Kainate-Triggered Currents in *Xenopus* Oocytes Injected with Chick Retinal Membrane Fragments: Effect of Guanine Nucleotides

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Purpose. To electrophysiologically characterize α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate receptors in chick retinal membrane fragments, incorporated into *Xenopus* oocytes by direct microinjection.

Methods. A 6-day retinal membrane suspension was injected into *Xenopus* oocytes by use of an electronic nanoliter injector. Fifteen to 40 hours after injection, the oocytes were assayed for kainate-elicited inward currents, under voltage-clamp conditions (membrane potential held at −70 mV). The structural incorporation of the retinal membrane fragments into the oocyte membrane was verified by specific immunofluorescent staining.

Results. Chick retinal membrane fragments were efficiently grafted onto *Xenopus* oocytes after microinjection, with 22.9% ± 7.6% of the oocyte membrane being stained with anti-chick retina antibody. Part of the retinal material was seen as patches of relatively uniform size (292.1 ± 72.3 μm^2). Bath-applied kainate induced dose-dependent (EC_{50}: 64 ± 7 μM), nondesensitizing inward currents (15–90 nA) in the chimeric *Xenopus* oocytes. Sham-injected oocytes did not respond to kainate. Kainate-driven currents were blocked by 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 1-(4-aminopropyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine hydrochloride (GYKI 52466), but not by γ-D-glutamylaminomethyl sulfonic acid (GAMS) or aminophosphonoheptanoate (AP7), suggesting the involvement of AMPA receptors in the observed responses. Guanine nucleotides (GNs) also blocked kainate currents in a concentration-dependent manner.

Conclusions. An alternative oocyte microinjection technique to analyze the electrophysiological properties of glutamate receptors in chick retinal membranes is described. The results show the functional activity of putative AMPA-preferring receptors from chick retina and confirm, in the chick retinal model, the antagonistic behavior of guanine nucleotides toward glutamate receptors and their potential role as neuroprotective agents under excitotoxic conditions. (*Invest Ophthalmol Vis Sci.* 2003;44:3124–3129) DOI:10.1167/iovs.02-0669

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The ex vivo chick embryonic retinal preparation has won wide acceptance as a convenient and versatile model system for excitotoxicity and neuroprotection studies.[1–10] In this experimental context, we have recently shown that guanine nucleotides protect against kainate- and *N*-methyl-D-aspartate (NMDA)–induced damage, and block AMPA- and NMDA-driven $^{45}Ca^{2+}$ influx, in 13-day chick embryonic retinal explants.[7,8] These results add to the accumulated evidence on the antagonistic behavior of guanine nucleotides (GNs) at ionotropic glutamate receptors, in very diverse experimental setups, including agonist displacement, electrophysiological recording and neuroprotection paradigms.[7,8,11–20] To further characterize the antagonistic behavior of GNs toward ionotropic glutamate receptors in chick retina we injected newborn chick retinal membrane microfragments into *Xenopus* oocytes and measured the effect of GNs on currents elicited by kainate under voltage-clamp conditions. This novel oocyte microinjection technique has been shown to result in a quick and efficient incorporation of foreign receptor channels into the oocyte membrane, in their own natural molecular environment, so that inward cationic current responses are readily recorded on exposure of the chimeric oocyte to the specific agonist.[21,22]

Materials and Methods

Chick Retinal Membrane Preparation

All experiments with animals (chicks, *Xenopus*, and rabbits) followed our institutional guidelines for care and handling of laboratory animals, in full agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Six-day-old white leghorn chicks were used as the source of retinal membrane fragments. Lysed membranes, prepared as described,[7] were resuspended in 10 mM HEPES (pH 7.4), containing 140 mM KCl and 20 mM NaCl, at a protein concentration of 2 mg/mL, stored in liquid nitrogen, and thawed and sonicated for 10 seconds in an ice water bath just before injection. A control solution without membranes was similarly processed and used for sham-injected control chicks.

Oocyte Preparation and Injection

Mature female *Xenopus laevis* were obtained from the Centre d'Elevage des Xénopes, CRBM (Montpellier, France), and kept in chlorine-free fresh water, at 22°C. Discrete ovary portions were removed from anesthetized frogs[23] and stage-V/VI oocytes[24] were individually dissected and kept, at 15°C to 17°C, in sterile modified Barth's solution (10 mM HEPES [pH 7.4], 88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, and 2.4 mM $NaHCO_3$) supplemented with penicillin (100 IU/mL) and streptomycin (0.1 mg/mL). Oocytes were further treated with collagenase (clostridiopeptidase A: EC3.4.24.3; type IA, 0.5 mg/mL; Sigma-Aldrich, St. Louis, MO), for 50 minutes at room temperature, to remove enveloping cells.[25]

Healthy-looking oocytes from different donors were microinjected with 100 nL of a chick retinal membrane suspension, prepared as just described, by use of an electronic nanoliter injector (model A203XVZ; WPI, Sarasota, FL).

Preparation of Polyclonal Antibodies against Chick Retinal Membranes

A 2-mg/mL suspension of chick retinal membranes[7] was mixed (1:1) with Freund's adjuvant (complete for the first and incomplete for successive injections), sonicated for 10 seconds, and injected intradermally to two rabbits (preimmune serum samples were obtained just before immunization). The rabbits received three additional subcutaneous antigen-adjuvant injections, at monthly intervals, and blood samples were then extracted by venous puncture. After coagulation (1 hour at room temperature plus 24 hours at 4°C) and centrifugation, the immune (and preimmune) sera were preabsorbed with chick liver acetone powder[26] and frozen in small aliquots.

Identification of Chick Retinal Membrane Fragments Incorporated into the Oocyte Membrane

Oocytes for immunocytochemistry were prepared as described, except that, after collagenase treatment and injection, they were incubated for 24 hours in Barth's solution and for another 24 hours in the same solution containing 1 mg/mL bovine serum albumin (BSA), always at 15°C. Oocytes were then fixed in a 4% paraformaldehyde/0.1 M cacodylate buffer [pH 7], solution, for 30 minutes at 4°C, extensively washed with Barth's/BSA (5 mg/mL), incubated with the anti-chick retina antibody (1:100 in Barth's/BSA)—or the pre-immune control serum—washed again, and further incubated with fluorescein isothiocyanate-conjugated goat anti-rabbit antibody, for 1 hour at 4°C. The oocytes were thoroughly washed, as above, and mounted with phosphate-buffered saline-glycerol (1:9, pH 9), containing 10 mg/mL *p*-phenylenediamine, on glass slides, and coverslipped for confocal microscopic observation (Radiance 2000 Laser Scanning Unit; Bio-Rad Laboratories, Hemel Hempstead, UK/Axiovert S100 TV inverted microscope; Carl Zeiss Meditech, Oberkochen, Germany). For quantitative determinations, oocytes were processed in pairs. Both were injected as described, one of them was stained with the specific anti-chick retina antibody and the other with the rabbit preimmune serum (control). Ten such pairs were used for the statistical analyses described in the Results section. Fresh oocytes ranged from 1.2 to 1.5 mm in apparent diameter, and from 1.4 to 1.7 mm after fixation and coverslipping. Confocal sections were taken every 15.1 μm, down to a depth of 377.5 μm, approximately halfway between the upper pole and the equator. Quantitative estimates are therefore extrapolated from approximately 25% of the total oocyte surface. Images shown are integrated projections of all sections on the frontal plane. A number of patches in the central area of the image were selected for planimetric area measurement (Photoshop; Adobe Systems, Mountain View, CA; and Metamorph; Universal Imaging, West Chester, PA), whereas the total chick retinal material grafted onto the oocyte surface was estimated by total color intensity (fluorescein green) determination. In this way all patches (central or peripheral, large or small) were taken into account, and the effect of parallax errors in the more peripheral patches was avoided (higher color intensity compensating for smaller projected area).

Electrophysiology

Membrane current recordings were performed at room temperature (20–22°C), 15 to 40 hours after membrane injection. Oocytes were placed in a 120 μL chamber that was continually perfused with Ringer's solution (5 mM HEPES [pH 7.0], 115 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$). Agonist-induced currents were recorded using a two-electrode voltage clamp configuration.[23] Intracellular electrodes (0.5–5 MΩ resistance) were filled with 3 M KCl for voltage recording and with 3 M K^+ acetate for current injection. The oocyte membrane potential was held at −70 mV. Currents were amplified (Geneclamp 500 Amplifier; Axon Instruments, Burlingame, CA), low-pass filtered at 10 Hz and recorded using either pClamp 5 (Axon Instruments) or the Whole Cell Analysis Program (generously provided by John Dempster, University of Strathclyde, Strathclyde, UK), after sampling with a digitizer (Digidata 1200A; Jessup, MD) at twice the filter frequency.

Dose–response and concentration-effect data were adjusted to a sigmoid curve by nonlinear regression on computer (SigmaPlot; SPSS Sciences, Chicago, IL). Data are expressed as the mean ± SD.

Results

Identification of Chick Retinal Membrane Patches in the Oocyte Plasma Membrane

To verify the structural incorporation of chick retinal membrane fragments into the oocyte plasma membrane we stained the oocyte surface, using standard immunocytochemical procedures, with a polyclonal antibody raised in rabbits against the same chick membrane preparation used for the microinjections. Figure 1A shows patches of fluorescent, antibody-stained plasma membrane, thus confirming the successful integration of the heterologous membrane fragments into the oocyte membrane. Sham-injected oocytes (buffer without the chick membranes) stained with the chick-specific antibody or chick-membrane-injected oocytes stained with rabbit preimmune serum yielded images such as that in Figure 1B.

The stained patches were more or less randomly distributed over the oocyte surface. A more detailed observation of the center field, to minimize curvature-related distortion (Fig. 1C), revealed the presence of a number of rather large patches of comparable size and many smaller stained spots. Planimetric area determinations in 220 large patches taken from all 10 oocytes resulted in a quasi-Gaussian distribution of sizes (Fig. 2), with a mean of 292.1 ± 72.3 μm^2 (n = 220). To put Figure 2 into perspective, we must say that the selected large patches for each oocyte accounted for just 12.1% ± 3.4% (n = 10) of the total grafted chick membrane in this center field. Although the selection was somewhat arbitrary (well-defined patches, with discernible contours, not superimposed), and therefore of limited quantitative value, it appears that much of the chick material was present as clouds of more diffuse, smaller spots. So, to ascertain the total contribution of chick retina to the chimeric oocyte membrane we measured the total color intensity of the image (see the Methods section). In this way, the chick material accounted for 22.9% ± 7.6% (n = 10) of the total oocyte membrane surface.

Functional Incorporation of Kainate-Driven Channels into the Oocyte Plasma Membrane: Effect of Antagonists Including GNs

The correct polarity and functional preservation of the chick membrane microinserts (Fig. 1) was then checked by electrophysiological techniques. Bath application of kainate (KA) to the oocytes microinjected with retinal membranes typically elicited nondesensitizing current responses of amplitude proportional to kainate concentration (Fig. 3, insets). Application of kainate to oocytes injected with the vehicle solution did not produce any responses. Currents recorded in the presence of 100 μM kainate, at a holding potential of −70 mV, varied between 15 and 90 nA for different oocytes. Twenty oocytes from eight different donors were used altogether in the study. Responses to different kainate concentrations, for a given oocyte, have been expressed as a percentage of the response at 100 μM. A kainate dose–response curve obtained in this way

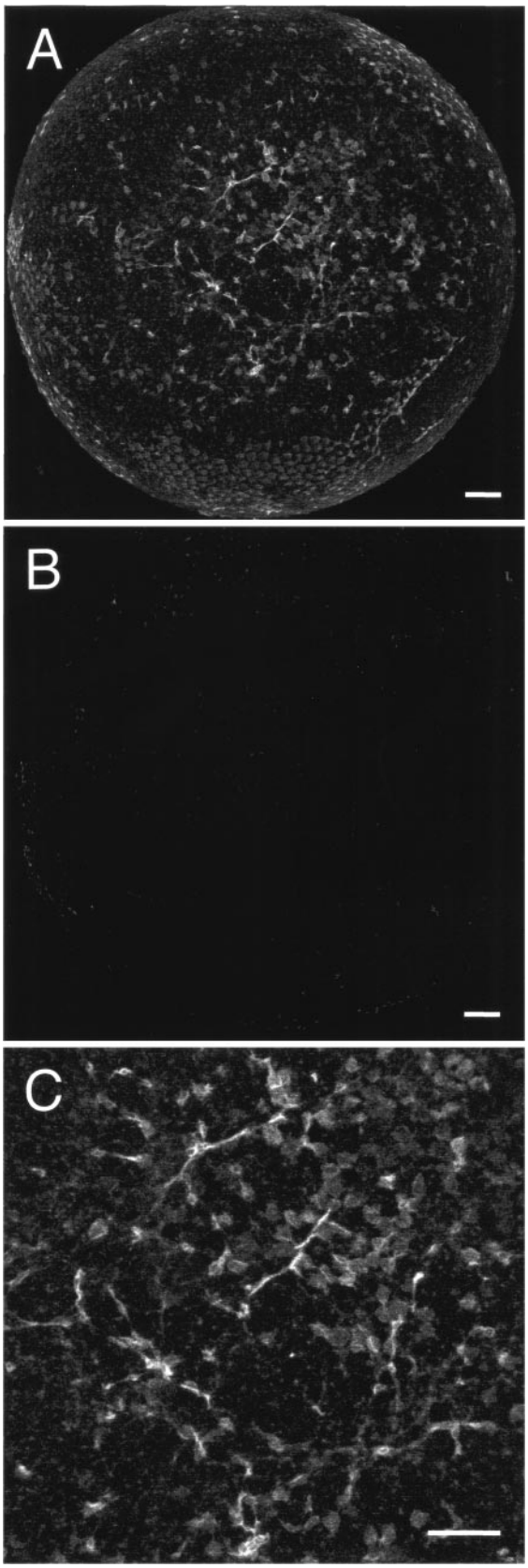

FIGURE 1. Visualization of chick retinal membrane fragments, integrated into the *Xenopus* oocyte plasma membrane on direct membrane microinjection, by specific immunofluorescent staining. (**A**) Integrated projection of confocal sections of a typical injected oocyte. Abundant fluorescent chick retinal membrane patches and small spots, stained with the specific polyclonal anti-chick retina antibody. (**B**) Control oocyte stained with rabbit preimmune serum. (**C**) Magnification ×2 of center field in (**A**). Scale bar, 100 μm.

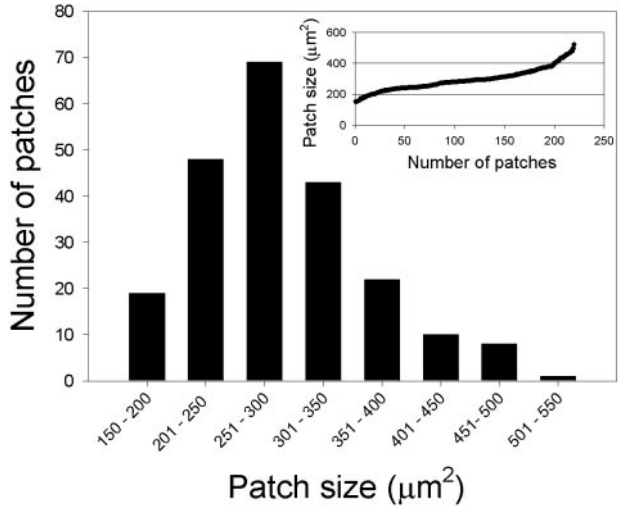

FIGURE 2. Statistical distribution of the areas of 220 large patches selected from the center fields (as in Fig. 1C) of 10 oocytes. Areas were directly measured by planimetric methods. Patch size: 292.1 ± 72.3 μm^2 (mean: ± SD). *Inset*: continuous plot of all measured areas to show better the concentration of values in the 200 to 400 μm^2 range.

from five oocytes is shown in Figure 3. The EC_{50} was estimated at 64 ± 7 μM.

Responses to kainate were totally blocked by DNQX (IC_{50} = 0.4 μM) and GYKI 52466 (IC_{50} = 32 μM), very slightly by GAMS,[27] and not at all by AP7 (Fig. 4).

Altogether, the nondesensitizing responses to kainate[28] and the observed antagonists profile suggest the preferential activation by kainate of AMPA receptor channels.[29,30] This was additionally confirmed by the absence of response to 100 μM methyl glutamate, a selective agonist at kainate receptors[31] (not shown). Direct application of AMPA to the bath did not, however, elicit any detectable response, presumably due to the very fast desensitization of AMPA receptors by their selective agonist.[29,30]

The antagonistic effect of GNs toward kainate-triggered responses is illustrated in Figure 5. GNs with different degrees of phosphorylation (mainly slowly hydrolyzable analogues) consistently blocked responses to 100 μM kainate in a concentration-dependent manner, with IC_{50}s in the 150 μM to 400 μM range.

DISCUSSION

Not only is the neural retina a significant part of the central nervous system (CNS) but, being to some extent a self-contained entity, with sophisticated circuitry and complex pharmacology, it has been often considered an appropriate and useful model of the whole CNS.[6] More to the point, the chick retinal ex vivo preparation affords a simple and versatile experimental system to analyze excitatory amino acid receptor activation as the structural and functional substrate for excitotoxic phenomena, and to assay for novel antagonists with neuroprotective potential.[1–10]

Excitotoxic neuronal cell death has actually been linked to acute (stroke) and chronic neurodegenerative diseases such as Parkinson and Alzheimer diseases and pathologic conditions of

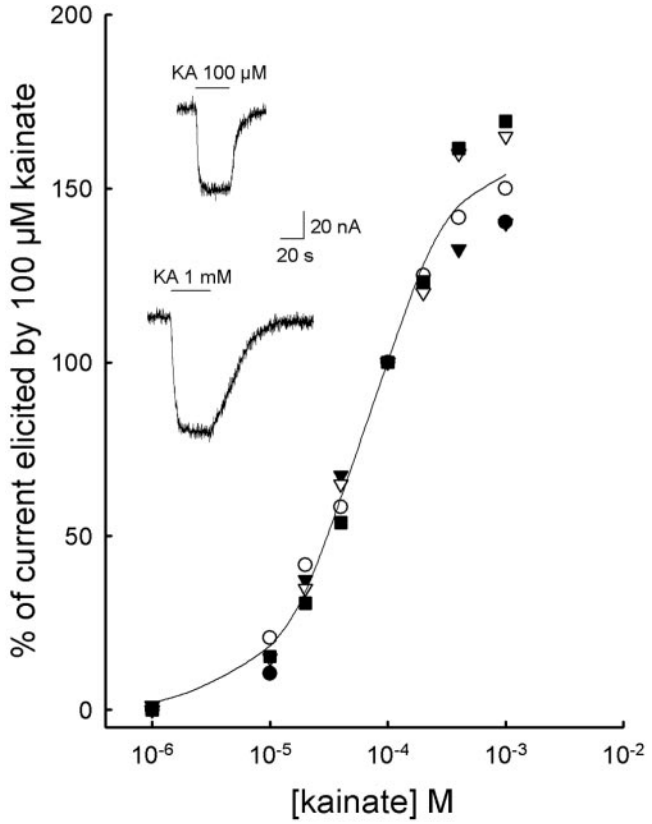

FIGURE 3. Kainate (KA) dose-response relationship in *Xenopus* oocytes injected with chick retinal membranes. Different *symbols* represent different oocytes. *Insets*: Sample current recordings at two kainate concentrations. Kainate application is indicated by *bars*. Downward deflections denote inward currents.

the CNS.[32] In the case of retina (especially the chick retina[1–10,33,34]), most experimental studies using glutamate and analogues as experimental tools address rather basic questions on the mechanisms of excitotoxicity, including ionic dependence, time-course of the toxic process, and the underlying subcellular phenomena. Other studies, more relevant to retinal disease, have shown that excitotoxicity may play a decisive role in the pathogenesis of well-known retinal diseases such as myopia[35] and ischemia- and glaucoma-related neuronal loss.[36]

To extend the validity of the chick retinal model to include the electrophysiological characterization of glutamate receptors and their putative agonists and antagonists, we have taken advantage of a new oocyte injection technique based on the direct injection of neural membrane microfragments which, after a short delay, become an integral—and apparently functional—part of the oocyte membrane.[21–23] In our experiments, some 23% of the total oocyte surface was stained with the anti-chick retina-specific antibody. This is not a substitute for other recording techniques using dissociated retinal cells or slices,[37,38] but a complementary strategy especially useful at the receptor, rather than cell or circuit, level of analysis.

In choosing the retinal membrane donor chicks we departed from the 13-day embryonic stage that is most suitable for the dissection of the neural retina as a single, intact sheet, which is a prerequisite for a successful ex vivo long-term culture. We preferred to use more developed retinas, with considerably higher receptor density, even if they had to be broken into pieces to detach them from the pigment epithelium. Six-day hatched chicks provided a good compromise between ease of dissection and the ability of the method to detect agonist-triggered ionic currents. By use of this novel injection protocol, the chick retinal membrane fragments became integrated into the oocyte plasma membrane in a seamless way (Fig. 1). Although much of the chick membrane was seen as diffuse clouds of small spots ($<20\ \mu m^2$), we could also identify a population of larger patches of comparable size (in the vicinity of 300 μm^2; Fig. 2). We could not tell, however, at this point, whether these patches originated from the confluence of the smaller spots or were generated as such in the ultrasonic fragmentation procedure.

On challenging these chimeric oocytes with kainate, we consistently observed concentration-dependent current responses (Fig. 3) that seemed to be mediated by AMPA receptors if we take into account their steady, nondesensitizing character, and the strong inhibition by GYKI 52466 but not by

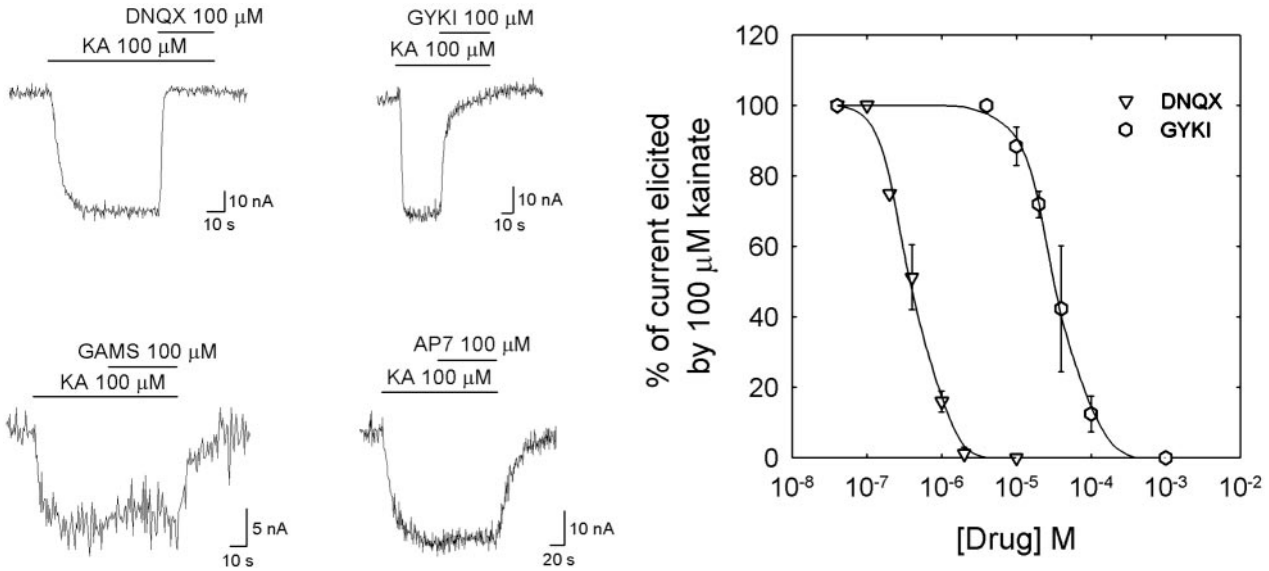

FIGURE 4. Effect of some glutamate antagonists on kainate-activated currents in oocytes injected with chick retinal membranes. *Left*: individual recordings for different antagonists. Drug application is indicated by *bars*. *Right*: Dose-dependent inhibition of kainate-driven currents for DNQX and GYKI 52466.

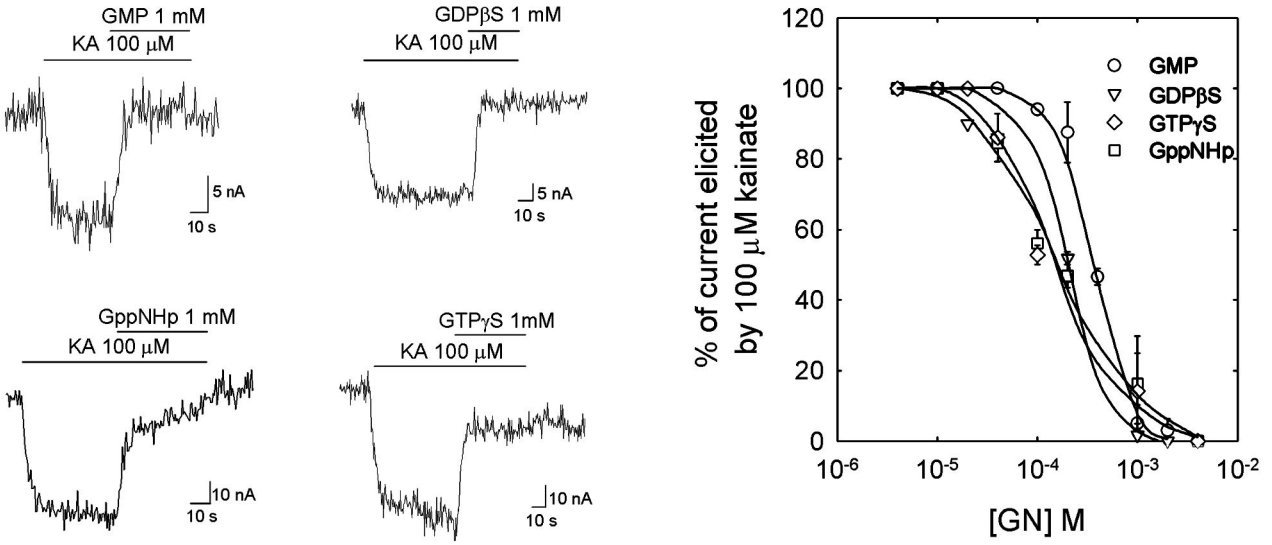

FIGURE 5. Effect of GNs on kainate-activated currents in oocytes injected with chick retinal membranes. *Left*: individual recordings for different GNs. Drug application is indicated by *bars*. *Right*: Dose-dependent inhibition of kainate-driven currents for the same GNs. GMP, guanosine 5′-monophosphate; GDP*β*S, guanosine 5′-*O*-(2-thiodiphosphate); GppNHp, 5′-guanylyl-imidodiphosphate; GTP*γ*S, guanosine 5′-*O*-(3-thiotriphosphate).

GAMS. This is not surprising, because it has already been shown, in the case of the chick retina, that kainate toxicity is mediated by AMPA receptors.[39] These retinal AMPA receptor channels mediate both Na^+ and Ca^{2+} currents[4,8,40] and can therefore be involved in both the early Na^+/Cl^-- and late Ca^{2+}-dependent modes of kainate excitotoxicity.[10]

The importance and interest of adapting this oocyte method to the chick retina is that we can, using the same neural cell populations, correlate the results of different approaches—namely, the protection against kainate-mediated excitotoxicity in the chick retinal ex vivo preparation,[7] the blockade of $^{45}Ca^{2+}$ fluxes in the same preparation,[8] and the electrophysiological approach described in the present paper, to the characterization of the behavior of potential glutamate antagonists. The detailed knowledge of the basic properties of glutamate receptors, at both the pharmacologic and physiological levels, including the critical concentrations of excitotoxins that activate the different channels, obtained in simple models such as the one described herein, should be of help in devising experiments geared to a more direct analysis of specific pathologic conditions, as remarked earlier in this discussion.

GNs have been shown to displace kainate in binding experiments,[7,14–16,19] to block AMPA- and NMDA-driven Ca^{2+} fluxes,[8] to act as neuroprotective agents in several excitotoxicity paradigms,[7,18,20] and to act as antagonists in a similar preparation[22] of oocytes injected with cerebellar membranes. Not unexpectedly, they behaved also as kainate antagonists in the present work (Fig. 5).

GNs are present in a much higher concentration in the retina than in brain,[41] and they could therefore play a physiological role in controlling the activity of ionotropic receptors. Alternatively, GMP, or some new molecule designed to interact with AMPA/KA receptors in an identical manner,[42] may be used to protect the retina from excitotoxic insults, either experimentally exerted or associated to a pathologic process.

References

1. Gibson BL, Reif-Lehrer L. *In vitro* effects of kainate on embryonic and post-hatching chick retina. *Dev Brain Res.* 1984;15:97–103.
2. Olney JW, Price MT, Samson L, Labruyere J. The role of specific ions in glutamate neurotoxicity. *Neurosci Lett.* 1986;65:65–71.
3. Zeevalk GD, Hyndman AG, Nicklas WJ. Excitatory amino acid-induced toxicity in chick retina: amino acid release, histology and effects of chloride channel blockers. *J Neurochem.* 1989;53:1610–1619.
4. Allcorn S, Catsicas M, Mobbs P. Developmental expression and self-regulation of Ca^{2+} entry via AMPA/KA receptors in the embryonic chick retina. *Eur J Neurosci.* 1996;8:2499–2510.
5. Chen Q, Olney JW, Lukasiewicz PD, Almli T, Romano C. Ca^{2+}-independent excitotoxic degeneration in isolated retina, an intact neural net: a role for Cl^- and inhibitory transmitters. *Mol Pharmacol.* 1998;53:654–572.
6. Romano C, Chen Q, Olney JW. The intact isolated (ex-vivo) retina as a model system for the study of excitotoxicity. *Prog Retinal Eye Res.* 1998;17:465–483.
7. Burgos JS, Barat A, Souza DO, Ramírez G. Guanine nucleotides protect against kainate toxicity in an *ex vivo* chick retinal preparation. *FEBS Lett.* 1998;430:176–180.
8. Burgos, JS, Barat A, Ramírez G. Guanine nucleotides block agonist-driven $^{45}Ca^{2+}$ influx in chick embryo retinal explants. *Neuroreport.* 2000;11:2303–2305.
9. Burgos JS, Barat A, Ramírez G. Cl^--dependent excitotoxicity is associated with ^3H_2O influx in chick embryonic retina. *Neuroreport.* 2000;11:3779–3782.
10. Burgos JS, Barat A, Ramírez G. Ca^{2+}-dependent kainate excitotoxicity in the chick embryonic neural retina *ex vivo. Neuroreport.* 2000;11:3855–3858.
11. Monahan JB, Hood WF, Michel J, Compton, RP. Effects of guanine nucleotides on N-methyl-D-aspartate receptor-ligand interactions. *Mol Pharmacol.* 1988;34:111–116.
12. Baron BN, Dudley MW, McCarty DR, Miller FP, Reynolds IJ, Schmidt CJ. Guanine nucleotides are competitive inhibitors of *N*-methyl-D-aspartate at its receptor site both *in vitro* and *in vivo. J Pharmacol Exp Ther.* 1989;250:162–169.
13. Budson AE, Jackson PS, Lipton SA. GDP*β*S antagonizes whole-cell responses to excitatory amino acids. *Brain Res.* 1991;548:346–348.
14. Souza DO, Ramírez G. Effect of guanine nucleotides on kainic acid binding and on adenylate cyclase in chick optic tectum and cerebellum. *J Mol Neurosci.* 1991;3:39–45.
15. Gorodinsky A, Paas Y, Teichberg VI. A ligand binding study of the interactions of guanine nucleotides with non-NMDA receptors. *Neurochem Int.* 1993;23:285–291.
16. Paas Y, Devillers-Thiéry A, Changeux J-P, Medevielle F, Teichberg VI. Identification of an extracellular motif involved in the binding

of guanine nucleotides by a glutamate receptor. *EMBO J.* 1996;15: 1548–1556.

17. Dev KK, Roberts P, Henley JM. Characterisation of the interaction between guanyl nucleotides and AMPA receptors in rat brain. *Neuropharmacology.* 1996;35:1583–1593.
18. Malcon C, Achaval M, Komlos F, et al. GMP protects against quinolinic acid-induced loss of NADPH-diaphorase-positive cells in the rat striatum. *Neurosci Lett.* 1997;225:145–148.
19. Ramos M, Souza DO, Ramírez G. Specific binding of [^{3}H]GppNHp to extracellular membrane receptors in chick cerebellum: possible involvement of kainic acid receptors. *FEBS Lett.* 1997;406:114–118.
20. Regner A, Ramírez G, Belló-Klein A, Souza D. Effects of guanine nucleotides on glutamate-induced chemiluminescence in rat hippocampal slices submitted to hypoxia. *Neurochem Res.* 1998;23: 523–528.
21. Marsal J, Tigyi G, Miledi R. Incorporation of acetylcholine receptors and Cl^- channels in *Xenopus* oocytes injected with *Torpedo* electroplaque membranes. *Proc Natl Acad Sci USA.* 1995;92: 5224–5228.
22. Aleu J, Barat A, Burgos JS, Solsona C, Marsal J, Ramírez G. Guanine nucleotides, including GMP, antagonize kainate responses in *Xenopus* oocytes injected with chick cerebellar membranes. *J Neurochem.* 1999;72:2170–2176.
23. Canals JM, Ruiz-Avila L, Cantí C, Solsona C, Marsal J. Functional reconstitution of KCl-evoked, Ca^{2+}-dependent acetylcholine release system in *Xenopus* oocytes microinjected with synaptic plasma membranes and synaptic vesicles. *J Neurosci Res.* 1996;44: 106–114.
24. Dumont JN. Oogenesis in *Xenopus laevis* (Daudin). *J Morphol* 1972;136:153–180.
25. Sumikawa K, Parker I, Miledi R. Expression of neurotransmitter receptors and voltage-activated channels from brain mRNA in *Xenopus* oocytes. *Methods Neurosci.* 1989;1:30–63.
26. Harlow E, Lane D. *Antibodies. A Laboratory Manual.* New York: Cold Spring Harbor Laboratory; 1988.
27. Zhou N, Hammerland LG, Parks TN. γ-D-glutamylaminomethyl sulfonic acid (GAMS) distinguishes kainic acid- from AMPA-induced responses in *Xenopus* oocytes expressing chick brain glutamate receptors. *Neuropharmacology.* 1993;8:767–775.
28. Kiskin NI, Krishtal OA, Tsyndrenko AY. Excitatory amino acid receptors in hippocampal neurons: kainate fails to desensitize them. *Neurosci Lett.* 1986;63:225–230.
29. Barnard EA, Henley JM. The non-NMDA receptors: types, structure and molecular biology. *Trends Pharmacol Sci.* 1990;11:500–507.
30. Michaelis EK. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. *Prog Neurobiol.* 1998;54:369–415.
31. Zhou LM, Gu ZQ, Costa AM, et al. (2S, 4R)-4-methylglutamic acid (SYM 2081): a selective, high affinity ligand for kainate receptors. *J Pharmacol Exp Ther.* 1997;280:422–427.
32. Rothstein JD. Excitotoxicity hypothesis. *Neurology.* 1996; 47(suppl 4):S19–S25.
33. Fischer AJ, Seltner RL, Poon J, Stell WK. Immunocytochemical characterization of quisqualic acid- and N-methyl-D-aspartate-induced excitotoxicity in the retina of chicks. *J Comp Neurol.* 1998;393:1–15.
34. Ientile R, Macaione V, Teletta M, Pedale S, Torre V, Macaione S. Apoptosis and necrosis occurring in excitotoxic cell death in isolated chick embryo retina. *J Neurochem.* 79:71–78, 2001.
35. Fischer AJ, Seltner RL, Stell WK. N-methyl-D-aspartate-induced excitotoxicity causes myopia in hatched chicks. *Can J Ophthalmol.* 1997;32:373–377.
36. Dreyer EB, Zurakowski D, Schumer RA, Podos SM, Lipton SA. Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. *Arch Ophthalmol.* 1996;114:299–305.
37. Massey SC, Redburn DA. Transmitter circuits in the vertebrate retina. *Prog Neurobiol.* 1987;28:55–96.
38. Huba R, Hofmann H-D. Transmitter-gated currents of GABAergic amacrine-like cells in chick retinal cultures. *Vis Neurosci.* 1991;6: 303–314.
39. Ferreira IL, Duarte CB, Carvalho AP. Kainate-induced retina amacrine-like cell damage is mediated by AMPA receptors. *Neuroreport.* 1998;9:3471–3475.
40. Bennet MVL, Pellegrini-Giampetro DE, Gorter JA, Aronica E, Connor JA, Zukin RS. The GluR2 hypothesis: Ca^{2+}-permeable AMPA receptors in delayed neurodegeneration. *Cold Spring Harbor Symp Quant Biol.* 1996;61:373–384.
41. Klethi J, Urban PF, Mandel P. Free nucleotides in retina. *FEBS Lett.* 1970;8:119–121.
42. Mendieta J, Ramírez G, Gago, F. Molecular dynamics simulations of the conformational changes of the glutamate receptor ligand-binding core in the presence of glutamate and kainate. *Proteins.* 2001; 44:460–469.