Inhibitor of Glutamate Transport Alters Synaptic Transmission at Sensorimotor Synapses in *Aplysia*

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Chin, Jeannie, John A. Burdohan, Arnold Eskin, and John H. Byrne. Inhibitor of glutamate transport alters synaptic transmission at sensorimotor synapses in *Aplysia*. *J Neurophysiol* 87: 3165–3168, 2002; 10.1152/jn.00333.2001. *Aplysia* sensory neurons possess high-affinity glutamate uptake activity that is regulated by serotonin. To gain insight into the physiological role of glutamate uptake in sensory neurons, we examined whether blockade of glutamate transport altered synaptic transmission. We also examined whether glutamate transport affected homosynaptic depression and posttetanic potentiation (PTP). In the presence of DL-threo-β-hydroxyaspartic acid (THA), previously shown to block glutamate uptake in *Aplysia*, the duration of unitary excitatory postsynaptic potentials (EPSPs) was significantly increased and their amplitude was significantly reduced. Similar effects were observed in the properties of summated EPSPs. However, no effect on the induction of homosynaptic depression or PTP was observed. Although it is unclear whether THA exerted its effect by modulating neuronal and/or glial mechanisms, at least one target of THA was neuronal, as the duration of unitary EPSPs measured in cultured sensorimotor synapses was also increased in the presence of THA. These results support the hypotheses that glutamate is the transmitter released by the sensory neurons and that glutamate transport plays an important role in regulating features of synaptic transmission in *Aplysia*.

**Methods**

*Aplysia californica* (150–300 g, Alacrity, Marine Specimens, and Marimus) were housed in aquaria at 15°C on a 12 h:12 h light:dark cycle. Pleural-pedal ganglia were removed and desheathed in a 1:1 solution of isotonic MgCl₂ and artificial seawater (ASW, pH 7.5) containing the following (in mM): 450 NaCl, 10 KCl, 30 MgCl₂, 20 MgSO₄, 10 CaCl₂, 2.5 NaHCO₃, 10 HEPES. Drug, vehicle, and wash solutions for these experiments were mixed in high-divalent artificial seawater (HD ASW, pH 7.5) containing the following (in mM): 300 NaCl, 10 KCl, 130 MgCl₂, 20 MgSO₄, 30 CaCl₂, 2.5 NaHCO₃, 10 HEPES. For experiments using cultured sensory and motor neurons (SN and MN, respectively), culturing procedures followed those described in Chin et al. (1999). Culture media was exchanged for ASW prior to recording.

In ganglia experiments, a single-tail SN in the pleural ganglion and a single-tail MN in the adjacent pedal ganglion were impaled with one and two microelectrodes, respectively, containing 3 M KAc with 100 mM KCl. Excitatory postsynaptic potentials (EPSPs) were evoked in MNs, held at −80 mV, by single action potentials (APs) elicited in SNs. Summated EPSPs were evoked in MNs by bursts of SN APs (20 Hz, 400-ms trains of 10-ms pulses). This paradigm was a modified version of those used in previous studies designed to challenge the transmitter release machinery in *Aplysia* (Eliot et al. 1994; Walters and Byrne 1984).

Each experiment consisted of six blocks of trials, each block (Fig. 1A) consisting of 10 single APs elicited in a SN with a 30-s interstimulus interval (ISI). The interval between each block of trials was 5 min. A burst of APs between the fifth and sixth APs evoked a summated EPSP in MNs (at −80 mV) after each set of trials by injecting 1 s, −0.4 nA current pulses. Preparations were used if cells maintained resting potentials of −35 to −70 mV for the duration of an experiment.

In experiments with cultured neurons (Fig. 3), the MN was impaled with a single microelectrode. Nine EPSPs were evoked in 10-min intervals by extracellularly stimulating the SN. During measurements, the MN was held at −80 mV. After the third EPSP, 20 ml of 10 mM THA or vehicle (−10 volume changes) was perfused at −2 ml/min. THA was washed out after the sixth EPSP.

Data are expressed as means ± SD. Unless otherwise indicated, values are normalized to the first measurement at the beginning of an experiment. Statistical tests were performed using a treatment by trial
analysis of variance (ANOVA). For the analysis of unitary EPSP duration and amplitude, the values included in the ANOVA represented the average of the 10 EPSPs in each block. A single ANOVA was performed to analyze the effect of THA over the three TEST blocks, or over the two WASH blocks.

RESULTS

Inhibition of glutamate transport altered unitary and summated EPSPs

The possible effects of THA on synaptic communication in Aplysia were tested at the sensorimotor synapse in the pedal ganglion. THA increased the duration of unitary EPSPs (Fig. 1B and 2A), measured as the time from EPSP onset to the time at which it decayed to half of its maximum amplitude. The concentration of THA used in these experiments was its IC$_{50}$ for inhibition of glutamate uptake in isolated ganglia of Aplysia (Levenson et al. 2000a). The average EPSP duration of the THA-treated group increased to 280% (TEST 1), 377% (TEST 2), and 378% (TEST 3) that of the vehicle control (TEST 1–3: $F$(1,36) = 56.99, $P < 0.001$). After washout, the EPSP duration partially returned to control levels but was still significantly longer (WASH 1: 150%, WASH 2: 142%) than that of the control for both blocks of trials (WASH 1–2: $F$(1,24) = 24.79, $P < 0.001$). THA suppressed the amplitude of unitary EPSPs (Figs. 1B and 2B). The EPSP amplitude of the THA-treated group was reduced to 44% (TEST 1), 34% (TEST 2), and 32% (TEST 3) that of the control (TEST 1–3: $F$(1,36) = 77.78, $P < 0.001$). Following washout, the EPSP amplitude returned to control levels (WASH 1: 94%, WASH 2: 111%; WASH 1–2: $F$(1,24) = 0.0049, $P = 0.95$).

The effect of inhibition of glutamate transport on sEPSPs was also examined. The increased glutamate release during the burst might further challenge the transport mechanisms. Indeed, THA increased the duration of summated EPSPs (Fig. 1B). The duration of sEPSPs was measured as the time from EPSP onset to the time at which it decayed to half of its peak amplitude.
amplitude. The average duration of summated EPSPs of the THA-treated group increased to 191% that of the control (TEST 1–3: $F(1,36) = 72.46, P < 0.001$). Following washout, summated EPSP duration returned to 112% of control levels when averaged over both sets of trials (WASH 1–2: $F(1,24) = 3.91, P = 0.06$). THA also reduced the average peak amplitude of summated EPSPs to 59% that of the control (TEST 1–3: $F(1,36) = 26.59, P < 0.001$). Following washout, the summed EPSP amplitude returned to 116% of control levels when averaged over both blocks of trials (WASH 1–2: $F(1,24) = 3.04, P = 0.09$). THA also affected the rise time of the sEPSP, measured as the time from EPSP onset to the time it reached peak amplitude. THA significantly increased the rise time to 264% (TEST 1), 253% (TEST 2), and 231% (TEST 3) that of vehicle controls (TEST 1–3: $F(1,36) = 19.53, P < 0.001$). The rise time of the sEPSP returned to control levels on wash-out of THA (WASH 1: 120%; WASH 2: 108%; WASH 1–2: $F(1,24) = 1.17, P = 0.29$).

Inhibition of glutamate transport did not affect homosynaptic depression or PTP

Glutamate uptake may affect the size of the readily releasable pool of transmitter. If so, inhibitors of glutamate uptake might affect pool size and thereby affect forms of synaptic plasticity, such as homosynaptic depression and PTP, which can depend on pool size. However, THA had no effect on either PTP, defined as the amplitude of the sixth EPSP divided by the amplitude of the fifth EPSP of that block, or homosynaptic depression (amplitude of the fifth EPSP divided by the amplitude of the first EPSP of that block) (Fig. 2B). The degree of PTP in the THA-treated group was 95% that of the vehicle control when averaged over the three blocks of trials following the infusion of drug or vehicle. The average PTP in the THA-treated group was 166 ± 8% of pretetanus EPSP amplitude and the average PTP in the vehicle-treated group was 176 ± 3% of pretetanus EPSP amplitude. This difference was not significant (TEST 1–3: $F(1,36) = 0.552, P = 0.46$). The degree of homosynaptic depression in the THA-treated group was 93% that of the vehicle control when averaged over the three blocks of trials following the application of drug or vehicle. On average, EPSPs in the THA-treated group depressed to 47 ± 1% of initial values and EPSPs in the vehicle-treated group depressed to 50 ± 3% of initial values. This difference was also not significant (TEST 1–3: $F(1,36) = 0.68, P = 0.42$).

THA modulates neuronal glutamate uptake

If THA indeed acts by blocking glutamate transporters, it may do so by acting on neuronal and/or glial mechanisms. Since the isolated ganglion contains glia in close proximity to the sensorimotor synapse, it is not possible to determine whether THA is altering synaptic transmission by blocking neuronal or glial uptake of glutamate. To examine whether THA affects neuronal glutamate uptake, we cultured a single SN with a single MN and examined THA’s ability to modulate transmission at this synapse. This culture system does not contain glia or any cells other than the SN and MN. Thus any effect of THA on the shape of the EPSP can be directly attributed to a neuronal mechanism.

THA increased EPSP duration in cultured neurons by approximately 50% (Fig. 3A, $F(1,41) = 6.816, P < 0.05$). After washout, there was no difference between EPSP duration measured in THA- or vehicle-treated cultures ($P > 0.5$). THA appeared to reduce the amplitude of EPSPs measured in cultured neurons but this effect was not significantly different from controls ($F(1,45) = 0.206, P > 0.2$, Fig. 3B).

DISCUSSION

The increase in EPSP duration produced by THA is most likely a direct consequence of decreased glutamate uptake. A decreased rate of clearance would lead to glutamate remaining in the synaptic cleft for a longer period of time, prolonging the synaptic response. Similar observations have been made in mammalian CNS (e.g., Barbour et al. 1994; Otis et al. 1996). Although the effects of THA may result from modulation of neuronal and/or glial transporters, at least one target of THA is neuronal, as THA increased EPSP duration in neurons cultured in the absence of glia. The magnitude of THA’s effect on EPSP duration was greater in the ganglion than in the culture system, which may represent differences in the contribution of diffusion to the clearance of glutamate from the cleft in the two preparations and/or possible contributions of glial glutamate uptake.

![THA affects the duration of EPSPs in cultured sensorimotor synapses.](http://jn.physiology.org/)

**FIG. 3.** THA affects the duration of EPSPs in cultured sensorimotor synapses. A: examples of EPSPs evoked in the MN by stimulation of the SN. For clarity, the 5th EPSP (dotted line, in the presence of THA/vehicle) is shown overlaid on the 1st EPSP (solid line, before treatment with THA/vehicle). For vehicle and THA groups, the 5th EPSP has been vertically scaled by a factor of 219 and 210%, respectively, to match the 1st EPSP. B: summary data showing that THA significantly prolonged the duration of EPSPs. C: summary data showing that THA tended to reduce EPSP amplitude, but this effect was not statistically significant.
The difference in the effects of THA in ganglia and culture on EPSP duration may also be related to the different effects on amplitude in the two systems. For example, the increase in duration of glutamate within the cleft in ganglia might result in the activation of receptors, such as presynaptic metabotropic glutamate receptors (mGluRs) that influence the release properties of the presynaptic neuron (Cartmell and Schoepp 2000; Fitzimonds and Dichter 1996; Maki et al. 1994; Scanziani et al. 1997). In the culture system, diffusion may play a greater role in clearing glutamate from the cleft than in the isolated ganglion due to the fact that the sensory and motor neurons, as well as their processes, are completely exposed to the surrounding media. Thus it is possible in the culture system for glutamate to modulate EPSP amplitude because glutamate diffuses away from the cleft before significant desensitization can occur.

Despite the pronounced effects of THA on EPSP amplitude and duration, no significant change in PTP or homosynaptic depression was observed. Both PTP and homosynaptic depression are thought to occur primarily due to presynaptic mechanisms and would not be expected to change if the reduction in glutamate clearance induces only postsynaptic effects such as receptor desensitization. THA’s ability to modulate depression may be frequency-dependent, however. For example, THA increased depression only at frequencies exceeding 20 Hz in neurons of the cochlear nucleus magnocellularis (Turecek and Trussell 2000). The depression examined in this study was induced by 0.03-Hz stimulation. Therefore depression of the sensorimotor synapse induced by higher frequency stimulation might be affected by glutamate uptake.

The results from this study demonstrate that glutamate uptake plays an important role in shaping the time course of EPSPs and thus modulates synaptic efficacy. Although glial uptake may also be involved, neuronal glutamate uptake is important for synaptic transmission and is therefore a possible site of regulation by stimuli or treatments that induce changes in synaptic efficacy.

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