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# TRANSIENT COUPLING OF NMDA RECEPTOR WITH $ip_3$ PRODUCTION IN CULTURED CELLS OF THE AVIAN RETINA

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**Abstract**—The mobilization of inositol triphosphate $ip_3$ by *N*-methyl D-aspartate (NMDA) and kainate, two excitatory amino acid EAA receptor agonists, was studied in cultured chick retina cells as a function of culture differentiation. Kainate ($EC_{50}$ = 30 $\mu$M) stimulated from 6 to 9-fold the production of [$^3$H]$ip_3$ between E8C3 (embryonic day 8 plus 3 days *in vitro*) and E8C13. The kainate response was blocked by CNQX (100 $\mu$M) by more than 80% until stage E8C9. MK-801, however, was totally ineffective in preventing the kainate induced $ip_3$ generation. [$^3$H]$ip_3$ production evoked by NMDA was increased 4-fold above basal levels at E8C3. As cultures differentiated, [$^3$H]$ip_3$ production promoted by NMDA decreased to 2.5-fold at E8C6 to 1.6-fold the basal levels in cultures at later stages of differentiation. The removal of $Mg^{2+}$ from the incubating medium at E8C3 increased the NMDA mediated [$^3$H]$ip_3$ production by 80%. However, at more differentiated stages of the cultures, when cells were not responsive to NMDA, removal of $Mg^{2+}$ plus the addition of 1 mM glycine did not change the pattern of the response. Although NMDA mediated $ip_3$ production is almost absent in more differentiated cultures, NMDA is able to induce [$^3$H]GABA release in E8C3 and E8C13 cultures with characteristics that reflect typical NMDA receptor activation: it is highly potentiated by the absence of $Mg^{2+}$ and by the presence of glycine. The NMDA induced production of [$^3$H]$ip_3$ at E8C3 was entirely blocked by MK-801 (100 $\mu$M) and APV (100 $\mu$M) but not by CNQX. Cultured cells stimulated with kainate or NMDA in the absence of extracellular $Ca^{2+}$ and in the presence of 2 mM EGTA were unable to mobilize [$^3$H]$ip_3$. The present study shows that NMDA, but not kainate induced $ip_3$ production, is present only in undifferentiated cultures, suggesting the existence of an embryological window during which NMDA receptors, coupled to $ip_3$ production, might signal differentiation cues during retina development.

Glutamate and aspartate are considered the major excitatory neurotransmitters acting in the central nervous system (Monaghan *et al.*, 1989). In the last decade, several groups established the existence of at least five different receptors for the excitatory amino acids (EAA). These receptors can be divided into two groups based on their mode of action, a fast acting ionotropic receptor, and a slow acting metabotropic receptor. In the first group there are at least three subtypes of receptors that respond selectively to the agonists NMDA (*N*-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate, each associated with different ion channels responsible for fast depolarizing ionic currents. In the second group there is evidence of receptors that respond to quisqualate or *trans*-ACPD generating second messengers in several areas of the CNS. However, the effects of these two groups can be interchangeable (Récasens *et al.*, 1991). Thus, ionotropic receptors can generate second-messengers and metabotropic receptors modulate changes in ionic conductances (Lester and Jahr, 1990; Schoepp *et al.*, 1990).

One of the glutamate metabotropic receptors (Tanabe *et al.*, 1992) is coupled to a second messenger generating system dependent on phospholipase C activation. This results in cleavage of the membrane phospholipid phosphatidylinositol-4,5-biphosphate and production of $ip_3$, which modulates the increase of intracellular $Ca^{2+}$ from endogenous sources (Berridge

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