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SHORT COMMUNICATION

INITIATION OF SWIMMING ACTIVITY IN THE MEDICINAL LEECH BY GLUTAMATE, QUISQUALATE AND KAINATE

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The excitatory amino acid, glutamate, and several of its agonists are known to produce locomotory activity in the lamprey (Brodin *et al.* 1985), rat (Kudo and Yamada, 1987) and the embryos and larvae of amphibians (Dale and Roberts, 1984; McClellan and Farel, 1985). It is hypothesized that glutamate acts as a neurotransmitter in the neuronal pathway that generates locomotion in these systems. In both the central and peripheral nervous systems of invertebrates, glutamate also acts as a neurotransmitter. For example, at many crayfish neuromuscular junctions, exogenously applied glutamate mimics the effects of the endogenous excitatory transmitter (Takeuchi and Takeuchi, 1964; Kawagoe *et al.* 1981). In other invertebrates, application of glutamate to central neurons produces a variety of responses depending on the neuron stimulated and the preparation (Walker *et al.* 1980; Shinozaki, 1988). However, in no invertebrates has glutamate been implicated in eliciting any centrally generated motor patterns.

In the medicinal leech, *Hirudo medicinalis*, local application of L-glutamate (L-glu) onto a segmental ganglion produces prolonged excitation of the Retzius cell (James and Walker, 1979). This L-glu-induced excitatory response is provocative, given that Tr1 stimulation, which can initiate swimming activity in the ventral nerve cord (Brodfuehrer and Friesen, 1986), elicits a similar type of sustained excitation in the Retzius cells prior to the onset of swimming. These similarities suggest that L-glu could act as a neurotransmitter in the initiation of leech swimming. In this paper we tested whether pressure ejection of L-glu can elicit the swim motor pattern in the isolated leech nerve cord and determined which of the three glutamate receptor subtypes (quisqualic acid, kainic acid or *N*-methyl-D-aspartic acid receptors) mediate this effect.

To determine if L-glu and its agonists can elicit swimming in an isolated nerve cord (head ganglion or segmental ganglion 2–19), a micropipet containing L-glu or an agonist dissolved in leech physiological saline (pH 7.40±0.03) was positioned

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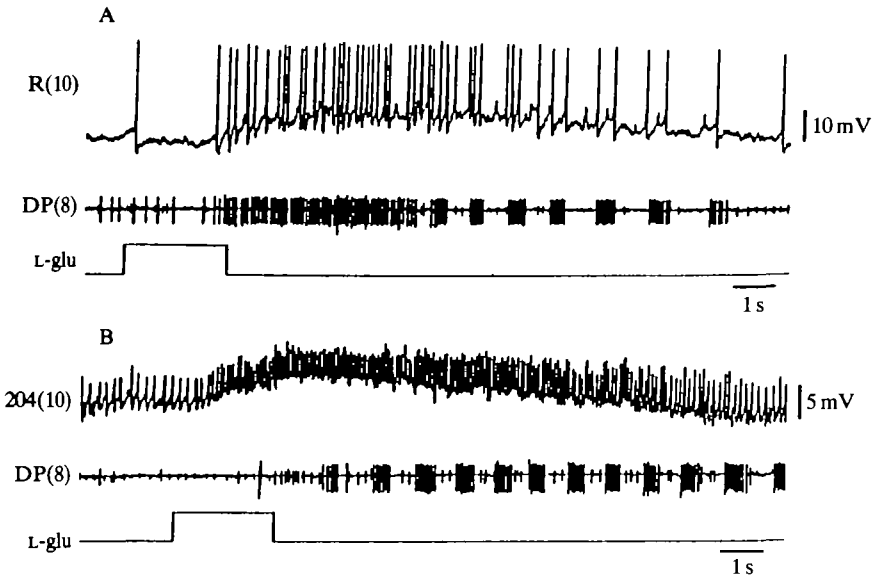

Fig. 1. Initiation of swimming activity by L-glutamate (L-glu). Pressure ejection of 10^{-3} mol l^{-1} L-glu onto segmental ganglion 10 depolarizes and increases the firing frequency of the Retzius cell (A) and cell 204 (B) in ganglion 10 prior to the onset of swimming activity (indicated by the rhythmic bursts of impulses in the dorsal posterior, DP, nerve recording). R, Retzius cell; 204, cell 204; the number in parentheses indicates the segmental ganglion from which the cell and nerve root recordings were made. (A and B) Top trace, intracellular recording; middle trace, extracellular recording of DP nerve activity; bottom trace, timing of the pressure ejection pulse. A and B are from different preparations.

slightly above the surface of a desheathed segmental ganglion or the subesophageal ganglion. The substances were delivered to the preparation by applying pressure pulses to a micropipet mounted in an electrode holder designed for micropressure injection (WP Instruments). The micropipet tip was broken by brushing it against the bottom of the recording dish to increase its diameter, so that when L-glu was released from the micropipet it covered approximately 5–10 neurons. During a pressure pulse the preparation was continuously superfused with saline. The final concentration of a substance at the neuropil level, therefore, was significantly diluted compared to its concentration in the micropipet. The duration of the pulse applied to the micropipet was controlled by a stimulator connected to a solenoid-activated valve (General Valve).

In an isolated nerve cord, a pulse of L-glu pressure ejected onto a single segmental ganglion evoked swimming activity along the nerve cord (Fig. 1A). Similarly, two known glutamate agonists, quisqualate (QQ) and kainate (KA), when pulsed onto a segmental ganglion also initiated swimming activity, while the glutamate agonist *N*-methyl-D-aspartate (NMDA) led to swimming activity in only one of five preparations. The swim-initiating abilities of QQ and KA were different from that of L-glu in two respects. First, both QQ and KA were

equipotent at eliciting swimming activity and substantially more effective than L-glu. Swimming usually occurred following pressure ejection of 10^{-4} mol l^{-1} QQ and KA from the micropipet onto a segmental ganglion, while application of L-glu at 10^{-3} mol l^{-1} was generally necessary to initiate swimming activity. Second, QQ and KA initiated swimming in 67% (8/12) and 64% (9/14) of the nerve cords tested, respectively, while L-glu only initiated swimming in 39% (7/18) of the nerve cords. These results are consistent with effects in other invertebrate and vertebrate preparations where the glutamate agonists are often more potent than L-glu itself (Walker, 1976; Roberts and Walker, 1982; Brodin *et al.* 1985; Shinozaki, 1988). The lack of an NMDA-induced response in the leech nervous system is additional evidence that there are fundamental differences between glutamate receptors in vertebrates, where NMDA is a potent agonist of L-glu, and in invertebrates (Shinozaki, 1988).

In response to L-glu, QQ and KA, ganglia in the nerve cord did not initiate swimming equally. Except for segmental ganglion 4 (0/4 preps), swim episodes occurred following stimulation of all ganglia tested [segmental ganglia 8 (1/1 prep.), 10 (16/18 preps), 11 (8/10 preps), 12 (3/3 preps), 13 (3/3 preps), 18 (3/4 preps) and the subesophageal ganglion (3/4 preps)]. However, even though swimming activity could be evoked following application of L-glu, QQ or KA to segmental ganglion 18 and the subesophageal ganglion, stimulation of these ganglia was less reliable at initiating swimming compared to stimulation of midbody ganglia (10–13). These segmental differences in swim responsiveness to pulses of L-glu, QQ and KA suggest that there may be regional differences in the pathway or mechanism by which these substances activate the swim oscillator along the nerve cord. Moreover, as reported by Hashemzadeh and Friesen (1989), bath application of L-glu (3 preps) was ineffective at eliciting swimming activity.

Since sustained depolarization of a single cell 204, a segmental swim-initiating interneuron, is sufficient to evoke swimming activity in the isolated nerve cord (Weeks and Kristan, 1978), we tested whether cell 204, like the Retzius cell, is excited by L-glu. As is shown in Fig. 1B, L-glu did excite cell 204. The observed depolarization was similar to that seen in the Retzius cell (compare Fig. 1A and B). In response to a pulse of L-glu the membrane potential of cell 204 was depolarized by more than 5 mV, and its firing frequency greatly increased prior to the onset of swimming activity. We also observed, as reported for the Retzius cell (Mat Jais *et al.* 1983), that L-glu occasionally elicited a biphasic response in cell 204 in which its membrane potential was first hyperpolarized and then exhibited a sustained depolarization (not shown).

QQ and KA, but not NMDA, also strongly excited cell 204 and were generally more potent than L-glu. This effect is illustrated in Fig. 2, where pressure ejection of 10^{-3} mol l^{-1} L-glu produced only a slight increase in the firing frequency of cell 204, while a pulse of 10^{-4} mol l^{-1} KA produced strong excitation of cell 204 and led to a swim episode. In fact, 10^{-4} mol l^{-1} QQ (12/12 preps) and 10^{-4} mol l^{-1} KA (13/14 preps) consistently excited the Retzius cells and cell 204, while 10^{-4} mol l^{-1} L-glu excited only the Retzius cell and cell 204 in 10/18 preparations. It is

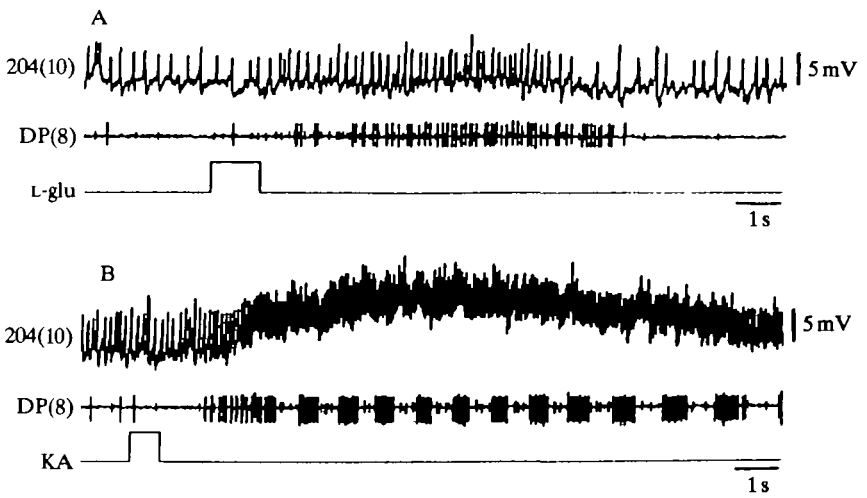

Fig. 2. Differential sensitivity of cell 204 to L-glutamate and kainate. (A) Pressure ejection of 10^{-3} mol l^{-1} L-glu (bottom trace) only weakly excites cell 204 (top trace; intracellular) and does not initiate a swim episode (middle trace; extracellular DP nerve), while (B) a 10^{-4} mol l^{-1} pulse of kainate (KA, bottom trace) strongly depolarizes the same cell 204 (top trace) and elicits a swim episode (middle trace).

interesting that, although both QQ and KA always strongly excited cell 204, swimming was not always elicited.

The excitatory effect of L-glu, QQ and KA on Retzius cells and cell 204 appears direct because it occurred even when the isolated nerve cord was bathed in leech saline containing an elevated concentration of Mg^{2+} (20 mmol l^{-1}) and zero Ca^{2+} to block disynaptic connections (Nicholls and Purves, 1970) (Fig. 3A). However, pressure ejection of L-glu, QQ and KA onto processes of cell 204 that extend into segmental ganglia other than the one containing its soma did not cause cell 204 to spike. This was true even for cell 204 processes in ganglia immediately adjacent to the segmental ganglion containing its soma (Fig. 3B). Thus, it appears that most of the glutamate receptors are located on the processes of cell 204 within the same segmental ganglion as its soma.

In this paper, we have shown that L-glu, QQ and KA can elicit swimming in the isolated leech nerve cord. The mechanism by which these substances initiate swimming is unknown. Our results, however, are consistent with the possibility that glutamate is a neurotransmitter in the pathway by which Tr1 stimulation triggers swimming activity, because L-glu, like the action of Tr1, produces a long-term depolarization of cells 204 prior to the onset of swimming. Direct physiological proof that Tr1 stimulation triggers swimming *via* the release of glutamate awaits a specific glutamate receptor blocker that functions in the leech nervous system. Alternatively, glutamate could be a transmitter in a pathway, other than *via* Tr1, by which swimming is elicited in the leech. Preliminary observations indicate that glutamate-like immunoreactivity is located in the leech central

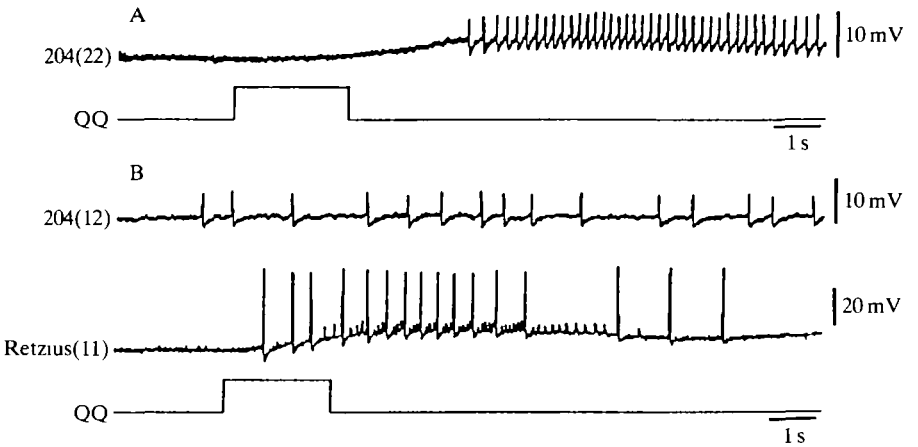

Fig. 3. Direct effect of quisqualate (QQ) on cell 204 and the Retzius cell. Preparation bathed in leech saline containing elevated levels of Mg^{2+} (20 mmol l^{-1}) and zero Ca^{2+} to block disynaptic connections. (A) Pressure ejection of 10^{-4} mol l^{-1} QQ onto segmental ganglion 10 (bottom trace) depolarizes cell 204 (top trace; intracellular) in segmental ganglion 10 sufficiently for it to spike. (B) Pressure ejection of 10^{-4} mol l^{-1} QQ on segmental ganglion 11 (bottom trace) excites the Retzius cell in that same ganglion (middle trace; intracellular), but does not increase the spiking frequency of cell 204 (top trace; intracellular) in ganglion 12. The membrane potential of cell 204 in ganglion 12 was slightly depolarized with 0.4 nA of injected current.

nervous system (Brodfuehrer and Cohen, 1990). Thus, it appears that glutamate potentially is a neurotransmitter in the leech nerve cord and one of its functions may be associated with the generation of swimming.

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