Published in final edited form as:

J Neurophysiol. 1994 November ; 72(5): 2503–2516.

Differential Role of Two Ca^{2+} -Permeable Non-NMDA Glutamate Channels in Rat Retinal Ganglion Cells: Kainate-Induced Cytoplasmic and Nuclear Ca^{2+} Signals

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SUMMARY AND CONCLUSIONS

1. The permeability of non-*N*-methyl-D-aspartate (non-NMDA) glutamate channels to divalent cations and specifically the entry of Ca^{2+} and subsequent elevations in cytoplasmic and nuclear Ca^{2+} signals were investigated in cultured neonatal rat retinal ganglion cells using the whole cell patch-clamp technique and Ca^{2+} imaging with confocal microscopy. In addition, divalent-permeable non-NMDA receptor channels were studied in retinal slices using a Co^{2+} staining technique.
2. Using Ca^{2+} (2.5 mM) as the only permeable cation in the external solution, stimulation with 100 μM kainate produced nondesensitizing, nonselective cation currents with either low or high Ca^{2+} permeability. Both currents were reversibly blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Neurons with the low divalent-permeable currents (*type 1*) had reversal potentials of -41.5 ± 4.4 mV (mean $\pm$ SD), and neurons with the high divalent-permeable currents (*type 2*) had reversal potentials of -22.6 ± 5.5 mV. The permeability ratio $P_{\text{Ca}}/P_{\text{Cs}}$ was 3.3 for the *type 1* currents and 8.5 for the *type 2* currents, indicating a 2.5-fold greater permeability to Ca^{2+} for the *type 2* non-NMDA glutamate channels.
3. Both types of non-NMDA glutamate channels showed relatively little selectivity between Ca^{2+} and Co^{2+} . The *type 1* neurons had a slightly higher permeability to Co^{2+} than to Ca^{2+} , whereas the *type 2* neurons were equally permeable to both divalent cations. The *type 2* neurons had a much higher permeability for both divalent cations compared with the *type 1* neurons.
4. Staining for Co^{2+} uptake through kainate-stimulated non-NMDA glutamate channels in retinal slices provided additional evidence for the presence of the two ganglion cell populations. Activation of the neurons by kainate in conditions isolating the non-NMDA glutamate channel caused differential uptake of Co^{2+} . In contrast, depolarization in the presence of the non-NMDA antagonist CNQX failed to cause Co^{2+} influx.
5. Imaging experiments using confocal microscopy showed that kainate stimulation induced an increase in intracellular Ca^{2+} in both types of retinal ganglion cells, but only the *type 2* neurons showed a substantial increase in cytoplasmic and nuclear Ca^{2+} signals. Kainate-induced Ca^{2+} signals in the *type 2* neurons were almost nine times greater than those of the *type 1* neurons.

6. When intracellular Ca^{2+} stores were depleted by brief treatment with thapsigargin, kainate-induced Ca^{2+} signals in the *type 1* neurons were unchanged. However, in the *type 2* neurons kainate no longer induced large Ca^{2+} signals in the cytoplasm and nucleus, despite normal influx of Ca^{2+} . After thapsigargin treatment, kainate-induced Ca^{2+} signals in the *type 2* neurons were reduced to the levels of the treated or untreated *type 1* neurons. Calcium influx via the *type 2* receptor channels therefore appears capable of triggering the release of intracellular Ca^{2+} stores.
7. The two types of non-NMDA glutamate channels described here are likely to contribute to a variety of Ca^{2+} -dependent intracellular processes. Glutamate may differentially affect intracellular Ca^{2+} in retinal ganglion cells. In particular, it appears that Ca^{2+} entry via the high divalent-permeable *type 2* non-NMDA glutamate channel can trigger Ca^{2+} -induced Ca^{2+} release and thereby amplify intracellular Ca^{2+} signals.

INTRODUCTION

Changes in intracellular Ca^{2+} concentration, $[\text{Ca}^{2+}]_i$, are important in regulating many cellular functions. In neurons, increased $[\text{Ca}^{2+}]_i$ can initiate a variety of cellular events from neurosecretion to gene expression. Elevations in $[\text{Ca}^{2+}]_i$ can arise from Ca^{2+} influx via voltage-gated Ca^{2+} channels or receptor-operated Ca^{2+} -permeable ion channels. This influx can in turn release Ca^{2+} from internal stores.

Studies using confocal fluorescence microscopy have shown changes in nuclear Ca^{2+} signals in different neuronal types upon depolarization of the cell membrane (Birch et al. 1992; Hernandez-Cruz et al. 1991; Przywara et al. 1991). It has been proposed that altered nuclear Ca^{2+} levels might be involved in the regulation of gene transcription (Przywara et al. 1991), particularly because increased $[\text{Ca}^{2+}]_i$ has been shown to be a key event in gene expression (Lerea et al. 1992; Morgan and Curran 1986; Zafra et al. 1990). Many studies of gene expression have used depolarization to activate voltage-gated Ca^{2+} channels, but Ca^{2+} influx and corresponding increases in gene expression also can occur with activation of either *N*-methyl-D-aspartate (NMDA) or non-NMDA receptors (Lerea et al. 1992; Zafra et al. 1990). Although non-NMDA receptor activation is known to cause Ca^{2+} influx indirectly via voltage-gated Ca^{2+} channels, recent studies have shown that Ca^{2+} can enter neurons through non-NMDA glutamate channels as well (Gilbertson et al. 1991; Iino et al. 1990), indicating a novel Ca^{2+} influx pathway that may potentially contribute to the initiation of gene expression.

In a recent *in vivo* study of gene activation, the non-NMDA glutamate agonist kainate was found to induce the expression of a *c-fos*-like protein in a subpopulation of ganglion cells in the rabbit retina (Osborne and Barnett 1992). It is unclear whether kainate acted directly or indirectly to activate Ca^{2+} influx in this study. Distinct protein subunits that can complex to form Ca^{2+} -permeable non-NMDA glutamate channels have been identified in various layers of the rat retina using *in situ* hybridization (Hamassaki-Britto et al. 1993). Using Ca^{2+} imaging techniques and pharmacological tools, we previously have observed large Ca^{2+} signals in cultured neonatal rat retinal neurons after stimulation with kainate (Kocsis et al. 1993), indicating Ca^{2+} influx through non-NMDA glutamate channels. In rat retinal ganglion cells, kainate produces a nondesensitizing current, which has an almost linear current-voltage relationship (Aizenman et al. 1988). This current also has been shown to be nonselective for cations and to be blocked by the non-NMDA antagonist kynureate but not by the NMDA antagonist 2-amino-5-phosphonovalerate (APV). In a subpopulation of salamander bipolar cells, kainate-induced currents have a high Ca^{2+} permeability (Gilbertson et al., 1991). However, in retinal ganglion cells the presence of Ca^{2+} -permeable non-NMDA receptor channels has only been indicated via Ca^{2+} imaging techniques (Kocsis et al., 1993).

We are interested in elucidating the mechanisms that lead from activation of cell surface receptors to activation of genomic events (Birch et al. 1992; Kocsis et al. 1993, 1994). Using the whole cell patch-clamp technique, we now report that neonatal rat retinal ganglion cells possess either low Ca^{2+} -permeable (*type 1*) or high Ca^{2+} -permeable (*type 2*) nondesensitizing non-NMDA receptor currents. Using Ca^{2+} imaging with confocal microscopy, we also report that Ca^{2+} entry via the *type 2* receptor channel can induce substantial increases in cytoplasmic and nuclear Ca^{2+} signals. These signals are shown to be caused not only by Ca^{2+} influx into the cell, but also by the release of Ca^{2+} from intracellular stores. The functional properties of these two non-NMDA glutamate receptor types have important implications for potential activation of Ca^{2+} -mediated gene expression. Some of these results have been published in abstract form (Leinders-Zufall et al. 1994).

METHODS

Freshly dissected retinas from postnatal day 2–3 (P2–P3) neonatal Wistar rats were incubated for 10 min at 37°C in Hanks' balanced salt solution without Ca^{2+} and Mg^{2+} (Gibco) containing 0.25% papain (2× crystallized, Sigma), and 0.04% EDTA (Sigma). Retinas then were mechanically dispersed by trituration through a glass pipette and cells were plated on polyornithine and laminin-coated glass coverslips. The culture medium consisted of 45% Dulbecco's modified Eagle's medium (Gibco), 45% F-12 nutrient mixture (Gibco), 10% fetal calf serum and gentamicin (50 $\mu\text{g}/\text{ml}$). Culture medium was replaced every 2–3 days and cells were used for experiments after 3–6 days in culture. Retinal ganglion cells initially were identified by labeling with anti-Thy-1 (kindly provided by Dr. C. J. Barnstable) as previously described (Barnstable and Dräger 1984; Leifer et al. 1984). All cells that had triangular somas > 10 μm wide, several short branched processes, and a single long fine process were found to be Thy-1 immunoreactive (data not shown). Cells fitting these morphological criteria exclude small retinal ganglion cells and all amacrine cells (Perry 1981). Retinal ganglion cells also are distinguished from amacrine and bipolar cells by the amplitude of their sodium currents (see Huba et al. 1992; Karschin and Wässle 1990), which are much larger in terms of absolute value and in relation to the outward currents (using K^{+} instead of Cs^{+} internally) when compared with amacrine cells. Sodium currents therefore were measured and used in addition to the morphological criteria to unequivocally identify retinal ganglion cells for the imaging and electrophysiological experiments.

Ionic currents were measured using the whole cell voltage-clamp technique. Patch electrodes were pulled from thick-walled borosilicate glass (A-M Systems) and had a resistance of 4–8 M Ω . Currents were recorded to videotape using an Axoclamp-2A amplifier; for off-line analysis the signals were digitized and stored on an IBM 386 computer using PCLAMP software (Axon Instruments). All solutions were prepared from milli-Q purified water (Millipore). Internal solution contained (mM): 140 CsCl, 20 *N*-2-hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (HEPES), 3 ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), and 0.661 CaCl_2 . The pH was adjusted to 7.2 with CsOH and the osmolarity was adjusted to 300 mOsm with sucrose. Normal external solution contained (mM): 140 NaCl, 20 HEPES, 5 KCl, 2.5 CaCl_2 , and 1 MgCl_2 . The pH was adjusted to 7.4 with NaOH and the osmolarity was adjusted to 330 mOsm with glucose. External choline solution contained (mM): 145 choline-Cl, 20 HEPES, and either 2.5 CaCl_2 or 2.5 CoCl_2 . The pH was adjusted to 7.4 with *N*-methyl-D-glucamine and the osmolarity was adjusted to 330 mOsm with glucose. External solutions containing either 100 μM kainate or 100 μM kainate and 25 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, Cambridge Research Biochemicals) were delivered directly to individual cells via a multibarrel micropipette using a picospritzer (General Valve Corp.). Thapsigargin (Research Biochemicals International) was dissolved in ethanol, and small amounts of stock solution were dispersed in the external medium, yielding a nominal concentration of 400 nM thapsigargin and 0.2% (vol/vol) of solvent. Recordings

were made at room temperature. Unless otherwise stated all chemicals were obtained from Sigma.

The apparent permeability ratios P_{Ca}/P_{Cs} and P_{Co}/P_{Cs} were calculated as described by Iino et al. (1990) from the various values of reversal potentials using ion concentrations. The permeability ratios also were estimated, using ionic activities instead of ion concentrations. Ionic activities for Ca^{2+} and Cs^{+} were calculated by multiplying their concentrations by their activity coefficients, which were estimated from Shatkay (1968) and Bates et al. (1970).

Cells were loaded with Ca^{2+} indicator dye by adding fluo-3AM (Molecular Probes) at a final concentration of 5 μM and incubating for 35 min, as described previously (Kocsis et al. 1993). The fluo-3 loaded cells were imaged with a Bio-Rad MRC-500 confocal system and an inverted microscope (Nikon Diaphot) using a $\times 40$, 1.3 numeric aperture objective. An argon ion laser was used for excitation illumination at 488 nm. Confocal scanning settings (black level, gain, detection aperture, and neutral density filter) were kept the same over the series of experiments. Fluorescence intensities were measured with NIH Image (Wayne Rasband, National Institutes of Health). For all experiments 8–12 images were taken at 3-s intervals before, during, and after kainate application for 4 s with the multibarrel micropipette. The relative increase in fluorescence was calculated by dividing the difference between the image having the peak response during stimulation and the image before stimulation (control image) by the control image.

Cobalt uptake by kainate stimulation was examined in retinal slices using a modification of a method used to identify divalent cation-permeable kainate receptors in cerebellum and hippocampus (Pruss et al. 1991). Whole retinas were dissected rapidly and carefully transferred to petri dishes. The retinas were washed twice in uptake medium containing (in mM): 145 choline-Cl, 20 HEPES; pH adjusted to 7.4 with *N*-methyl-D-glucamine and osmolarity adjusted to 330 mOsm with glucose. The retinas then were stimulated for 30 min at room temperature in uptake medium supplemented with either 100 μM kainate, 2.5 mM $CoCl_2$, and 100 μM APV or 50 mM KCl, 2.5 mM $CoCl_2$, 100 μM APV, and 100 μM CNQX. After stimulation the retinas were rinsed once in uptake medium, then in uptake medium containing 2 mM EDTA to remove nonspecifically bound Co^{2+} , and again in uptake medium; the intracellular Co^{2+} was then precipitated with 1.2% $(NH_4)_2S$ for 5 min. After fixation with ice-cold 4% paraformaldehyde for ≥ 1 hour, retinas were embedded into 4% agar/phosphate-buffered saline (PBS) solution and cut into 50 or 100 μm slices with a Vibratome. The individual slices were transferred to microscope slides, after which a silver enhancement of the CoS precipitate was performed using the intenSEM kit from Amersham.

Data were analyzed with statistical software (StatView 4.0 and SuperANOVA 1.1, Abacus Software, Berkeley, CA; SAS/STAT, SAS Institute, Cary, NC). The least significant difference (LSD) test was used as a post hoc comparison of the analysis of variance (ANOVA). Results are presented as means $\pm$ SD.

RESULTS

Identification of two types of kainate-induced currents in subpopulations of retinal ganglion cells

Kainate (50 and 100 μM) evoked nondesensitizing currents in all dissociated neonatal rat retinal ganglion cells (Fig. 1 A). Currents induced by 100 μM kainate were inward at negative potentials and had a mean value of -514 ± 181 pA ($n = 17$) at -80 mV. Peak currents measured at several holding potentials were used to generate current-voltage relationships which did not differ from linearity in normal external solution ($P = 0.97 - 0.99$; Fig. 1 B). The average reversal potential for the kainate-induced currents was 0.93 ± 2.84 mV ($n = 5$). The responses to 100

μM kainate were completely and reversibly blocked by 25 μM CNQX, a selective antagonist of non-NMDA receptors (Fig. 1C).

To test the kainate-induced current for Ca^{2+} permeability, we used a Na^+ , K^+ -free solution (choline solution) supplemented with 2.5 mM Ca^{2+} . Under these conditions kainate induced small inward Ca^{2+} currents at -80 mV that could be blocked completely and reversibly by 25 μM CNQX (Fig. 2A). Inward currents were abolished completely using 145 mM choline with no other cations in the external solution. Comparison of the kainate-induced inward current in the presence and absence of Na^+ showed that under normal physiological conditions most of the inward current is carried by an inward Na^+ flow.

The current-voltage relationship, measured in the presence of external choline only, demonstrated a complete outward rectification and a shift in the reversal potential as expected for a cation conductance (Fig. 2B; $\blacktriangle$). When the choline solution was supplemented with 2.5 mM Ca^{2+} , two types of inward Ca^{2+} currents within the population of retinal ganglion cells were distinguished. The two populations had different reversal potentials and current amplitudes at -80 mV. The current-voltage relationships in 18 out of 36 cells had a slight outward rectification and a reversal potential of -41.5 ± 4.4 mV (Fig. 2B, $\circ$). At -80 mV these kainate-induced Ca^{2+} currents had a mean value of -50.6 ± 15.4 pA. In the other 18 retinal ganglion cells, the current-voltage relationships showed a double rectification with a reversal potential of -22.6 ± 5.5 mV (Fig. 2B, $\bullet$) and currents of -74.3 ± 17.4 pA. Statistical analysis indicated that the two types were significantly distinct (ANOVA: $P < 0.001$). We refer to the low-permeable and high-permeable kainate responses as *type 1* and *type 2*, respectively, and used a reversal potential of -30 mV as the cut-off between them (see also Fig. 4E). Figure 2A gives an example of a *type 2* response and Fig. 3A shows a *type 1* response. The two types of Ca^{2+} -permeable kainate currents did not change between day 3 and 6 of culture (LSD: $P = 0.13 - 0.99$; Fig. 2C), indicating that they differentiated before this time.

Because cations, which block voltage-gated Ca^{2+} channels, are able to move through divalent-permeable receptor channels (Iino et al. 1990; Ogura et al. 1990; Pruss et al. 1991), the determination of the reversal potential was repeated with choline solution supplemented with Co^{2+} instead of Ca^{2+} . Before the reversal potential of the kainate-induced Co^{2+} current was measured, we determined whether the ganglion cells belonged to the *type 1* or *type 2* Ca^{2+} response using Ca^{2+} in the external solution as described above. The kainate-induced Co^{2+} peak current was always slightly higher than the Ca^{2+} peak current (Fig. 3A). Ganglion cells classified as having a *type 1* response had Co^{2+} currents of -52.2 ± 8.2 pA ($n = 3$) and reversal potentials of -38.5 ± 3.1 mV ($n = 3$). Ganglion cells classified as having a *type 2* response had Co^{2+} currents of -84.1 ± 21 pA ($n = 3$) and reversal potentials of -26.5 ± 4.0 mV ($n = 3$). For both types of cells, the shape of the current-voltage relationship was similar for Co^{2+} and Ca^{2+} (Fig. 3, B and C). The two types of Co^{2+} reversal potentials were not different from their corresponding Ca^{2+} reversal potentials (LSD: $P = 0.63$ for *type 1* and $P = 0.06$ for *type 2*), but they were significantly different from each other (ANOVA: $P < 0.001$; Fig. 3, B and C). We were able to take advantage of the differential influx of Co^{2+} to stain the two populations of retinal ganglion cells in the whole retina (see below).

To estimate the permeability of the non-NMDA receptor channels to Ca^{2+} and to Co^{2+} , the constant field equation was used as described by Iino et al. (1990). The permeability coefficients to Ca^{2+} and Cs^+ ($P_{\text{Ca}}/P_{\text{Cs}}$) were calculated to be 3.3 ± 0.6 ($n = 18$) for *type 1* and 8.5 ± 2.5 ($n = 18$) for *type 2*, revealing a 2.5 times higher permeability of the *type 2* non-NMDA receptor channel to Ca^{2+} (see Table 1). The permeability coefficients for Co^{2+} and Cs^+ ($P_{\text{Co}}/P_{\text{Cs}}$) were 3.8 ± 0.5 ($n = 3$) for the *type 1* and 6.8 ± 1.4 ($n = 3$) for the *type 2* responses, revealing a 1.8 times greater permeability of the *type 2* non-NMDA receptor channel to Co^{2+} . The permeability ratios for Co^{2+} relative to Ca^{2+} were calculated from the shift in the reversal

potential measured in the same cell, assuming constant P_{Cs} . The relative permeabilities were 1.34 ± 0.30 and 0.94 ± 0.09 for the *type 1* and *type 2* responses, respectively. Thus the *type 1* response was more permeable to Co^{2+} than to Ca^{2+} whereas the *type 2* response was equally permeable to both divalent cations. However, the *type 2* response had a much higher permeability for both divalent cations compared with the *type 1* response (LSD: $P < 0.05$).

Kainate stimulation increases cytoplasmic and nuclear Ca^{2+} signals

The method of loading Ca^{2+} indicator dye into cells can influence the measurement of nuclear Ca^{2+} signals (Rand et al. 1994a,b). In the present study, retinal ganglion cells were loaded using the nondisruptive, acetoxymethylester (AM) form of fluo-3. Confocal measurements of kainate-induced Ca^{2+} signals were made for each retinal ganglion cell before patch clamping to avoid prestimulating the neurons with the patch pipette. When we attempted to combine imaging and patch-clamp techniques, the cells appeared to be activated at the moment of breaking the membrane to voltage clamp the cell. Even though we were careful to preset the amplifier to the average resting potential (-50 mV) to avoid activation of voltage-sensitive Ca^{2+} channels and buffered the intracellular solution to 10 nM Ca^{2+} , imaging the neurons at the moment of membrane disruption indicated that an influx of Ca^{2+} occurred in most of the cells. This resulted in elevated nuclear Ca^{2+} signals at baseline that lasted for many minutes. When the neurons then were stimulated with kainate, only small increases in the already elevated Ca^{2+} signals occurred. By imaging the neurons before disturbing them with the patch pipette we were able to avoid this problem.

After loading retinal neurons with fluo-3AM, retinal ganglion cells had low Ca^{2+} signals at rest (Fig. 4, A and C). Upon 4 s of kainate stimulation Ca^{2+} signals rose rapidly, peaked ~ 10 s later, and then recovered over a period of ~ 40 s to the original baseline. Kainate induced an increase in intracellular Ca^{2+} signals in both types of retinal ganglion cells, but only those with *type 2* kainate-induced currents showed a substantial increase in cytoplasmic and nuclear Ca^{2+} signals (Fig. 4, B and D).

Confocal microscopy measurements of fluo-3-loaded retinal ganglion cells confirmed the results showing Ca^{2+} permeation through kainate-gated channels, and showed a dramatic difference in the effect of Ca^{2+} influx through the two types of non-NMDA channels. Retinal ganglion cells were imaged before and during application of $100 \mu\text{M}$ kainate while they were perfused with choline solution containing 2.5 mM Ca^{2+} . All imaged neurons subsequently had their reversal potentials determined. Figure 4, A–D, shows two retinal ganglion cells before kainate application and at peak fluorescence during kainate application. Two types of Ca^{2+} responses were observed. One population of retinal ganglion cells had a relatively low increase in fluorescence (as shown in Fig. 4, A and B), whereas the other had a substantial increase in cytoplasmic and nuclear Ca^{2+} signals upon kainate stimulation (as shown in Fig. 4, C and D). These two types of elevated Ca^{2+} signals were exclusively associated with the presence of the *type 1* or *type 2* kainate-induced Ca^{2+} currents (Fig. 4E). Neurons with fluorescence increases $>50\%$ fit the classification of *type 2* neurons, and were clearly distinct from the *type 1* neurons. To verify this, a discriminant analysis was used to test the hypothesis that there were two different groups (as opposed to a continuum of responses) based on reversal potentials and fluorescence changes; the result was significant at $P < 0.02$ ($\chi^2 = 10.33$), with 100% concordance between tested and predicted group membership.

The imaged neurons had reversal potentials of -39.6 ± 2.6 mV ($n = 8$) for the *type 1* and -22.0 ± 5.3 mV ($n = 9$) for the *type 2* responses. The *type 1* ganglion cells had a relatively low increase in fluorescence, whereas the *type 2* cells showed a considerable increase in Ca^{2+} signals (Fig. 5A). The range of increases in fluorescence was 0.9 – 38% for *type 1* and 83 – 207% for *type 2* responding cells. The two types were very different in their Ca^{2+} -induced fluorescence (ANOVA: $P < 0.001$). The enhancement in Ca^{2+} signals for *type 2* ganglion cells was almost

nine times higher than the *type 1* ganglion cells. Glutamate might therefore differentially affect $[Ca^{2+}]_i$ in retinal ganglion cells via these two types of non-NMDA-gated ion channels.

In both types of retinal ganglion cells, nuclear Ca^{2+} signals exceeded those of the cytoplasm upon stimulation with kainate (Fig. 5B). The ratio of nuclear to cytoplasmic Ca^{2+} signals was 1.50 ± 0.66 ($n = 5$) for *type 1* neurons and 1.60 ± 0.30 ($n = 6$) for *type 2* neurons. However, the *type 2* neurons had much larger Ca^{2+} signals in both intracellular compartments.

The kainate-induced Ca^{2+} signals may have been due to Ca^{2+} entering the cell and triggering the release of Ca^{2+} from intracellular stores. To investigate possible amplification of the signals by Ca^{2+} -induced Ca^{2+} release (CICR), 400 nM thapsigargin was applied to the bath for ≥ 10 min. Thapsigargin specifically inhibits the family of Ca^{2+} -ATPases that move Ca^{2+} into the lumen of the sarcoplasmic or endoplasmic reticulum, and consequently depletes intracellular Ca^{2+} stores (Thastrup et al. 1990). We used the same imaging and electrophysiological procedures as described above and compared the thapsigargin-treated neurons to the previously described ganglion cells (control). Fluorescence signals in the cytoplasm and nucleus did not noticeably change during thapsigargin treatment and remained approximately equal in the two compartments. Figure 6 shows control and thapsigargin-treated retinal ganglion cells at peak fluorescence during kainate application. After thapsigargin treatment, only *type 2* ganglion cells had a substantially lower increase in Ca^{2+} signals upon kainate stimulation (LSD: $P < 0.001$). For all thapsigargin-treated *type 1* ganglion cells the stimulation-induced fluorescence was not significantly different from the control *type 1* ganglion cells (LSD: $P = 0.63$; Fig. 5A and C). Notably, the increase in fluorescence of the *type 2* ganglion cells was only 11–90% instead of 83–207% and did not differ from the small increase in fluorescence of the *type 1* ganglion cells (LSD: $P = 0.27$; Fig. 5C). The reduced increase in Ca^{2+} signals in *type 2* ganglion cells after thapsigargin treatment was not due to an inhibition of the kainate current. Comparison of the kainate-induced Ca^{2+} currents in the absence and presence of thapsigargin revealed no change in the peak currents (LSD: $P = 0.17$ and 0.52 for *type 1* and *type 2*, respectively). The reversal potentials also did not differ from their corresponding values in the control situation (LSD: $P = 0.36$ and 0.91 for *type 1* and *type 2*, respectively). The relatively large Ca^{2+} currents through the *type 2* receptor channel therefore appeared to trigger CICR and lead to an amplification of the intracellular Ca^{2+} signals.

After thapsigargin treatment, nuclear Ca^{2+} signals still exceeded those of the cytoplasm in both types of neurons upon kainate stimulation (Fig. 5D). The increase for the Ca^{2+} signals in the nucleus relative to the cytoplasm was 1.26 ± 0.15 ($n = 5$) for the *type 1* neurons and 1.15 ± 0.07 ($n = 4$) for the *type 2* neurons. However, the *type 2* neurons no longer had much larger Ca^{2+} signals in both intracellular compartments. Nuclear-to-cytoplasmic fluorescence ratios for control and thapsigargin-treated cells upon kainate stimulation were not significantly different irrespective of conductance type (LSD: $P = 0.07$ – 0.68).

Kainate-induced cobalt uptake in retinal slices

Fresh whole retinas were stimulated with kainate in conditions that isolated Co^{2+} influx via the non-NMDA receptor channels to determine if the two populations of retinal ganglion cells having high and low divalent-permeable channels were present in the intact retina. This was done because the channel properties of the two populations observed in vitro might have been altered by the techniques of dissociation and culture. Because the *type 2* response had a much higher permeability to Co^{2+} than the *type 1* response, we attempted to see a difference in Co^{2+} staining in cells within the ganglion cell layer of the whole retina.

Because of the possibility of glutamate release in the whole retina, all Co^{2+} staining experiments were carried out in the presence of the NMDA antagonist APV to prevent Co^{2+} entry through NMDA glutamate channels. As a control, whole retinas were depolarized with

50 mM K^+ in the presence of the kainate antagonist CNQX, to ensure that Co^{2+} influx did not occur through voltage-gated Ca^{2+} channels during the period of kainate stimulation (30 min). Depolarization failed to induce Co^{2+} uptake under these conditions (Fig. 7A). Cobalt uptake during kainate stimulation in the presence of APV therefore would be expected to be caused by influx through divalent-permeable non-NMDA receptor channels.

When 100 μ M kainate was used to stimulate the retinas of P3 animals, many retinal neurons took up Co^{2+} , indicated by a brown precipitate in the cell bodies (Fig. 7B). The darkest-stained cells were found in the ganglion cell layer and in the part of the inner nuclear layer that borders the inner plexiform layer. The staining in the ganglion cell layer was heterogeneous, and either dark or lightly-stained cells were observed (Fig. 7B). Differential staining always was observed when retinas were stimulated for ≤ 30 min, but after 45 min saturated staining occurred. These results indicate that Co^{2+} influx was responsible for the differential staining. There was also no discernible edge artifact; as can be seen in Fig. 7B, cells near the inner plexiform layer were differentially stained as well as cells at the edge of the ganglion cell layer. High-power magnification (not shown) revealed that cells with almost no staining were not empty of cytoplasmic material but only less-darkly stained, and dark-stained neurons were single cells, not overlapping cells. Only a few neurons at the edge of the tissue appeared to be washed out during the staining procedure, and they were easily identified at high-power magnification.

Although we cannot exclude the possibility that some of the darkly stained cells in the ganglion cell layer are displaced amacrine cells, the size of the cells and their location within the ganglion cell layer indicate that they are likely to be ganglion cells and not displaced amacrine cells. Displaced amacrine cells have been found in the ganglion cell layer of adult rat retina (Perry 1981). However, at postnatal day 3, 70–85% of the cells in the ganglion cell layer are retinal ganglion cells (Perry et al. 1983). Furthermore, displaced amacrine cells at P3 are located predominantly at the border of the ganglion cell layer and the inner plexiform layer. The likelihood of amacrine cells accounting for the differential staining in the ganglion cell layer at P3 therefore seems remote. The heterogeneous staining of the retinal ganglion cell layer supports the idea that the two types of retinal ganglion cell responses in vitro were not a characteristic of the acute dissociation.

The dark-stained cells at the inner border of the inner nuclear layer (Fig. 7B) are likely to be amacrine cells, and they appear to have the high Ca^{2+} -permeable non-NMDA glutamate channel. Cells in the outer nuclear layer of the rat retinal slices also were stained and appear to be rod photoreceptors. The intensity of the staining indicates that they too may have Ca^{2+} -permeable non-NMDA glutamate channels at this point in development. Although not clearly shown in Fig. 7B, we frequently have observed darkly stained cells at regular distances along the outer border of the inner nuclear layer, proximal to the not-yet-formed outer plexiform layer. These may be horizontal cells, which previously have been shown to have Ca^{2+} -permeable non-NMDA glutamate channels (Linn and Christensen 1992).

DISCUSSION

Two populations of retinal ganglion cells were identified by the presence of kainate-induced currents with either a low (*type 1*) or a high (*type 2*) divalent cation permeability. The *type 2* kainate-gated ion channels had a higher permeability to Ca^{2+} than the *type 1* kainate-gated ion channels. The presence of these two populations of ganglion cells in the retina was supported by a Co^{2+} staining technique modified from Pruss et al. (1991).

In both types of ganglion cells, Ca^{2+} signals were elevated after stimulation with kainate. In ganglion cells possessing the *type 1* response there was only a slight increase in intracellular Ca^{2+} signals. The larger Ca^{2+} currents mediated by the *type 2* receptor ion channels induced a

dramatic elevation in cytoplasmic and nuclear Ca^{2+} signals. A substantial portion of these cytoplasmic and nuclear Ca^{2+} signals was due to the release of Ca^{2+} from intracellular stores. These results establish a direct relationship between kainate-induced currents and elevated nuclear Ca^{2+} signals in retinal ganglion cells.

Divalent cation-permeable ion channels activated by kainate

On the basis of ligand affinity, non-NMDA receptors are classified as α -amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptors and kainate receptors (Keinänen et al. 1990; Seeburg 1993; Sommer and Seeburg 1992). The family of AMPA receptors comprises four different protein subunits, GluR1 to GluR4, which when assembled show nondesensitizing currents to kainate stimulation (Keinänen et al. 1990). In contrast, high-affinity kainate receptor ion channel complexes are formed from protein subunits GluR5 to GluR7 as well as KA1 and KA2, and show desensitizing currents upon kainate activation (Herb et al. 1992). In addition, AMPA can activate receptors formed by particular combinations of the high-affinity kainate receptor subunits (see Seeburg 1993; Sommer and Seeburg 1992). The concentration of kainate used in the present study was likely sufficient to activate both types of non-NMDA glutamate channels. The kainate-induced currents in our experiments showed no desensitization, indicating that they belong to the family of AMPA receptors. Consistent with these data, Hamassaki-Britto et al. (1993) found that there was more labeling for protein subunits of the AMPA receptor family in the ganglion cell layer than for the subunits of the high-affinity kainate receptor.

The Ca^{2+} permeability and the current-voltage relationship of recombinant non-NMDA glutamate channels is dominated by the presence of the GluR2 subunit (Burnashev et al. 1992b; Hollmann et al. 1991; Nakanishi et al. 1990; Verdoorn et al. 1991). In the presence of physiological external solution, recombinant AMPA glutamate channels coassembled with GluR2 protein subunits show either linear or simple outward rectification and lose their Ca^{2+} permeability. In the present study, both types of non-NMDA receptor currents had linear current-voltage relationships in normal external solution, implying that the retinal ganglion cell receptors have GluR2 subunits. We therefore would expect the retinal ganglion cells to have low Ca^{2+} permeability; in contrast, we found that they had relatively large kainate-induced Ca^{2+} currents. Hamassaki-Britto et al. (1993) reported that patches of cells within the rat retinal ganglion cell layer were labeled heavily by the GluR2 subunit probe. Our results suggest that this subpopulation of retinal ganglion cells might possess the low Ca^{2+} -permeable *type 1* response, whereas the remainder of the ganglion cells might underexpress or lack the GluR2 subunit, conferring the high Ca^{2+} permeability of the *type 2* response. In fact, a study using recombinant ion channel subunit expression has shown that a greater amount of GluR2 coinjected for heteromeric ion channel expression decreases the Ca^{2+} permeability of the resultant receptor ion channel (Hollmann et al. 1991).

In most neurons, non-NMDA glutamate channels have linear or outwardly rectifying current-voltage relationships and exhibit low permeability to Ca^{2+} (Mayer and Westbrook 1987), but in some neurons and glia cells the non-NMDA receptor channels are highly permeable to Ca^{2+} (Burnashev et al. 1992a; Gilbertson et al. 1991; Iino et al. 1990). Table 1 shows reported Ca^{2+} /monovalent ion permeability ratios ($P_{\text{Ca}}/P_{\text{mono}}$) of NMDA and non-NMDA glutamate channels in various tissues, as well as those found in the present study. In hippocampal neurons, two types of non-NMDA receptors have been observed. *Type 1* receptors have a low permeability ratio and exhibit a linear current-voltage relationship, whereas the *type 2* receptors have a high permeability ratio and exhibit a strong inward rectifying current-voltage relationship (Iino et al. 1990). In contrast, non-NMDA receptors in salamander bipolar cells exhibit a high permeability ratio and show a linear current-voltage relationship (Gilbertson et al. 1991). The two types of non-NMDA receptors reported here resemble those of bipolar cells,

in that they are Ca^{2+} permeable and have linear current-voltage relationships. The *type 1* receptor described in the present study has a permeability ratio in the same range as that previously reported for non-NMDA receptors with high permeability to Ca^{2+} (Table 1). The *type 2* receptor had a much higher permeability to Ca^{2+} and even exceeded the permeability ratios reported for the NMDA receptor channel (Table 1). The high Ca^{2+} permeability of the two types of non-NMDA glutamate channels indicates that they may contribute to a variety of Ca^{2+} -dependent processes in the retina. Although we used a maximal or near-maximal dose of kainate in the present study (see Murphy et al. 1987; Ozawa et al. 1991), it is possible that differential agonist sensitivity might account for the two types of divalent-permeable responses.

In contrast to voltage-gated Ca^{2+} channels, Ca^{2+} -permeable non-NMDA receptor channels have been reported to be permeable to a wide range of other divalent cations including Ba^{2+} , Mg^{2+} , Sr^{2+} , and Mn^{2+} (Burnashev et al. 1992b; Hollmann et al. 1991; Iino et al. 1990) and have been shown indirectly to be permeable to Co^{2+} (Pruss et al. 1991). Our results demonstrate that the non-NMDA receptor is highly permeable to Co^{2+} in retinal ganglion cells. The permeability sequence of the *type 1* and *type 2* responses was $\text{Co}^{2+} > \text{Ca}^{2+}$ and $\text{Co}^{2+} = \text{Ca}^{2+}$, respectively. Because the *type 2* Co^{2+} permeability was almost two times greater than the *type 1* Co^{2+} permeability, we used a Co^{2+} staining technique to identify the two populations of retinal ganglion cells in retinal slices, supporting the electrophysiological and Ca^{2+} imaging data. The existence of physiologically different populations of retinal ganglion cells has been implicated by measurements of cGMP-gated cation channels in a subpopulation of these cells (Ahmad et al. 1994) and by measurements of glutamate currents in retinal whole mount preparations (Röhrig and Grantyn 1993).

Apart from heterogeneous Co^{2+} staining in the ganglion cell layer, the inner third of the inner nuclear layer also was stained heavily by Co^{2+} . This is the same region that has strong labeling for GluR3 and GluR4 subunit probes (Hamassaki-Britto et al. 1993), supporting the presence of high divalent-permeable AMPA receptors in a subpopulation of bipolar cells (Gilbertson et al. 1991) and possibly amacrine cells. In addition, Hamassaki-Britto et al. (1993) report that GluR3 and GluR4 labeling appeared to be concentrated in some cell bodies in the outer third of the inner nuclear layer, where horizontal cells are located, and Ca^{2+} permeable non-NMDA glutamate channels have been reported in horizontal cells of the fish retina (Linn and Christensen 1992). In the present study, scattered cell bodies in this region had strong Co^{2+} staining, supporting the presence of high Ca^{2+} -permeable receptor ion channels in a subset of horizontal cells.

Increased Ca^{2+} signals after kainate stimulation

Both types of retinal ganglion cells responded to kainate with elevations in cytoplasmic and nuclear Ca^{2+} , but the *type 2* neurons had substantially greater Ca^{2+} signals. There are several possible explanations for the different magnitudes of the Ca^{2+} signals. First, they might be exclusively due to the amount of Ca^{2+} influx. Alternatively, because Ca^{2+} can cause the release of Ca^{2+} from intracellular stores (Holliday et al. 1991; Lipscombe et al. 1988), the amount of Ca^{2+} influx might trigger or modulate the amount of Ca^{2+} released from intracellular stores. To assay the possible contribution of CICR to the Ca^{2+} signals, we treated neurons with thapsigargin, which depletes intracellular Ca^{2+} stores (Thastrup et al. 1990). This manipulation allowed us to generally distinguish Ca^{2+} influx from the combination of Ca^{2+} influx and subsequent CICR. After thapsigargin treatment, the Ca^{2+} signals of *type 2* ganglion cells were diminished to the level of the *type 1* ganglion cells. These results indicate that Ca^{2+} entry via the *type 2* non-NMDA glutamate channels evoked CICR. The latency to the peak of the kainate-induced Ca^{2+} signals in untreated neurons (~6 s after the end of stimulation) also indicates the release of Ca^{2+} from intracellular stores. In retinal horizontal cells of the rabbit retina (Linn

and Christensen 1992) and in spinal neurons of *Xenopus* embryos (Holliday et al. 1991), Ca^{2+} entry has been reported to be amplified by CICR. In rat retinal neurons, caffeine pretreatment decreased intracellular Ca^{2+} signals (probably by depletion of Ca^{2+} stores) and removal of extracellular Ca^{2+} greatly reduced kainate-induced intracellular Ca^{2+} signals (Kocsis et al. 1993). The kainate-induced intracellular Ca^{2+} signals therefore appear to be dependent on Ca^{2+} influx and subsequent amplification via CICR. Interestingly, in the present study the kainate-induced Ca^{2+} signals of the *type 1* ganglion cells did not change after thapsigargin treatment. These results suggest that either Ca^{2+} entry in the *type 1* neurons is not sufficient to activate CICR or that these neurons do not have large accessible Ca^{2+} stores. Regarding the latter possibility, depolarization caused Ca^{2+} signals of the same magnitude as the *type 2* neurons (data not shown), indicating that the *type 1* neurons have similar Ca^{2+} stores.

Although it has been suggested that spatially distinct sites of Ca^{2+} entry (Lerea and McNamara 1993) or the mode of Ca^{2+} entry (Bading et al. 1993) might differentially affect transcriptional activation, it seems likely that the amount of Ca^{2+} entry also might modulate activation of a subset of cellular events. The two Ca^{2+} -permeable non-NMDA glutamate channels described here therefore may confer different physiological and genomic responses to glutamate stimulation depending on the differential expression and composition of GluR protein subunits. Burnashev et al. (1992b) have suggested that the assembly of several protein subunits into a functional ion channel may depend on their respective mRNA levels in the cell and may generate a range of different whole cell current-voltage relationships and divalent permeabilities. Our observations of two distinct populations of Ca^{2+} -permeable non-NMDA receptors indicate a more restricted expression or assembly of GluR protein subunits for retinal ganglion cells.

Nuclear Ca^{2+} signals in retinal ganglion cells

Several studies of neurons using AM Ca^{2+} indicator dyes and confocal microscopy have found that nuclear Ca^{2+} signals increase more than those in the cytoplasm upon depolarization (Birch et al. 1992; Hernandez-Cruz et al. 1991; Holliday et al. 1991; Przywara et al. 1991). AM Ca^{2+} -indicator dyes are trapped metabolically by cells, loading them in a nondisruptive manner. A study using micropipette loading of Ca^{2+} indicator dye in neurons has suggested that depolarization-induced Ca^{2+} signals are not higher in the nucleus compared with the cytoplasm (Al-Mohanna et al. 1994). However, we have found recently that micropipette dye loading can elevate baseline nuclear Ca^{2+} signals (Rand et al. 1994a, b), confounding measurements of $[\text{Ca}^{2+}]_i$ relative to baseline. In the present study, we initially found that patch clamping the retinal ganglion cells raised their intracellular Ca^{2+} signals, particularly in the nucleus, and it was therefore necessary to image kainate-induced Ca^{2+} signals in individual neurons before mechanically perturbing them with the patch pipette.

Regardless of whether nuclear Ca^{2+} signals are greater than cytoplasmic Ca^{2+} signals, Ca^{2+} consistently has been shown to increase in both intracellular compartments upon stimulation (Al-Mohanna et al. 1994; Birch et al. 1992; Hernandez-Cruz et al. 1991; Holliday et al. 1991; Przywara et al. 1991). Observations of stimulation-induced Ca^{2+} transients imply that a critical level of $[\text{Ca}^{2+}]_i$ must occur in either the cytoplasm, nucleus, or both before transcription of a subset of genes, given that increased $[\text{Ca}^{2+}]_i$ has been shown to be important for various types of gene expression (Lerea et al. 1992; Morgan and Curran 1986; Zafra et al. 1990). In the present study, fluo-3 AM showed that nuclear Ca^{2+} signals always increased more than those in the cytoplasm upon kainate stimulation. Independent of thapsigargin treatment or type of retinal ganglion cell response, nuclear-to-cytoplasmic Ca^{2+} signal ratios were relatively constant, despite the fact that control *type 2* neurons had signals almost nine times greater than *type 1* neurons or thapsigargin-treated neurons. This is noteworthy because it indicates that the

processes underlying differential signals in the cytoplasm and nucleus might be independent of CICR.

Physiological function of Ca^{2+} -permeable non-NMDA receptors

In the retina glutamate is regarded as a major excitatory neurotransmitter for NMDA and non-NMDA glutamate channels. Although NMDA receptors are abundant in the inner retina, they mediate only a minor portion of the light-driven input to ganglion cells in rabbit retina (Massey and Miller 1990). Therefore, it is likely that signal transmission from bipolar cells to ganglion cells is carried primarily by non-NMDA receptors. Activation of non-NMDA receptors may be involved in *c-fos* expression in the ganglion cell layer. Under a physiological light/dark cycle, *c-fos* mRNA levels in a subpopulation of retinal ganglion cells increased transiently for 30 min immediately after the onset of the light period (Yoshida et al. 1993). In addition, an intraocular injection of kainate induced the expression of *c-fos*-like protein in a subpopulation of ganglion cells in the rabbit retina (Osborne and Barnett 1992). These reports support the involvement of non-NMDA receptors in gene expression in the retinal ganglion cell layer.

In the present study, extracellular sodium was absent to isolate Ca^{2+} influx via the non-NMDA glutamate channel. Although these conditions were not physiological, we have shown previously that Ca^{2+} influx and amplification occur with kainate stimulation in the presence of extracellular sodium and that removal of extracellular Ca^{2+} greatly reduces kainate-induced intracellular Ca^{2+} signals (Kocsis et al. 1993). The contribution of Ca^{2+} influx via the non-NMDA glutamate channel in normal extracellular solution also has been directly measured in septal neurons having similar linear conductance properties (Schneggenburger et al. 1993). This study found that the fractional contribution of Ca^{2+} to the currents was relatively large, consistent with the results reported here.

We have shown that there are two populations of rat retinal ganglion cells with distinct types of divalent cation-permeable non-NMDA glutamate channels. The two types of non-NMDA responses produce different levels of intracellular Ca^{2+} signals and might differentially modulate Ca^{2+} -dependent gene expression. In *type 2* retinal ganglion cells, one pathway for Ca^{2+} signaling appears to be CICR upon Ca^{2+} influx via the non-NMDA receptor ion channel resulting in high cytoplasmic and nuclear Ca^{2+} signals. In *type 1* retinal ganglion cells, we did not find evidence for activation of CICR via this pathway. Although Ca^{2+} influx was smaller in the *type 1* than the *type 2* retinal ganglion cells, it was still able to elevate intracellular Ca^{2+} signals. Calcium influx through the non-NMDA receptor in retinal ganglion cells, and particularly the *type 2* non-NMDA receptor channel, therefore might contribute to a variety of Ca^{2+} -dependent processes in the retina, analogous to what has been suggested for the NMDA receptor in the hippocampus (Bliss and Collingridge 1993; Goodman and Shatz 1993). In this regard, the role of Ca^{2+} influx through non-NMDA glutamate channels may have been underestimated previously (see Schneggenburger et al. 1993).

Acknowledgements

We thank H. F. Mi for technical assistance in staining the retina slices, Dr. Frank Zufall for discussion and helpful comments on the manuscript, and Dr. Colin J. Barnstable for kindly supplying Thy-1 antibody and for help with the anatomy of the Co^{2+} -stained retinal slices.

This investigation was supported by the Medical Research Service, Department of Veterans Affairs, and the National Institutes of Health.

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FIG. 1.

A: kainate (KA) at a concentration of $100\ \mu\text{M}$ produced nondesensitizing currents as shown at various membrane potentials in normal external solution. *B:* KA-induced currents are plotted as a function of the membrane potential. Reversal potential was 0 mV. *C:* effect of the non-*N*-methyl-D-aspartate (NMDA) antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) on the KA-induced current. In the control situation the retinal ganglion cell was stimulated by $100\ \mu\text{M}$ kainate. Subsequent exposure of the same cell to $100\ \mu\text{M}$ KA and $25\ \mu\text{M}$ CNQX resulted in complete block of the current, which was reversible. The holding potential was $-80\ \text{mV}$.

FIG. 2.

A: in all retinal ganglion cells, 100 μ M KA was able to induce a current in Na^+ , K^+ -free (choline) solution with 2.5 mM Ca^{2+} as the only permeable cation present. KA-induced Ca^{2+} current could be reversibly blocked by 25 μ M CNQX. Holding potential was -80 mV. **B:** normalized current-voltage relationships of KA-induced currents from 3 different cells in choline solution only (Δ) or in choline solution supplemented with 2.5 mM Ca^{2+} ($\circ$ and $\bullet$). KA-induced currents in the presence of Ca^{2+} were classified into two types having a reversal potential either near -41 mV (*type 1*; $\circ$) or near -22 mV (*type 2*; $\bullet$). Current shown in (A) belonged to a *type 2* response. **C:** The two types of Ca^{2+} -permeable KA-induced currents in P2–P3 rat retinal ganglion cells did not change between day 3 and 6 in culture. Number of cells shown in parentheses.

FIG. 3.

A: in retinal ganglion cells, 100 μ M KA induced a current in Na^+ , K^+ -free (choline) solution supplemented with 2.5 mM Ca^{2+} as well as in Na^+ , K^+ -free (choline) solution supplemented with 2.5 mM Co^{2+} as the only permeable cation present. Holding potential was -80 mV. B: normalized current-voltage relationships of the *type 1* KA-induced currents from a single cell in the presence of 2.5 mM Ca^{2+} ($\blacktriangle$) or 2.5 mM Co^{2+} ($\triangle$) as the only permeable cation in the external choline solution. C: normalized current-voltage relationships of the *type 2* KA-induced currents from a single cell in the presence of 2.5 mM Ca^{2+} ($\bullet$) or 2.5 mM Co^{2+} ($\circ$) as the only permeable cation in the external choline solution.

FIG. 4.

Retinal ganglion cells loaded with fluo-3 were imaged using confocal microscopy before and during application of 100 μ M K. A while perfused with Na⁺, K⁺-free (choline) solution supplemented with 2.5 mM Ca²⁺. Type 1 retinal ganglion cells imaged before kainate application (A) and at peak fluorescence during kainate application (B) typically responded with a relatively low increase in Ca²⁺-induced fluorescence. In contrast, type 2 retinal ganglion cells imaged before KA application (C) and at peak fluorescence during kainate application (D) typically responded with large increases in Ca²⁺-induced fluorescence. Scale bar = 10 μ m. E: scatterplot of reversal potentials vs. increases in Ca²⁺-induced fluorescence for all imaged retinal ganglion cells. Neurons with low divalent cation permeability (type 1); reversal

potentials less than -30 mV) are clearly distinguished from neurons with high divalent cation permeability (*type 2*; reversal potentials greater than -30 mV).

FIG. 5.

Both *type 1* and *type 2* retinal ganglion cells responded with an increase in Ca^{2+} signals upon kainate stimulation. *A*: retinal ganglion cells having high Ca^{2+} -permeable *type 2* currents with reversal potentials near -22 mV had a considerably greater increase in Ca^{2+} -induced fluorescence compared with retinal ganglion cells having low Ca^{2+} -permeable *type 1* currents with reversal potentials near -41 mV. *B*: nuclear Ca^{2+} signals exceeded those of the cytoplasm, and neurons with *type 2* responses had much larger Ca^{2+} signals than those with *type 1* responses. *C*: after thapsigargin treatment, *type 1* and *type 2* retinal ganglion cells could not be differentiated by their increase in fluorescence upon kainate stimulation. *D*: after thapsigargin treatment, nuclear Ca^{2+} signals still exceeded those of the cytoplasm, but neurons with *type 2* responses had Ca^{2+} signals that were reduced to the same level as neurons with *type 1* responses. Thapsigargin treatment did not affect the magnitude of KA-induced Ca^{2+} signals in *type 1* neurons. The dashed lines indicate the mean reversal potentials of the 2 types.

FIG. 6.

Thapsigargin-treated retinal ganglion cells loaded with fluo-3 were imaged using confocal microscopy during application of 100 μM KA in Na^+ , K^+ -free (choline) solution supplemented with 2.5 mM Ca^{2+} . A *type 1* retinal ganglion cell imaged at peak fluorescence during K A application before (A) and during thapsigargin treatment (B) responded with approximately the same low increase in Ca^{2+} -induced fluorescence. A *type 2* retinal ganglion cell imaged at peak fluorescence during KA application responded with a large increase in Ca^{2+} -induced fluorescence before treatment with thapsigargin (C), and a small increase in Ca^{2+} -induced fluorescence after exposure to thapsigargin (D). Scale bar = 10 μm .

FIG. 7.

Cobalt uptake in radial sections from P3 rat retina induced by (A) depolarization using 50 mM KCl in the presence of 100 μ M CNQX or (B) stimulation with 100 μ M kainate. Both conditions used 145 mM choline solution supplemented with 2.5 mM Co^{2+} and included 100 μ M 2-amino-5-phosphonovalerate to prevent activation of NMDA receptor channels. Cobalt staining was detected only in retinal slices stimulated by K.A, as indicated by the appearance of brown precipitate in the cell bodies. The ganglion cell layer shows lighter (arrow) and darker stained cells (arrowhead). GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer.

TABLE 1

Apparent permeability ratios for glutamate-gated ion channels

P_{Ca}/P_{mono}		Receptor	Tissue	Reference
1	2			
0.12	0.15	Non-NMDA	Mouse hippocampus and spinal cord	Mayer and Westbrook 1987
<0.18		Type 1 non-NMDA	Rat hippocampus	Iino et al. 1990
2.3	5.3	Type 2 non-NMDA		
	3.2	Non-NMDA	Salamander bipolar cells	Gilbertson et al. 1991
1.4		Non-NMDA	Fusiform glia cells (~ Bergman glia)	Burnashev et al. 1992a
3.3	7.1	Type 1 non-NMDA	Rat retinal ganglion cells	Present study
8.5	17.5	Type 2 non-NMDA		
4.0	10.6	NMDA	Mouse hippocampus and spinal cord	Mayer and Westbrook 1987
6.2	14.3	NMDA	Rat hippocampus	Iino et al. 1990

The apparent Ca^{2+} /monovalent ion permeability ratios (P_{Ca}/P_{mono}) were calculated from reversal potentials using the constant field equation with either ionic concentrations (1) or ionic activities (2).