Harreveld, *J. Neurochem.*, 3 (1959) 300.
4 A. van Harreveld and M. Mendelson, *J. Cell. Comp. Physiol.*, 54 (1959) 85.
5 A. Takeuchi and N. Takeuchi, *J. Physiol.*, 170 (1964) 296.
6 R. Miledi, *Nature*, 223 (1969) 1284.
7 J. Dudel and S.W. Kuffler, *J. Physiol.*, 155 (1961) 514.
8 B.L. Ginsborg, *Pharmacol. Rev.*, 19 (1967) 289.
9 M. Ozeki, A.R. Freeman and H. Grundfest, *J. Gen. Physiol.*, 49 (1966) 1335.
10 E.R. Kandel and L. Tauc, *J. Physiol.*, 183 (1966) 287.
11 P. Fatt and B. Katz, *J. Exp. Biol.*, 30 (1953) 433.
12 W. Burke and B.L. Ginsborg, *J. Physiol.*, 132 (1956) 599.
13 J. del Castillo and B. Katz, *J. Physiol.*, 132 (1956) 630.
14 R.K. Orkand, *J. Physiol.*, 164 (1962) 103.
15 R. Beranek and P.L. Miller, *J. Exp. Biol.*, 49 (1968) 83.
16 H. Grundfest and J.P. Reuben, in E. Florey, *Nervous Inhibition*, Pergamon Press, Oxford, 1961, p. 102.