Existence of Ionotropic Glutamate Receptor Subtypes in Cultured Rat Retinal Ganglion Cells Obtained by the Magnetic Cell Sorter Method and Inhibitory Effects of 20-Hydroxyecdysone, a Neurosteroid, on the Glutamate Response

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ABSTRACT—Glutamate and neurosteroids are known to exist in retinal ganglion cells (RGC). Therefore, patch clamp studies using the whole-cell recording method were performed to determine whether or not ionotropic glutamate receptor subtypes, i.e., N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors, were present on RGC obtained by the magnetic cell sorter (MACS) method and cultures. In addition, the effects of 20-hydroxyecdysone (20-HE), a neurosteroid, on inward currents induced by NMDA, AMPA and kainate were examined at a holding potential of −60 mV. The current-voltage relationship for NMDA in the presence of glycine and Mg²⁺-free, as well as those for AMPA and kainate were linear, with a reversal potential of around 0 mV. NMDA-induced currents were blocked by MK-801, while both AMPA- and kainate-induced currents were blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Application of 20-HE in the bath resulted in significant inhibitions on NMDA-, AMPA- and kainate-induced currents. Thus, NMDA, AMPA and kainate receptors were confirmed to exist on MACS-separated cultured RGC. Moreover, 20-HE inhibited NMDA receptor-mediated currents most prominently and AMPA- and kainate-mediated currents moderately, suggesting that neurosteroids may be playing a role in modulating glutamate-mediated transmission in RGC, and 20-HE might be useful for preventing glutamate neurotoxicity.

Keywords: Culture, Retinal ganglion cell, Patch clamp, Ionotropic glutamate receptor subtype, 20-Hydroxyecdysone

Glaucoma is a serious disease that lead to visual field disturbance because of retinochoroidal circulation disturbance or increased intraocular pressure. In addition, the glaucomatous optic nerve disturbance is known to be associated with apoptosis of retinal ganglion cells (RGC) (1, 2). Glutamate is commonly an excitatory neurotransmitter in the central nervous system. However, excessive levels of glutamate induce RGC damage (3, 4). In fact, the intra-vitreous glutamate concentration in glaucoma patients is 2 times higher than that in non-glaucoma patients (5). In general, it is difficult to dissociate a culture solely of the RGC of mammalian animals. Previously, we have developed a new method in culturing and dissociation of rat neonatal RGC using a magnetic cell sorter (MACS) (6); therefore, it is of interest to know whether or not glutamate receptor subtypes such as N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors are present in such cultured RGC, although the existence of NMDA and/or non-NMDA receptors in RGC has been reported using histochemical, cytochemical and electrophysiological methods (7 – 9). Thus, using the whole-cell patch clamp method, we performed studies to determine if ionotropic glutamate receptor subtypes were present on viable RGC that had been separated by the MACS method.

Many neuroprotective agents such as Zn²⁺ (10), pituitary adenylate cyclase activating polypeptide (PACAP) (11),
B vitamins (12), vasoactive intestinal polypeptide (VIP) (13), eliprodil (14, 15), and acidic condition (16) to inhibit glutamate-induced retinal damage have been reported. Moreover, dehydroepiandrosterone (DHEA), a neurosteroid, was reported to reduce NMDA-induced neurotoxicity of hippocampal neurons (17). However, neurosteroids reportedly have diverse modulation of NMDA and non-NMDA receptor functions; for example, pregnenolone potentiates NMDA-induced currents and inhibits non-NMDA responses, but pregnanolone inhibits these glutamate subtype responses of recombinant receptors in oocytes (18). Therefore, some neurosteroids may be useful for neuroprotection via inhibition of NMDA receptors (17, 19).

20-Hydroxyecdysone (20-HE), a neurosteroid, which was first found in plants and insects, is a biologically active endogenous ecdysteroid hormone involved in metamorphosis of some insects (20, 21). The ecdysone binding sites are known to exist in the brain. Previously, we have demonstrated that 20-HE potentiates the GABA-induced current in cultured cortical neurons (22) and vestibular nucleus neurons (23) and inhibits epileptic seizures in spontaneously epileptic rats (SER) (24). Thus, we examined the effects of 20-HE on the glutamate receptor subtypes of RGC before studying protective effects of 20-HE against glutamate-induced neurotoxicity in RGC.

MATERIALS AND METHODS

Retinal cultures

MACS-separated primary RGC cultures were obtained from 4- to 5-day-old Wistar rats. Isolation of RGC was performed as reported previously (6). Briefly, retinal cell suspension was incubated with biotinylated anti-rat Thy-1 antibody (Pharmingen, San Diego, CA, USA). After they were rinsed in Dulbecco’s modified eagle medium: nutrient substance mixture F-12 (DMEM/F-12; Gibco Laboratories Life Technologies Inc, Gibco, Grand Island, NY, USA), they were incubated with Streptavidin MicroBeads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) for 15 min to make the biotin-avidin complex with the antibody to RGC and then washed with DMEM/F-12. The cell pellet was resuspended in DMEM/F-12 and applied onto the MS2 column (Miltenyi Biotec GmbH) in the magnetic fields of a MiniMACS (Miltenyi Biotec GmbH). The column was washed with DMEM/F-12 and then removed from the magnetic fields. Cell attached on the column were flushed out with gentle pressure by 1 ml DMEM/F-12 supplemented with 10% heat-inactivated horse-serum (HS, Gibco), 10% heat-inactivated fetal calf-serum (FCS, Gibco) and nutriment substances. Following these treatment, 31.0 ± 1.6% (mean ± S.E.M., n = 6) of the total population of cells examined were RGC (6). The retinal cell maintained in a consistent culture condition (37°C, 5%CO2) for 1 week were used for the present studies. The growth medium was supplemented with 10% FCS and 10% HS, brain-derived neurotrophic factor (40 ng/ml) (a gift of Sumitomo Pharmaceuticals, Osaka), forskolin (10 μM), basic fibroblast growth factor (10 ng/ml) (Sigma, St. Louis, MO, USA), and insulin (5 μg/ml) (Gibco). The culture medium was exchanged at 3-day intervals.

Whole-cell recordings

Using the whole-cell recording method, the currents induced by NMDA (Research Biochemical International (RBI), Natik, MA, USA), AMPA (RBI), or kainate (RBI) were recorded under voltage-clamp conditions at a holding potential of ~60 mV, except for experiments on the current-voltage relationship. An Axopatch 200A amplifier (Axon Instruments, Burlingame, CA, USA) was employed. The bath solution contained: 145 mM NaCl, 5 mM KCl, 2 mM CaCl2, 11 mM D-glucose, 5 mM HEPES and 0.0003 mM tetrodotoxin (Wako Chemicals, Osaka). The pH of the solution was adjusted to 7.3. NMDA-induced currents were recorded in the presence or absence of MgSO4 (1.1 mM) in the bathing solution. The composition of pipette solution was as follows: 80 mM CsCl, 80 mM CsF, 10 mM HEPES and 10 mM ethylene glycol bis (β-aminoethyl-ester)-N,N,N',N'-tetraacetic acid (EGTA), adjusted to 7.4. The patch electrode had a resistance of 3 to 8 MΩ, and the series resistance ranged from 5 to 25 MΩ. The peak amplitude of the current was measured with pCLAMP software version 6.0 (Axon Instruments). The decay time constant of the desensitization phase (τdes) of the NMDA- or AMPA-induced current was fitted to a double exponential with a fast (τfast) and a slow (τslow) component of decay (pCLAMP).

Drug application

NMDA, AMPA and kainate were applied to RGC for 2 s via a U-shaped tube placed 100 μm above the recording neurons as described previously (25). Glycine (10 μM, Wako Chemicals), an NMDA-receptor co-agonist, and strychnine sulfate (10 μM, Wako Chemicals), a glycine receptor antagonist, were added to the solution when NMDA-induced currents were examined. MK-801 (10 μM) (RBI), an NMDA-receptor antagonist, was applied concomitantly with NMDA, and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM) (RBI), a non-NMDA receptor antagonist, with AMPA and kainate. After adding 20-HE (Daisel Chemical, Tokyo) to the bath for 3 and 5 min, a NMDA, AMPA or kainate was added for 2 s in the presence of 20-HE. When the drug was applied in the bath using the perfusion system, an exchange of the solution was completed within 3 min.
Data analyses

Statistical significance was calculated by Student’s t-test. Results are presented as the mean ± S.E.M. For fitting the dose-response curves, Hill plots were used according to the following formula: $R_{\text{exp}} = R_{\text{max}} / \{1 + (\text{EC}_{50} / [A])^n\}$, where [A] is the concentration of the agonist, $^n$ is the Hill coefficient, $R_{\text{exp}}$ is the expected response, and $R_{\text{max}}$ is the maximum response.

The effects of 20-HE on NMDA-, AMPA- and kainate-induced currents were expressed in percent, defined as $(I'/I) \times 100\%$, where $I'$ and $I$ are the agonist-induced current in the presence and absence of 20-HE, respectively.

RESULTS

Glutamate receptor subtype agonist-induced current in rat RGC

NMDA at concentrations of 3 μM – 1 mM induced inward currents in a concentration-dependent manner (Fig. 1: A and B). NMDA (300 μM) evoked an inward current with

![Fig. 1. NMDA-induced current in RGC in the absence of Mg²⁺ and the presence of glycine (10 μM) and strychnine (10 μM). A: Typical current traces induced by various concentrations of NMDA under voltage clamp at −60 mV. The bar above the traces indicates the period of NMDA application. The mean peak amplitude of NMDA (300 μM)-induced current was 224.3 ± 64.6 pA (n = 10). The $\tau_{\text{fast}}$ and $\tau_{\text{slow}}$ of NMDA (300 μM)-induced currents were 134.1 ± 27.3 (n = 5) and 675.8 ± 174.5 ms (n = 5), respectively. B: The concentration-response relationship of NMDA-induced current. The abscissa and ordinate represent the concentration of NMDA on a logarithmic scale and the normalized amplitude, respectively. Each point and bar represent the mean and S.E.M. (n = 4 – 16). The peak amplitude of the current was normalized to the response of NMDA (300 μM) in each cell (dotted circle). The estimated EC$_{50}$ value was 32.4 μM. C: NMDA (300 μM)-induced current at various holding potentials. The bar above the traces indicates the period of NMDA application. D: Current-voltage relationship of NMDA-induced current. The abscissa and ordinate represent the holding potential (mV) and the peak amplitude (pA) of NMDA (300 μM)-induced current, respectively. The current-voltage relationship of the NMDA (300 μM)-induced current was linear between −80 and +60 mV, with an equilibrium potential of about 0 mV (closed circles). In Mg²⁺ (1.1 mM)-containing solution, the NMDA-induced current was inhibited at more hyperpolarized potentials than −20 mV (open circles).]
a mean peak amplitude of \(224.3 \pm 64.6 \, \text{pA} \) (\(n = 10\)) in the absence of Mg\(^{2+}\) at \(-60 \, \text{mV}\). The estimated EC\(_{50}\) was 32.4 \(\mu\text{M}\) (Fig. 1B). The current-voltage relationship of the NMDA-induced current was linear, with a reversal potential of around 0 mV in the absence of Mg\(^{2+}\) (Fig. 1: C and D). In the presence of Mg\(^{2+}\) (1.1 mM), the NMDA (300 \(\mu\text{M}\))-induced currents were blocked at more hyperpolarized potentials than \(-20 \, \text{mV} \) (\(n = 3\)) (Fig. 1D). The \(\tau_{\text{fast}}\) and \(\tau_{\text{slow}}\) of NMDA (300 \(\mu\text{M}\))-induced currents were 134.1 \(\pm\) 27.3 (\(n = 5\)) and 675.8 \(\pm\) 174.5 ms (\(n = 5\)), respectively. AMPA (0.3 \(\mu\text{M} - 10 \, \text{mM}\)) also induced concentration-dependent inward currents (Fig. 2: A and B). The maximum amplitude of the AMPA (3 mM)-induced inward currents was 823.7 \(\pm\) 265.1 pA (\(n = 7\)). The EC\(_{50}\) was estimated as 46.5 \(\mu\text{M}\) (Fig. 2B). The current-voltage relationship of the AMPA (300 \(\mu\text{M}\))-induced current was linear, with a reversal potential of around 0 mV (Fig. 2: C and D). The \(\tau_{\text{fast}}\) and \(\tau_{\text{slow}}\) of AMPA (300 \(\mu\text{M}\))-induced currents were 31.3 \(\pm\) 7.9 (\(n = 5\)) and 1460.9 \(\pm\) 526.9 ms (\(n = 5\)), respectively. Concentration-dependent inward currents was also observed with kainate (1 \(\mu\text{M} - 3 \, \text{mM}\)) (Fig. 3: A and B). The maximum current induced by 1 mM kainate was

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**Fig. 2.** AMPA-induced current in RGC under voltage clamp at \(-60 \, \text{mV}\). A: Typical current traces induced by various concentrations of AMPA. The bar above the traces indicates the period of AMPA application. The mean peak amplitude of AMPA (3 mM) was 823.7 \(\pm\) 265.1 pA (\(n = 7\)). The \(\tau_{\text{fast}}\) and \(\tau_{\text{slow}}\) of AMPA (300 \(\mu\text{M}\))-induced currents were 31.3 \(\pm\) 7.9 (\(n = 5\)) and 1460.9 \(\pm\) 526.9 ms (\(n = 5\)), respectively. B: The concentration-response relationship of AMPA-induced current. The abscissa and ordinate represent the concentration of AMPA on a logarithmic scale and the normalized amplitude, respectively. Each point and bar represent the mean and S.E.M. (\(n = 3 - 9\)). The peak amplitude of current was normalized to the response of AMPA (100 \(\mu\text{M}\)) in each cell (dotted circle). The estimated EC\(_{50}\) value was 46.5 \(\mu\text{M}\). C: AMPA (300 \(\mu\text{M}\))-induced current at various holding potentials. The bar above the traces indicates the period of AMPA application. D: Current-voltage relationship of AMPA-induced current. The abscissa and ordinate represent the holding potential (mV) and the peak amplitude (pA) of AMPA (300 \(\mu\text{M}\))-induced current, respectively. The current-voltage relationship of the AMPA (300 \(\mu\text{M}\))-induced current was linear between \(-120\) and \(+60 \, \text{mV}\), with an equilibrium potential of about 0 mV.
1001.3 ± 191.6 pA (n = 5), the estimated EC<sub>50</sub> being 43.9 μM (Fig. 3B). In a similar tendency to AMPA, the current-voltage relationship of the kainate (100 μM)-induced currents was linear with a reversal potential of around 0 mV (Fig. 3: C and D). When NMDA, AMPA and kainate was applied in the same 3 RGC, all 3 neurons responded to three glutamate-receptor subtype agonists. NMDA (200 μM)-induced currents were blocked by MK-801 (10 μM) in all 4 neurons tested, and complete recovery of the current was detected 5 – 6 min after washing with the external solution (Fig. 4A). The blockade of the AMPA (30 μM)- and kainate (30 μM)-induced currents was observed in the presence of CNQX (10 μM) in all 4 neurons tested, and recovery of the current was also obtained 5 – 6 min after washing with the external solution (Fig. 4: B and C).

**Inhibitory effect of 20-HE**

When 20-HE (100 μM) was added to the bath 3 min before the application of NMDA, the peak amplitude of the current induced by NMDA (100 μM) applied using U-tube was not affected during application of 20-HE. However, when 20-HE (100 μM) was added to the bath 5 min prior to the application of NMDA, the peak amplitude of NMDA (100 μM)-induced currents was significantly (P<0.01) reduced to 63.9 ± 5.9% (n = 7) of the control in the pres-

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**Fig. 3.** Kainate-induced current in RGC under voltage clamp at −60 mV. A: Typical current traces induced by various concentrations of kainate. The bar above the traces indicates the period of kainate application. The mean peak amplitude of kainate (1 mM)-induced current was 1001.3 ± 191.6 pA (n = 5). B: The concentration-response relationship of kainate-induced current. The abscissa and ordinate represent the concentration of kainate on a logarithmic scale and the normalized amplitude, respectively. Each point and bar represent a mean and S.E.M. (n = 3 – 9). The peak amplitude of current was normalized to the response of kainate (100 μM) in each cell (dotted circle). The estimated EC<sub>50</sub> value was 43.9 μM. C: Kainate (100 μM)-induced current at various holding potentials. The bar above the traces indicates the period of kainate application. D: Current-voltage relationship of kainate-induced current. The abscissa and ordinate represent the holding potential (mV) and the peak amplitude (pA) of kainate (100 μM)-induced current, respectively. The current-voltage relationship of the kainate (100 μM)-induced current was linear between −120 and +60 mV, with an equilibrium potential of about 0 mV.
ence of 20-HE (100 μM) (Fig. 5A). The inhibitory effect of 20-HE on the NMDA-induced current was dose-dependent, and the maximum effect was observed at 100 μM. However, although AMPA- and kainate-induced currents was also inhibited by 20-HE applied 5 min before AMPA and kainate application, inhibitory effects of 20-HE on AMPA- and kainate-induced current were less potent than that on NMDA-induced current: AMPA (300 μM)- and kainate (100 μM)-induced current were reduced to 82.3 ± 3.9% (n = 8) and 93.0 ± 1.6% (n = 7) of the control in the presence of 20-HE (100 μM), respectively (Fig. 5B).

**DISCUSSION**

Since methods for separation of RGC using MACS involve various complex procedures such as use of anti-Thy-1 antibody, iron beads and magnets, these procedures might impair receptor functions. However, in the present study, RGC thus separated and cultured were confirmed to possess NMDA, AMPA and kainate receptors that responded to the respective receptor agonists. The properties of the NMDA receptor of our RGC neurons were similar to those reported for RGC neurons previously (8). The NMDA-induced current was blocked by Mg²⁺ and MK-801. The EC₅₀ of NMDA for inducing current in the RGC (32.4 μM) was similar to that in cultured cortical neurons (22.5 μM).
and hippocampal neurons (40.8 – 88.6 μM) in Mg\(^{2+}\)-free medium (26, 27). Furthermore, the RGC neurons we isolated also responded to both AMPA and kainate in manners similar to those observed in RGC that were separated and cultured using other methods (7, 8). In addition, the findings that our RGC had both a fast and slow desensitization phase in NMDA-induced current are in line with the observations by others for the visual cortex (28), hippocampal neurons (26) and cerebellar granule cells (29). These findings suggest that the MACS method does not impair the function of the glutamate-receptor subtypes. Therefore, this separation method is useful for obtaining highly enriched RGC, as described previously (6).

In addition, the present study has demonstrated the coexistence of three glutamate-receptor subtypes, NMDA, AMPA and kainate receptors in the same neurons and respective neurons. NMDAR1/NMDAR2A have been reported to exist in the retinal ganglion cell layer (30) and possess high sensitivity to Mg\(^{2+}\) and MK-801 (31). Since the NMDA-induced current in our MACS-separated RGC was highly sensitive to Mg\(^{2+}\) and MK-801, these RGC may have NMDAR1/NMDAR2A.

A variety of neurosteroids such as pregnenolone, 3α5α-tetrahydrodeoxycorticosterone (THDOC) and DHEA are known to exist in the brain and to modulate brain function; and they also to exist in the retina, although the functional role of neurosteroids in the retina remains unknown (32). Neurosteroids show a variety of actions and sometimes opposite effects in neurons. For instance, 3α-ol-5β-pregn-20-one hemisuccinate (3α5βHIS) inhibits the NMDA-induced current in hippocampal neurons (33) and 3α-hydroxy-5β-pregn-20-one sulfate (5β3αS) also inhibits all NMDA-, AMPA- and kainate-induced currents in chick spinal cord neurons (34). However, pregnenolone enhances the NMDA-induced current and inhibits the AMPA- and kainate-induced currents in chick spinal cord neurons and recombinant receptor in oocytes (18, 35). However, there are no available data on neurosteroids effects on RGC. In the present study, 20-HE was first found to most potently inhibit the NMDA-induced current of our RGC, and they

![Fig. 5. Effects of 20-HE on glutamate receptor subtype agonists-induced currents in the presence of 20-HE (100 μM) application. A: Effects of 20-HE on NMDA-induced currents in RGC under voltage clamp at −60 mV. Bars above the traces indicate the periods of 20-HE and NMDA application. 20-HE (100 μM) was applied in the bath for 5 min, and then NMDA (100 μM) was applied for 2 s with 20-HE in the presence of glycine (10 μM) and strychnine (10 μM). B: Concentration-response relationships of inhibitory effects of 20-HE on NMDA (closed circles)-induced currents. Effects of 20-HE (100 μM) on AMPA (300 μM) (closed square) and kainate (100 μM) (open circle)-induced current are also shown. The abscissa and ordinate represent the concentration of 20-HE on a logarithmic scale and the percentage of the control value, respectively. Each point and bar represent the mean and S.E.M. (n = 4 – 8). *P<0.05, **P<0.01, significantly different from the control (paired t-test).]
were found to inhibit the AMPA- and kainate-induced currents with much less potency. Since NMDA receptors are known to possess neurosteroid-binding sites in the neurons (19), 20-HE may bind with such binding sites of NMDA receptors in our RGC and thereby inhibit the NMDA-induced current, which causes an increase in intracellular Ca\(^{2+}\) concentration, leading to the neuronal death. However, since a relatively long time (5 min) incubation of 20-HE for inhibiting the NMDA and/or non-NMDA-induced current was required, the possibility that 20-HE produced such inhibition via an intracellular, second messenger system could not completely be excluded. In conclusion, 20-HE may be useful for preventing glutamate-induced RGC death, which is one of the possible pathogenic causes of glaucoma, together with an enhancement of the effects of GABA. In the next step, it is necessary to determine the molecular mechanism underlying the inhibitory effects of 20-HE on NMDA-induced current as well as ability of 20-HE to inhibit the glutamate-induced neuronal death.

REFERENCES

1 Garcia-Valenzuela E, Shareef S, Walsh J and Sharma SC: Programmed cell death of retinal ganglion cells during experimental glaucoma. Exp Eye Res 61, 33 – 44 (1995)
2 Quigley HA, Nickells RW, Kerrigan LA, Pease ME, Thibault DJ and Zack DJ: Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. Invest Ophthalmol Vis Sci 36, 774 – 786 (1995)
3 Lucas DR and Newhouse JP: The toxic effect of sodium L-glutamate on the inner layers of the retina. Arch Ophthalmol 58, 193 – 201 (1957)
4 Olney JW, Ho OL and Rhee V: Cytotoxic effects of acidic and sulphur containing amino acids on the infant mouse central nervous system. Exp Brain Res 14, 61 – 76 (1971)
5 Dreyer EB, Zurakowski D, Schumer RA, Podos SM and Lipton SA: Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. Arch Ophthalmol 114, 299 – 305 (1996)
6 Shoge K, Mishima HK, Mukai S, Shinya M, Ishihara K, Kanno M and Sasa M: Rat retinal ganglion cells culture enriched with the magnetic cell sorter. Neurosci Lett 259, 111 – 114 (1999)
7 Leinders-Zufall T, Rand MN, Waxman SG and Koosis JD: Differential role of two Ca\(^{2+}\)-permeable non-NMDA glutamate channels in rat retinal ganglion cells: kainate-induced cytoplastic and nuclear Ca\(^{2+}\) signals. J Neurophysiol 72, 2503 – 2516 (1994)
8 Taschenberger H, Engert F and Grantyn R: Synaptic current kinetics in a solely AMPA-receptor-operated glutamatergic synapse formed by rat retinal ganglion neurons. J Neurophysiol 74, 1123 – 1136 (1995)
9 Hamassaki-Britto DE, Hermans-Borgmeyer I, Heinemann S and Hughes TE: Expression of glutamate receptor genes in the mammalian retina: The localization of GluR1 through GluR7 mRNAs. J Neurosci 13, 1888 – 1898 (1993)
10 Kikuchi M, Kashii S, Honda Y, Ujihara H, Sasa M, Tamura Y and Akaike A: Protective action of zine against glutamate neurotoxicity in cultured retinal neurons. Invest Ophthalmol Vis Sci 36, 2048 – 2053 (1995)
11 Shoge K, Mishima HK, Saitoh T, Ishihara K, Tamura Y, Shiomi H and Sasa M: Attenuation by PACAP of glutamate-induced neurotoxicity in cultured retinal neurons. Brain Res 839, 66 – 73 (1999)
12 Kaneda K, Kikuchi M, Kashii S, Honda Y, Maeda T, Kaneko S and Akaike A: Effects of B vitamins on glutamate-induced neurotoxicity in retinal cultures. Eur J Pharmacol 322, 259 – 264 (1997)
13 Shoge K, Mishima HK, Saitoh T, Ishihara K, Tamura Y, Shiomi H and Sasa M: Protective effects of vasoactive intestinal peptide against delayed glutamate neurotoxicity in cultured retina. Brain Res 809, 127 – 136 (1998)
14 Pang I-H, Wexler EM, Nawy S, DeSantis L and Kapin MA: Protection by eliprodil against excitotoxicity in cultured rat retinal ganglion cells. Invest Ophthalmol Vis Sci 40, 1170 – 1176 (1999)
15 Kapin MA, Doshi R, Scatton B, DeSantis LM and Chandler ML: Neuroprotective effects of eliprodil in retinal excitotoxicity and ischemia. Invest Ophthalmol Vis Sci 40, 1177 – 1182 (1999)
16 Saitoh T, Mishima HK, Shoge K, Ishihara K and Sasa M: Protection against glutamate neurotoxicity in retinal cultures by acidic conditions. Jpn J Pharmacol 76, 87 – 95 (1998)
17 Kimonides VG, Khatibi NH, Svendsen CN, Sofroniew MV and Herbert J: Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEAS) protect hippocampal neurons against excitatory amino acid-induced neurotoxicity. Proc Natl Acad Sci 95, 1852 – 1857 (1998)
18 Yaghoubi N, Malavay A, Russek SJ, Gibbs TT and Farb DH: Neurosteroid modulation of recombinant ionotropic glutamate receptors. Brain Res 803, 153 – 160 (1998)
19 Park-Chung M, Wu F-S, Purdy RH, Malavay AA, Gibbs TT and Farb DH: Distinct sites for inverse modulation of N-methyl-D-aspartate receptors by sulfated Steroids. Mol Pharmacol 52, 1113 – 1123 (1997)
20 Truman JW, Talbot WS, Fahrbach SE and Hogness DS: Ecdysone receptor expression in the CNS correlates with stage-specific responses to ecdysteroids during Drosophila and Manduca development. Development 120, 219 – 234 (1994)
21 Prugh J, Croce KD and Levine RB: Effects of the steroid hormone, 20-hydroxyecdysone, on the growth of neurites by identified insect motoneurons in vitro. Dev Biol 154, 331 – 347 (1992)
22 Tsujiyama S, Ujihara H, Ishihara K and Sasa M: Potentiation of GABA-induced inhibition by 20-hydroxyecdysone, a neurosteroid, in cultured rat cortical neurons. Jpn J Pharmacol 68, 133 – 136 (1995)
23 Okada M, Ishihara K, Sasa M, Izumi R, Yajin K and Harada Y: Enhancement of GABA-mediated inhibition of rat medial vestibular nucleus neurons by the neurosteroid 20-hydroxyecdysone. Acta Otolaryngol 118, 11 – 16 (1998)
24 Hanaya R, Sasa M, Ishihara K, Akimitsu T, Iida K, Amano T, Serikawa T, Arita K and Kurisu K: Antiepileptic effects of 20-hydroxyecdysone on convulsive seizures in spontaneously epileptic rats. Jpn J Pharmacol 74, 331 – 335 (1997)
25 Ujihara H and Albuerque EX: Ontogeny of N-methyl-D-aspartate-induced current in cultured hippocampal neurons. J Pharmacol Exp Ther 263, 859 – 867 (1992)
26 Ishihara K, Alkondon M, Montes JG and Albuquerque EX: Ontogenically related properties of N-methyl-D-aspartate receptors in rat hippocampal neurons and the age-specific sensitivity of developing neurons to lead. J Pharmacol Exp Ther 273, 1459 – 1470 (1995)
27 Mealing GAR, Lanthorn TH, Small DL, Black MA, Laferriere NB and Morley P: Antagonism of N-methyl-D-aspartate-evoked currents in rat cortical cultures by ARL 15896AR. J Pharmacol Exp Ther 281, 376 – 383 (1997)
28 Carmignoto G and Vicini S: Activity-dependent decrease in NMDA receptor responses during development of the visual cortex. Science 258, 1007 – 1011 (1992)
29 Overstreet LS, Kinney GA, Liu Y-B, Billups D and Slater NT: Glutamate transporters contribute to the time course of synaptic transmission in cerebellar granule cells. J Neurosci 19, 9663 – 9673 (1999)
30 Watanabe M, Mishina M and Inoue Y: Differential distributions of the NMDA receptor channel subunit mRNAs in the mouse retina. Brain Res 634, 328 – 332 (1994)
31 Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M, Akazawa C, Shigemoto R, Mizuno N, Masu M and Nakanishi S: Molecular characterization of the family of the N-methyl-D-aspartate receptor subunits. J Biol Chem 268, 2836 – 2843 (1993)
32 Guarneri P, Guarneri R, Cascio C, Pavasant P, Piccoli F and Papadopoulos V: Neurosteroidogenesis in rat retina. J Neurochem 63, 86 – 96 (1994)
33 Weaver CE, Marek P, Park-Chung M, Tam SW and Farb DH: Neuroprotective activity of a new class of steroidal inhibitors of the N-methyl-D-aspartate receptor. Proc Natl Acad Sci 94, 10450 – 10454 (1997)
34 Park-Chung M, Wu F-S and Farb DH: 3α-Hydroxy-5β-pregn-20-one sulfate: A negative modulator of the NMDA-induced current in cultured neurons. Mol Pharmacol 46, 146 – 150 (1994)
35 Wu F-S, Gibbs TT and Farb DH: Pregnenolone sulfate: A positive allosteric modulator at the N-methyl-D-aspartate receptor. Mol Pharmacol 40, 333 – 336 (1991)