mutants bind ICAM-1 (Fig. 4B). Our findings in COS cells with the LFA-1 β subunit, integrin β2, are paralleled by the effect of cytoplasmic domain truncation on integrin β1 localization to focal contacts in fibroblasts (*10*) and suggest the presence of both positive and negative regulatory regions in β subunit cytoplasmic domains that can be recognized in fibroblastic cells. Cytoplasmic domain truncation of several immunoglobulin superfamily adhesion molecules has no effect on their adhesiveness (*12*), whereas such truncation ablates adhesiveness of a member of the cadherin family (*13, 14*). Cadherins participate in Ca^{2+}-dependent cell-cell adhesion, and there is no evidence for cellular regulation of their avidity, in contrast to integrins such as LFA-1.

Our results show that the cytoplasmic domain of the β subunit, but not the α subunit, of LFA-1 is important for the regulation of adhesion. This finding correlates with the strong conservation during evolution of the cytoplasmic domain of the LFA-1 β subunit compared to that of the α subunit. The cytoplasmic domains of the human and mouse leukocyte integrin β (CD18) subunits are 96% conserved (*15–17*), whereas the human and mouse LFA-1 α (CD11a) subunit cytoplasmic domains show only 52% amino acid identity (*18, 19*). Similarly, the mouse and human Mac-1 α (CD11b) subunit cytoplasmic domains are only 68% identical (*20–22*). It therefore seems likely that the β subunit would also function in adhesion regulation of not only LFA-1 but also of the other leukocyte integrins that share it, Mac-1 and p150,95.

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Permeation of Calcium Ions Through Non-NMDA Glutamate Channels in Retinal Bipolar Cells

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The conduction of calcium ions through glutamate-gated channels is important in the induction of long-term potentiation and may trigger other cellular changes. In retinal bipolar cells, which lack the *N*-methyl-D-aspartate (NMDA) type of glutamate-gated channel, calcium permeability through non-NMDA channels was examined. Changes in extracellular calcium concentration unexpectedly affected the reversal potential for glutamate-induced currents in a manner consistent with these channels being highly permeable to calcium. External magnesium ions promote desensitization of these non-NMDA channels in a voltage-independent way. Thus, in addition to non-NMDA channels that conduct only sodium and potassium, there is a class that is also permeable to calcium.

RECEPTORS FOR GLUTAMATE IN THE vertebrate central nervous system (CNS) are classified according to whether NMDA, kainate, or quisqualate acts as an agonist. Channels gated by NMDA differ from non-NMDA channels in several physiologically important ways. NMDA channels are permeable to Ca^{2+} ions (*1–4*) but are blocked by Mg^{2+} ions (*5–7*), whereas non-NMDA channels are thought to have neither property (*2–4, 8*) but have faster kinetics (*9*).

Glutamate is the neurotransmitter at the synapse between the photoreceptors and bipolar cells of the vertebrate retina (*10*). Using the patch-clamp technique (*11*) in the whole-cell mode, we have examined the permeability of bipolar cell channels opened by glutamate.

L-Glutamate and the agonists kainate and quisqualate, when rapidly applied (*12*) to bipolar cells isolated from the salamander retina, elicited a conductance increase in 66% of cells examined (n = 642). These cells are thought to be the hyperpolarizing or off-center bipolar cells (*13*). As in other neurons (*9, 14, 15*), quisqualate-induced currents decayed over a time course of tens of milliseconds, whereas kainate-induced currents showed no desensitization (Fig. 1, A and B). In cells that responded to glutamate, kainate, or quisqualate, NMDA (100 to 200 μM) evoked no responses (Fig. 1C) (n = 28 cells) in the presence of glycine (1 to 5 μM), a potentiator of the effect of NMDA (*16*), and in the absence of external Mg^{2+}, which exerts a voltage-dependent block of NMDA-induced currents (*6, 7*). As a further check that NMDA receptors were absent from our cells, we compared the magnitude of glutamate responses in the presence and absence of 1 or 5 μM glycine (*17*). Of 24 cells examined, 21 showed less than 5% change in glutamate-evoked current in the presence of glycine, 2 showed small (<15%) increases, and 1 showed a small (<10%) decrease. The non-NMDA receptor blocker, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (*18*), at 10 μM blocked a majority of the glutamate current. Since the residual current in CNQX could be seen at −60 mV in the presence of 0.5 mM external Mg^{2+}, it is not an NMDA channel current. On the basis of these results, we concluded that NMDA channels are absent from salamander bipolar cells.

Responses to L-glutamate itself (200 μM) resembled those to quisqualate in that a large transient current rapidly decayed to a sustained current. Time constants (mean ± SD) (*19*) for desensitization between 5 and 13 ms (10.1 ± 1.8; n = 24) were recorded, but are likely to be overestimates since drug application was not instantaneous (*12*). Responses to glutamate, quisqualate, and kai-

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nate had reversal potentials in standard physiological solutions (*20*) of about −3 mV (glutamate, −1.9 ± 1.2, n = 23; quisqualate, −3.7 ± 2.3, n = 13; kainate, −3.6 ± 2.2, n = 22), suggesting (*21*) that these agonists gate a cation-selective channel for which Na^+ and K^+ have approximately equal permeability.

To determine whether Ca^{2+} ions permeate glutamate-gated channels, we have applied glutamate in external solutions containing Ca^{2+} concentrations from 0 to 48 mM while making small (5 mV) voltage steps around the reversal potential (Fig. 2 and Table 1).

In the first set of experiments we applied L-glutamate in external solutions (*22*) containing either 1 mM or 20 mM Ca^{2+}, while other ions were unchanged. A negative shift in the reversal potential for both the peak and sustained glutamate current of about 10 mV (mean shift ± SEM of −10.7 ± 0.9; n = 11) was seen after the transition from 20 mM to 1 mM Ca^{2+} (Fig. 2, A and B).

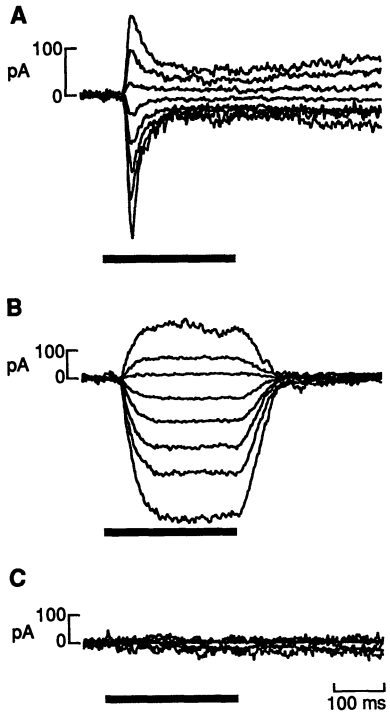

Fig. 1. Retinal bipolar cells contain only non-NMDA receptors. Whole-cell current records during a 250-ms application of 100 μM quisqualate (**A**), 20 μM kainate (**B**), and 100 μM NMDA (**C**). The traces in (B) and (C) are from the same cell. Individual traces represent the subtraction of interleaved control records from records with drug application, thereby removing any voltage-gated currents. Voltage steps from −95 mV to +45 mV lasting 1.8 s were applied in 20-mV increments. Standard internal and external solutions were used except in (C) where external Mg^{2+} was removed and glycine (5 μM) was added. Bars under current records indicate drug application. The delay before responses in (A) and (B) is largely attributable to the dead space between the drug pipette and the cell.

Similar shifts were seen for kainate responses (Fig. 2, C and D) (−10.4 mV ± 0.3; n = 6) and quisqualate responses (−11 mV ± 0.25; n = 2). In the second experiment, solutions of slightly different ionic composition (*23*) containing either 10 mM or no added Ca^{2+} were used. Under these conditions, the reversal potential shifted negative about 7 mV (−6.6 ± 0.8; n = 6) when the solution was changed from 10 mM Ca^{2+} to the nominally Ca^{2+}-free solution. In the third set of experiments, Ca^{2+} was increased from 3 to 48 mM while the Na^+ was reduced from 99 to 37 mM (*24*). If Ca^{2+} were impermeant, we would expect a reduction in external Na^+ concentration to move the reversal potential negative; instead, changing from low Ca^{2+} and high Na^+ to high Ca^{2+} and low Na^+ shifted the reversal potential of the glutamate-gated current slightly positive (+4.5 mV ± 0.9; n = 10). A similar result was obtained for the kainate-gated current (Table 1).

These experiments imply that Ca^{2+} ions carried some of the current induced by L-glutamate. To quantify the relative permeability of Ca^{2+} and monovalent cations, we used the Goldman-Hodgkin-Katz (GHK) equation, extended to include the contribution of divalent ions (*25*) and with calcium activities (*26*) rather than concentrations. All three sets of experiments are consistent with a permeability ratio P_{Ca}/P_{Na} of ~3.2 (*27*). This value is significantly greater than reported values for non-NMDA channels, where P_{Ca}/P_{Na} is estimated to be 0.15 (*3*), a value that gives a very poor fit to our data (Table 1). Our value of P_{Ca}/P_{Na} as 3.2 is less than that reported for the NMDA channel (10.6) (*3*), but this difference would be less if our estimate of P_{Ca} allowed for the decrease in Ca^{2+} activity coefficient with increasing Ca^{2+} concentration. A second reservation about this comparison is that a large negative surface charge at the mouth of the NMDA channel may inflate the estimate of its P_{Ca} by an unknown amount (*1*). Thus, qualitatively, both the NMDA channel and non-NMDA channels on hyperpolarizing bipolar cells have significant Ca^{2+} permeability, whereas the non-NMDA channels previously described (*2–4, 8*) do not.

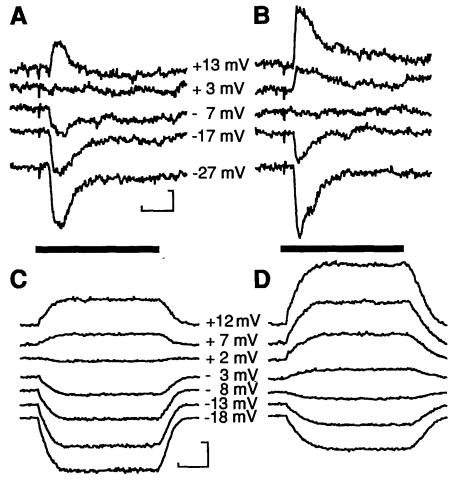

Fig. 2. Glutamate currents are partly carried by Ca^{2+} ions. (**A** and **B**) Current traces during a 200-ms application of 200 μM glutamate (bar) after control records have been subtracted. The external solutions were identical to the standard external solution except they contained 64.2 mM NaCl and either 20 mM $CaCl_2$ (A) or 1 mM $CaCl_2$ (B). In 20 mM Ca^{2+} the current reversed at +3 mV (A), but upon switching to 1 mM Ca^{2+} (B) the reversal potential shifted to −7 mV. Several cycles of solution change showed the effects of Ca^{2+} on the reversal potential to be reversible. (**C** and **D**) Sensitivity of reversal potential of kainate-gated current to Ca^{2+}. Responses of a bipolar cell to 20 μM kainate under the same conditions as above, in either 20 mM Ca^{2+} (C) or in 1 mM Ca^{2+} (D). In 20 mM Ca^{2+}, the kainate current reversed at +2.5 mV (C). After switching to 1 mM Ca^{2+} the reversal potential shifted to about −7 mV (D). Scale bars: (A and B) 50 ms, 20 pA; (C and D) 50 ms, 50 pA.

It is likely that we have examined different conducting states of the same channels as those investigated previously or else different subclasses of channels. Because in most neuronal cell types NMDA and non-NMDA channels coexist, classes of glutamate channel have been studied with the categorical agonists rather than glutamate itself (*3, 4, 8*). Glutamate may open channels with permeability properties different from those of channels gated by kainate or quisqualate; in fact, different agonists elicit preferred conductance states (*2, 28*). However, because we have seen no difference between glutamate- and kainate- or quisqualate-induced permeability properties, we do not favor this explanation.

Alternatively, different cell types may have different channel subclasses or mixtures of these. This view is supported by the cloning of a family of genes that encode non-NMDA channel subunits with mRNAs that show overlapping but differential distribution in the brain (*29*). Further physiological evidence for different subclasses of non-NMDA channel is suggested by a single channel study (*15*) and a report that, in hippocampal neurons, a fraction of the kainate channels have a high Ca^{2+} permeability, like those reported here, but show strong inward rectification (*30*).

The NMDA channel is blocked by external Mg^{2+} ions (*6, 7*). To examine whether our non-NMDA channels were blocked by external Mg^{2+}, we recorded glutamate responses in a nominally Mg^{2+}-free solution. The absence of Mg^{2+} augmented the steady-state current but left the peak current unaffected (n = 8) (Fig. 3). In the presence of Mg^{2+} the steady-state current was 14.4 ±

Table 1. Summary of calcium permeation experiments. In experiments 1 and 2, the only permeant ion that varied between the two solutions was Ca^{2+}. In experiment 3, Ca^{2+} was substituted for a fraction of the external Na^+. V_{rev}, reversal potential; QA, quisqualate; Glu, glutamate; KA, kainate.

Experiment no.	External Ca^{2+} (mM)	Agonist	Measured* V_{rev} (mV)	Predicted† V_{rev} (mV) for P_{Ca} of		n
				3.2	0.15	
1	20	Glu	+2.8 ± 3.3	+2.0	−8.3	11
	20	KA	+2.9 ± 0.7			6
	20	QA	+2.5 ± 0.7			2
	1	Glu	−8.7 ± 3.2	−8.3	−9.0	12
	1	KA	−7.3 ± 1.3			8
	1	QA	−8.5 ± 0.7			2
2	10	Glu	−7.0 ± 2.0	−6.2	−13.7	5
	0	Glu	−13.6 ± 2.2	−14.5	−14.5	6
3	48	Glu	+2.0 ± 3.1	+2.5	−25.3	10
	48	KA	+0.6 ± 4.0			5
	3	Glu	−1.9 ± 1.2	−2.0	−5.2	12
	3	KA	−3.6 ± 2.2			22

*Values are means ± SD. †Predicted reversal potentials (V_{rev}) were calculated by the extended GHK equation (*25*) with P_{Na}/P_K = 1.0 and P_{Ca}/P_K = 3.2 or P_{Ca}/P_K = 0.15 (*3*).

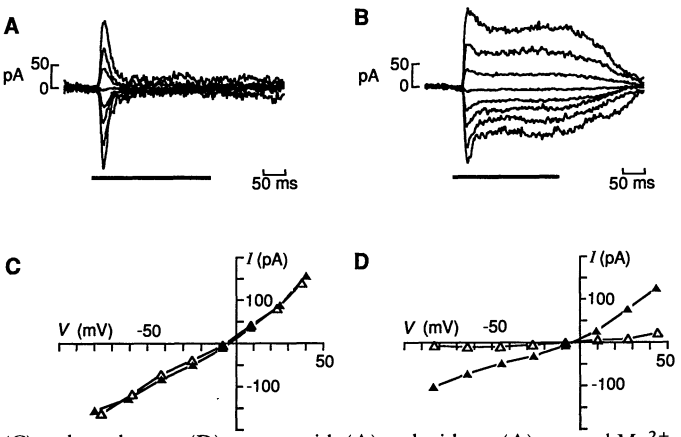

Fig. 3. External Mg^{2+} reduces the steady-state glutamate current but leaves the peak current unaffected. (**A** and **B**) Current responses to 200 μM glutamate in either 0.5 mM Mg^{2+} (A) or nominally zero Mg^{2+} (B) determined at a series of voltages. In this particular cell, steady-state currents were very small in 0.5 mM Mg^{2+}. Removal of Mg^{2+} (B) augments the sustained current at all voltages. (**C** and **D**) The current-voltage (*I-V*) relations for peak (C) and steady-state (D) current with (△) and without (▲) external Mg^{2+}. External Mg^{2+} has no effect on the magnitude of the peak current and has no effect on the reversal potential of either peak or steady-state currents. Unlike the NMDA receptor where Mg^{2+} blocks at a site deep inside the channel (*6*), Mg^{2+} here is probably binding to a site outside the channel.

4.1% (mean ± SD) of the peak current, whereas without Mg^{2+} the steady state was 68.8 ± 10.4% of the peak. Mg^{2+} may promote a transition from the open state into a longer lasting closed state; however, it is clear (Fig. 3D) that the current suppression by Mg^{2+} is not as strongly voltage-dependent as it is at the NMDA channel (*6*).

The current understanding of long-term potentiation (LTP) in the hippocampus implicates NMDA channels in the induction of LTP by virtue of their ability to admit external Ca^{2+} ions. Non-NMDA channels have been thought to play only a secondary role in induction (*31, 32*), consistent with their previously described low permeability to Ca^{2+}. Although this mechanism is supported for the best-studied synapse, that from Schaffer collateral fibers to CA1 neurons, our finding of high Ca^{2+} permeability for some non-NMDA channels raises the possibility that these channels might themselves be sufficient to induce LTP elsewhere in the brain.

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12. Calibration experiments showed that drug onset approximates a single exponential with a time constant of about 30 msec (mean ± SD = 33.1 ± 3.8; n = 5 cells).
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17. Experiments were performed in rapidly flowing Ringer solutions to prevent buildup of extracellular glycine from cells in the chamber.
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20. Internal solution: 83 mM potassium acetate, 10 mM KCl, 1 mM $CaCl_2$, 2mM $MgCl_2$, 13 mM Hepes, 11 mM EGTA, 1 mM adenosine triphosphate (ATP), 0.5 mM guanosine triphosphate (GTP); pH was adjusted to 7.00 with KOH. External solution: 88 mM NaCl, 1.5 mM KCl, 3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 15 mM glucose, 20 mM Hepes, 1 mM sodium pyruvate; pH was adjusted to 7.25 with NaOH.
21. This suggestion is consistent with a previous report (*3*) and is supported by our own measurements of the reversal potential in solutions of different Na^+, K^+, and Cl^- concentrations (T. A. Gilbertson and M. Wilson, unpublished data).
22. External solutions were as in (*20*) except that NaCl was 64 mM and $CaCl_2$ was either 1 or 20 mM. Sucrose was added to maintain the osmolarity.
23. External solutions as in (*22*) except that NaCl was 52 mM and $CaCl_2$ was either 0 or 10 mM.
24. One solution used in the experiment was the standard external solution (*20*) (high Na^+, low Ca^{2+}) while the other (low Na^+, high Ca^{2+}) had 37 mM NaCl and 48 mM $CaCl_2$, leaving other ions constant. Under these conditions, if Na^+ and not Ca^{2+} was permeant through these channels, upon switching from high Na^+ and low Ca^{2+} to low Na^+ and high Ca^{2+}, a large negative shift (−21 mV) in the reversal potential would result.
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