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Tech-Trends Application Note Volume 4, Series 8

Functional evidence for D-serine inhibition of non-N-methyl-D-aspartate ionotropic glutamate receptors in retinal neurons

D-Serine is an important signaling molecule throughout the central nervous system, acting as an N-methyl-D-aspartate (NMDA) receptor coagonist. This study investigated the D-serine modulation of non-NMDA ionotropic glutamate receptors expressed by inner retinal neurons.

It was first identified that the degradation of endogenous retinal D-serine, by application of D-amino acid oxidase, caused an enhancement of kainate- and a-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptor-mediated calcium responses from the ganglion cell layer of the isolated rat retina and light-evoked responses obtained by multi-electrode array recordings from the guinea pig retina. Approximately 30–45% of cells were endogenously inhibited by D-serine, as suggested by the effect of D-amino acid oxidase. Conversely, bath application of D-serine caused a reduction in multi-electrode array recorded responses and decreased kainate, but not potassium-induced calcium responses, in a concentration-dependent manner (IC50, 280 lm). Using cultured retinal ganglion cells to reduce network influences, D-serine reduced kainate-induced calcium responses and AMPA induced whole-cell currents. Finally, the inhibitory effect of D-serine on the kainate-induced calcium response was abolished by IEM 1460, thereby identifying calcium-permeable AMPA receptors as a potential target for D-serine. To the author's knowledge, this is the first study to address specifically the effect of D-serine on AMPA/kainate receptors in intact central nervous system tissue, to identify its effect on calcium permeable AMPA receptors and to report the endogenous inhibition of AMPA/kainate receptors.

ECM 830 Square Wave Generator
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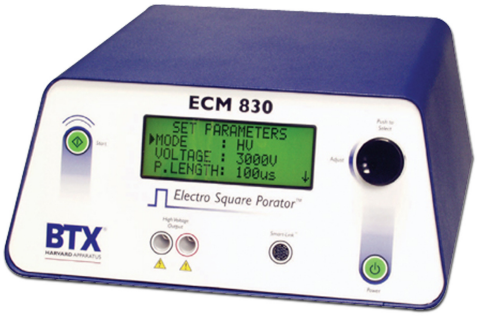

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Methods

Pre-electroporation:
For calcium imaging experiments, fura-2 pentapotassium salt (Invitrogen) solution was electroporated into the retina as described previously (Yu et al., 2009; Daniels & Baldridge, 2010). Eyes were removed quickly and 4 IL of 22 mm fura-2 was injected into the vitreous through the optic nerve head.

Electroporation protocol:
Tweezertrodes (BTX, Holliston, MA, USA) were positioned on the eye (anode on the anterior pole, cathode on the posterior pole) and five 40 V square-wave pulses were applied for 50 ms at 1 Hz using the ECM 830 electroporation system (BTX).

Post-electroporation:
The retina was dissected out under red light in room temperature Hank's balanced salt solution (HBSS) bubbled with 100% oxygen. Each retina was cut into two to four pieces and mounted separately onto black filter paper (Millipore, Bedford, MA, USA), ganglion cell layer up, and left in oxygenated HBSS for 15–30 min, to allow for recovery from the procedure, before transfer to the superfusion chamber for calcium imaging.

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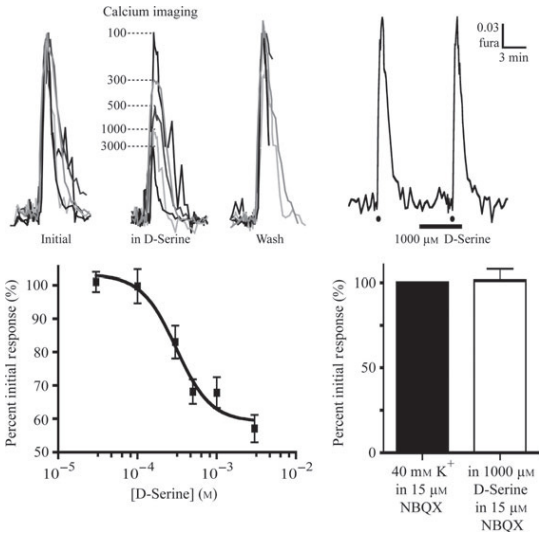
Results



Kainate- but not potassium-induced calcium responses are reduced by D-serine in ganglion cell layer cells in isolated rat retina. (A) Representative fura-2 traces, normalized to the initial 50 lm kainate-induced calcium response, from individual cells from separate experiments. The second response was obtained in the presence of D-serine (lm concentration is noted to the left of the peak of each trace). The third response was obtained after washout of D-serine. (B) Dose–response curve for D-serine inhibition of 50 lm kainate-induced calcium responses (mean ± SEM). Each concentration was tested in separate experiments with subsequent washout (i.e. third peak in A; n = 5–9, 102–195 cells/concentration). For graphing purposes, 10)4.5 m was used as the 0 M concentration and the data were fit with a variable slope sigmoidal dose–response function, r2 = 0.9663. (C) Example trace of consecutive 40 mm potassium-induced (20 s, black circles) calcium responses. The second potassium-induced response was obtained in the presence of 1000 lm D-serine (black bar). (D) Mean + SD normalized peak potassium-induced calcium responses in the absence (left) and presence (right) of 1000 lm D-serine. There was no significant difference between these two responses (P = 0.47, n = 5, from 138 cells, paired t-test). For potassium experiments, 15 lm NBQX was present in all solutions.

Conclusion

Glutamate, the primary excitatory neurotransmitter in the central nervous system, inhibits ON bipolar cells in the retina by acting at mGluR6 metabotropic glutamate receptors and has recently been shown to enhance glycinergic inhibitory currents in these cells (Liu et al., 2010), presumably by allosteric modulation. The findings presented here argue that D-serine can inhibit non-NMDAR iGluRs, opposing its well-documented excitatory effect on NMDAR-dependent glutamatergic neurotransmission.