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Research Paper

Suppression of γ-aminobutyric acid transporter current by activation of ionotropic glutamate receptors on retinal horizontal cells

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Abstract: In the present study, the modulatory effect of AMPA receptors on γ-aminobutyric acid (GABA) transporter current was investigated on enzymatically isolated horizontal cells of carp retina. The GABA transporter current elicited by 1 mmol/L GABA was decreased immediately after pre-application of AMPA (30 μmol/L or 3 mmol/L) for 50 s. Application of 10 mmol/L BAPTA in intracellular solution inhibited the suppression effect of AMPA on GABA transporter current. The suppression effect induced by co-

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one of our previous studies, it was found that Ca^{2+}-influx via *N*-methyl-*D*-aspartate (NMDA) receptors in horizontal cell of carp retina inhibited GABA transporter current[6]. In horizontal cell of carp retina, Ca^{2+} influx can occur through glutamate receptors, including both NMDA receptors[7] and Ca^{2+}-permeable AMPA receptors[8]. In the present study, the inhibition of GABA transporters by activation of AMPA receptors was also observed in horizontal cell of carp retina. The suppressive effect of GABA transporters was eliminated by application of 10 mmol/L BAPTA in intracellular solution. Since both AMPA receptors and NMDA receptors coexist in carp retinal horizontal cell[7, 8], suppression of GABA transporters was examined further when AMPA receptors and NMDA receptors were both activated at the same time. Further suppression of GABA transporters by co-application of AMPA and NMDA at high concentration was not observed, which suggests that the activation of AMPA receptors or NMDA receptors on the retinal horizontal cell inhibits GABA transporter-mediated current by affecting similar intracellular Ca^{2+} processes in the retinal horizontal cells.

1 MATERIALS AND METHODS

1.1 *Cell isolation*

Horizontal cells were enzymatically dissociated from retinas of adult carp (*Carassius auratus*, 15-20 cm body length), following the procedures previously described[8, 9]. Briefly, retinas were isolated from carp, and incubated for 20 min at room temperature in 4 mL Hank's solution with 25 U/mL papain and 4 mg *L*-cysteine. The retinas were rinsed and stored in the Hank's solution at 4 ºC. Cells were freshly dissociated from the retinal pieces by gentle trituration in Ringer's solution and the cell suspension was placed onto a plastic dish. H1 horizontal cell was identified by its characteristic morphology.

1.2 *Whole cell recording and drug application*

Whole cell recordings, voltage-clamped at -60 mV, were obtained using 5-8 MΩ patch pipette pulled from borosilicate glass (Sutter Instrument Inc., USA) using a horizontal puller (P87, Sutter Instrument Inc., USA). The pipette was filled with intracellular solution, mounted on a motor-driven micromanipulator (MC1000e, SD Instrument Inc., USA), and was connected to a patch amplifier (Axopatch 200B, Axon Instrument Inc., USA). An agar/NaCl bridge connected to the recording chamber with an Ag/AgCl wire inside was used as a reference electrode. Fast capacitance, cell capacitance transients, and 70% of the series resistance of the recording electrode were compensated. Data acquisition was performed using AxoScope software (Axon Instrument Inc., USA), with 1 kHz of sample rate and being lowpass filtered (0-1 kHz). The recorded data were analyzed by Clampfit 9.2 software (Axon Instrument Inc., USA).

For brief application of GABA, AMPA and NMDA, the superfusion system (DAD-12, ALA Scientific Instruments, USA) was used. Other chemicals were bath-applied with the perfusate at a flow rate of 1 mL/min. In all experiments, NMDA was always co-applied in Mg^{2+}-free Ringer's solution with 100 μmol/L glycine, which alone could not induce detectable current.

1.3 *Solutions*

Hank's solution contained (in mmol/L) 120.0 NaCl, 3.0 KCl, 0.5 $CaCl_2$, 1.0 $MgSO_4$, 1.0 Na-pyruvate, 1.0 NaH_2PO_4, 0.5 $NaHCO_3$, 20.0 HEPES and 16.0 glucose. Ringer's solution contained (in mmol/L) 120.0 NaCl, 5.0 KCl, 2.0 $CaCl_2$, 1.0 $MgSO_4$, 10.0 HEPES and 16.0 glucose. Picrotoxin (PTX), NMDA and GABA were dissolved in Ringer's solution. AMPA was prepared in dimethyl sulfoxide (DMSO) and diluted to its final concentrations in Ringer's solution (DMSO < 0.5%). The pH value of the perfusate was adjusted to 7.4 with NaOH. The intracellular solution for patch electrode contained (in mmol/L) 140.0 CsCl, 0.05 $CaCl_2$, 1.0 $MgSO_4$, 0.5 EGTA, 10.0 HEPES. The pH value was adjusted to 7.3 with CsOH. All the drugs were purchased from Sigma (St. Louis, MO, USA).

1.4 *Statistics*

Data were expressed as mean ± SD in main text and mean ± SEM in figures. Comparisons were made using paired *t*-test or unpaired *t*-test as stated. $P < 0.05$ was considered statistically significant.

2 RESULTS

2.1 Suppression of GABA transporter current by activation of AMPA receptors

Whole cell recordings were performed on isolated H1 horizontal cell which was voltage clamped at -60 mV. Application of 1 mmol/L GABA could elicit ionotropic GABA receptor-mediated current and GABA transporter-mediated current in the H1 cell. Figure 1 gives an example of the GABA-elicited current that recorded from an H1 cell (133.1 pA) in the presence of 300 μmol/L PTX (control), a potent antagonist of $GABA_A$ and $GABA_C$ receptors. This PTX-insensitive current was effectively suppressed (2.4 pA) by application of 200 μmol/L SKF-89976A, a GABA

transporter inhibitor[10]. This result indicates that the GABA-elicited PTX-insensitive current was mediated by GABA transporter.

To analyze possible modulatory effect that AMPA receptors exert on GABA transporters, PTX at a concentration of 300 μmol/L was applied to block the ionotropic GABA receptors, and GABA at a concentration of 1 mmol/L was given after the pre-application of 30 μmol/L AMPA in Ringer's solution.

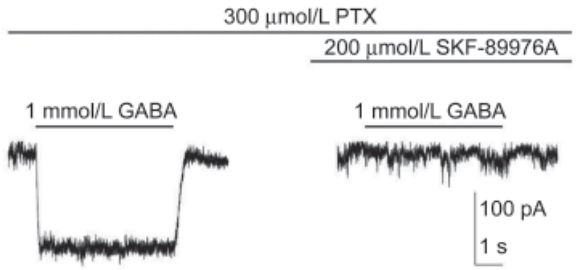

Fig. 1. Blockage of GABA transporter current by SKF-89976A. In the presence of 300 μmol/L PTX, application of 1 mmol/L GABA induced an inward current, which was blocked by 200 μmol/L SKF-89976A, a GABA transporter blocker.

In the example presented in Fig. 2*A*, application of GABA (1 mmol/L) in the presence of PTX elicited an inward current of 98.0 pA which was taken as control. Then 30 μmol/L AMPA was superfused for 50 s. The co-application of AMPA and GABA may cause more noise, and ions through AMPA receptors can disturb GABA transporter current. Therefore GABA (1 mmol/L) was applied again, after AMPA was washed out by the PTX-containing Ringer's solution for 5 s. The pre-application of AMPA decreased the GABA-elicited current to 76.0 pA. After superfusion with the PTX-containing Ringer's solution for another 4 min, the GABA-elicited current recovered to 98.5 pA. The white columns in Fig. 2*D* represent the normalized suppression effect of GABA transporter currents after pre-application of 30 μmol/L AMPA and recovery (n = 5). The pre-application of 30 μmol/L AMPA decreased GABA transporter current to (79.32±2.41)%

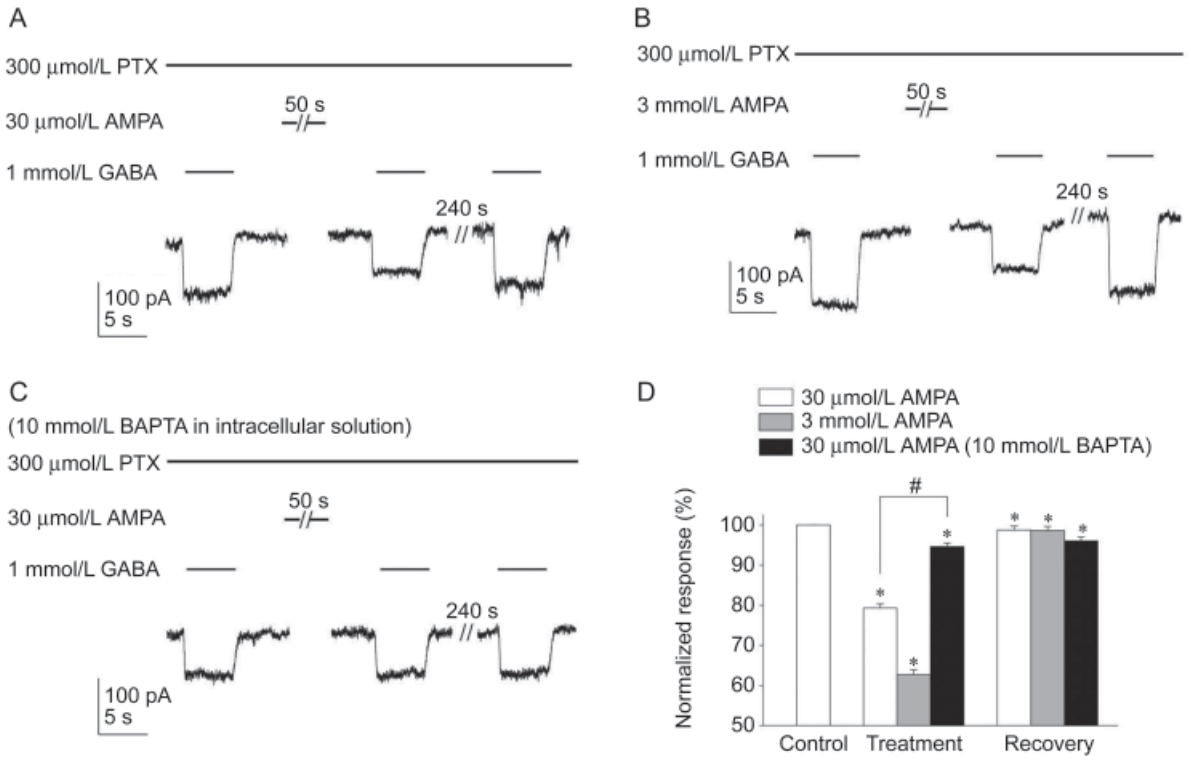

Fig. 2. Suppression of GABA transporter currents by activation of AMPA receptors. *A*: An example of the GABA transporter currents measured during control, after the pre-application of 30 μmol/L AMPA and recovery. *B*: The GABA transporter currents measured during control, after the pre-application of 3 mmol/L AMPA and recovery. *C*: The GABA transporter currents measured during control, after the pre-application of 30 μmol/L AMPA and recovery, with 10 mmol/L BAPTA added into the intracellular solution. *D*: The statistics for the GABA transporter currents (normalized against control, mean ± SEM, n = 5 for each) measured during control, after pre-application of AMPA, and recovery (white columns: 30 μmol/L AMPA; grey columns: 3 mmol/L AMPA; black columns: 30 μmol/L AMPA with 10 mmol/L BAPTA in intracellular solution). * $P < 0.05$ (paired t-test, as compared to control). $^{\#}$ $P < 0.05$ (unpaired t-test, comparison between data as indicated).

($P < 0.05$, paired *t*-test) of the control level. The recovery level was (98.74 ± 2.22)% ($P < 0.05$, paired *t*-test) as compared to control level.

Figure 2*B* gives an example from a cell in which GABA transporter currents were 137.1 pA, 82.6 pA, 136.8 pA (control, after pre-application of 3 mmol/L AMPA, recovery, respectively). The grey columns in Fig. 2*D* represent the normalized suppression effect of GABA transporter currents after pre-application of 3 mmol/L AMPA and recovery in five cells. The pre-application of 3 mmol/L AMPA decreased GABA transporter current to (62.71 ± 2.15)% ($P < 0.05$, paired *t*-test) of the control level. The recovery level was (98.61 ± 2.24)% ($P < 0.05$, paired *t*-test) as compared to control level.

2.2 Intracellular Ca^{2+} and the AMPA receptor-mediated modulation of GABA transporters

In one of our previous studies, it was reported that Ca^{2+}-impermeable AMPA receptors and Ca^{2+}-permeable AMPA receptors coexisted in carp retinal horizontal cells[8]. Our previous work also showed that Ca^{2+} influx via NMDA receptors in carp horizontal cell increased intracellular Ca^{2+} level[7]. When intracellular Ca^{2+} changes were attenuated by 10 mmol/L BAPTA in intracellular solution, NMDA modulation of GABA transporters was eliminated[6].

To further confirm that intracellular Ca^{2+} takes part in the AMPA modulation of GABA transporters, BAPTA at a concentration of 10 mmol/L was added into the Ca^{2+}-free intracellular solution. Figure 2*C* gives an example from a cell with 10 mmol/L BAPTA in intracellular solution, in which the GABA transporter currents were measured 77.9 pA, 73.0 pA, and 75.5 pA (control, after 30 μmol/L AMPA pre-application, and recovery, respectively). The black columns in Fig. 2*D* represent suppression effect of GABA transporter currents after pre-application of 30 μmol/L AMPA and recovery in five cells with 10 mmol/L BAPTA in the intracellular solution. After pre-application of 30 μmol/L AMPA, the GABA transporter current was decreased to (94.61 ± 1.97)% of the control level ($P < 0.05$, paired *t*-test). The reduction of GABA transporter current after pre-application of APMA was significantly attenuated as compared to that measured in normal intracellular solution ($P < 0.05$, unpaired *t*-test, comparison between the middle white column and the middle black column in Fig. 2*D*). The recovery level was (96.05 ± 2.16)% of the control level ($P < 0.05$, paired *t*-test).

2.3 Modulation of GABA transporters by activation of AMPA receptors and NMDA receptors

Since both AMPA receptors and NMDA receptors coexist in carp retinal horizontal cell[7,8], suppression of GABA transporters was examined further when NMDA and/or AMPA were applied at high concentration (3 mmol/L, Fig. 3).

Figure 3*A* gives an example from a cell in which GABA transporter currents were 93.4 pA, 62.5 pA, 90.3 pA (control, after pre-application of 3 mmol/L NMDA, recovery, respectively). The pre-application of 3 mmol/L NMDA

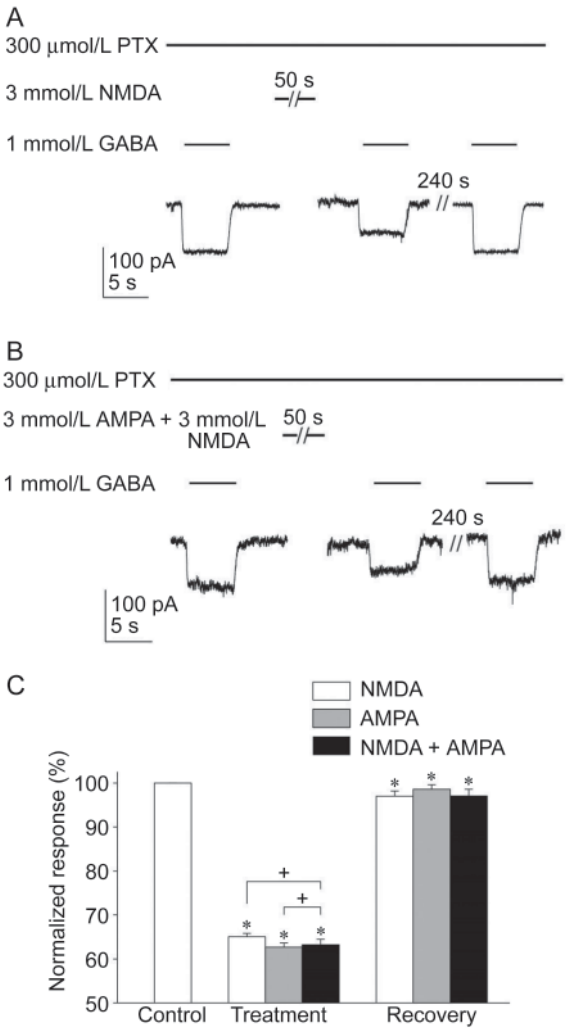

Fig. 3. Modulation of GABA transporters by activation of AMPA receptors and NMDA receptors. *A*: The GABA transporter currents measured during control, after the pre-application of 3 mmol/L NMDA and recovery. *B*: The GABA transporter currents measured during control, after the pre-application of 3 mmol/L AMPA + 3 mmol/L NMDA and recovery. *C*: The statistics for the GABA transporter currents (normalized against control, mean ± SEM, $n = 5$ for each) measured during control, after pre-application of AMPA or/and NMDA, and recovery (white columns: 3 mmol/L NMDA; grey columns: 3 mmol/L AMPA; black columns: 3 mmol/L NMDA + 3 mmol/L AMPA). $^{*}P < 0.05$ (paired *t*-test, as compared to control); $^{+}P > 0.05$ (unpaired *t*-test, comparison between data as indicated).

decreased GABA transporter current to (65.10 ± 1.66)% ($P < 0.05$, paired *t*-test) of the control level, and the recovery level was (97.02 ± 2.66)% ($P < 0.05$, paired *t*-test) as compared to control level, as illustrated by the white columns in Fig. 3*C* ($n = 5$). The grey columns in Fig. 3*C* represent the normalized suppression effect of GABA transporter currents after pre-application of 3 mmol/L AMPA and recovery ($n = 5$), which are identical to the grey columns in Fig. 2*D*.

To examine whether any further suppression of GABA transporter current can be induced by simultaneous activation of NMDA receptors and AMPA receptors on retinal horizontal cell, 3 mmol/L AMPA + 3 mmol/L NMDA was applied. Figure 3*B* gives an example from a cell in which GABA transporter currents were 81.7 pA, 52.7 pA, and 74.6 pA (control, after pre-application of 3 mmol/L AMPA + 3 mmol/L NMDA, recovery, respectively). The black columns in Fig. 3*C* represent the normalized suppression effect of GABA transporter currents after pre-application of 3 mmol/L AMPA + 3 mmol/L NMDA and recovery ($n = 5$). The pre-application of 3 mmol/L AMPA + 3 mmol/L NMDA decreased GABA transporter current to (63.27 ± 2.14)% ($P < 0.05$, paired *t*-test) of the control level. The recovery level was (97.07 ± 2.46)% ($P < 0.05$, paired *t*-test) as compared to control level. The decrease of GABA transporter current induced by 3 mmol/L AMPA + 3 mmol/L NMDA was similar to that by 3 mmol/L AMPA alone ($P > 0.05$, unpaired *t*-test), or 3 mmol/L NMDA alone ($P > 0.05$, unpaired *t*-test).

3 DISCUSSION

Kreitzer *et al*. reported that glutamate regulates GABA transporter current in horizontal cell of skate retina through a Ca^{2+}-dependent process[5]. Ca^{2+}-influx via AMPA receptors down-regulates the GABA uptake of the transporters[5]. This AMPA receptor-mediated suppression of GABA transporter function was also observed in carp retinal H1 horizontal cells in the present study. Application of BAPTA in intracellular solution attenuated the suppression of GABA transporter current induced by activation of AMPA receptors, which implies that the suppression effect is related to the increase of intracellular Ca^{2+}. In addition to AMPA receptors, NMDA receptors are expressed in carp retinal horizontal cells[11]. In our previous work, activation of NMDA receptors inhibits GABA transporters of carp retinal horizontal cells via intracellular Ca^{2+} process[6]. Such NMDA-mediated down-regulation of GABA transporter current has been also confirmed in the present study.

For the retinal horizontal cell, calcium is important for the modulation of GABA transporters[5], as well as many other physiological functions, including the modulation of gap junctions[12], voltage-gated channels[13], sodium channels[14], and even synaptic plasticity between horizontal cells and cones[15]. Extracellular calcium can enter the retinal horizontal cell through voltage-gated channels and glutamate receptors[16]. Because the horizontal cells were voltage-clamped at -60 mV in our experiments, the voltage-gated Ca^{2+} channels were not activated, therefore Ca^{2+} influx could only occur through glutamate receptors, i.e., the Ca^{2+}-permeable AMPA receptors[8] and NMDA receptors[17]. Therefore, it is reasonable to infer that Ca^{2+} may play a role as second messenger to modulate physiological functions of the cell, such as activities of GABA transporters.

Since AMPA receptors and NMDA receptors coexist in carp retinal horizontal cells, suppression effect of GABA transporters mediated by simultaneous activation of AMPA receptors and NMDA receptors was examined in the present study. The experimental results demonstrate that the suppression effect of 3 mmol/L AMPA + 3 mmol/L NMDA was similar to that of 3 mmol/L NMDA alone, which induced maximum decrease of GABA transporter current as reported in our previous work[6]. Additional activation of AMPA receptors failed to induce any further suppression of GABA transporter current in the present study, which suggests that the activation of AMPA receptors and NMDA receptors on the retinal horizontal cells inhibits GABA transporter-mediated current by affecting the same intracellular Ca^{2+} processes in the retinal horizontal cells.

Under physiological condition, AMPA receptors on horizontal cell can be activated by glutamate released from photoreceptors[18]. The subsequent membrane depolarization caused by the activation of AMPA receptors will relieve the Mg^{2+} block of NMDA receptors and result in the activation of NMDA receptors. Since AMPA receptors display very rapid and significant desensitization, while NMDA receptors do not desensitize as quickly or as fully, this activation of NMDA receptors will lead to more Ca^{2+} influx through NMDA receptors as compared to that through AMPA receptors. Therefore, more suppression of GABA transporters can be induced by the further increase of intracellular Ca^{2+}. Although both AMPA receptors and NMDA receptors take part in GABA transporter suppression by increasing intracellular Ca^{2+}, these two types of glutamate receptors may play different roles in triggering and signal amplifying respectively in physiological modulation of GABA transporters.

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