Cellular/Molecular

Q/R Site Editing Controls Kainate Receptor Inhibition by Membrane Fatty Acids

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RNA editing within the pore loop controls the pharmacology and permeation properties of ion channels formed by neuronal AMPA and kainate receptor subunits. Genomic sequences for the glutamate receptor 2 (GluR2) subunit of AMPA receptors and the GluR5 and GluR6 subunits of kainate receptors all encode a neutral glutamine (Q) residue within the channel pore that can be converted by RNA editing to a positively charged arginine (R). Receptors comprised of unedited subunits are permeable to calcium and display inwardly rectifying current–voltage relationships, because of blocking of outward current by intracellular polyamines. In contrast, receptors that include edited subunits conduct less calcium, resist polyamine block, and have relatively linear current–voltage relationships. We showed previously that cis-unsaturated fatty acids, including arachidonic acid and docosahexanoic acid, exert a potent block of native kainate receptors as well as homomeric recombinant receptors formed by transfection of heterologous cells with cDNA for the GluR6(R) subunit. Here, we show that fatty acid blockade of recombinant homomeric and heteromeric kainate receptors is strongly dependent on editing at the Q/R site. Recombinant channels that include unedited subunits exhibit significantly weaker block than channels made up of fully edited subunits. Inhibition of fully edited channels is equivalent at voltages from −70 to +40 mV and is noncompetitive, consistent with allosteric regulation of channel function.

Key words: arachidonic acid; docosahexanoic acid; polyamines; rectification; receptor; hippocampus

Introduction

The activity of voltage- and ligand-gated ion channels can be modulated by direct interactions with cis-unsaturated fatty acids, including arachidonic acid (AA) and docosahexanoic acid (DHA), which represent major constituents of brain membrane phospholipids (Salem et al., 2001). Normally esterified in the 2 position of phosphatidylserine (PS) and phosphatidylethanolamine (PE), AA and DHA can be released as free fatty acids through the action of phospholipase A2 (Six and Dennis, 2000; Murakami and Kudo, 2002). Channels known to be susceptible to direct modulation by free AA or DHA include voltage-gated potassium channels (Honore et al., 1994), members of the two-pore domain family of channels (Fink et al., 1996; Patel et al., 2001), GABA and glycine receptor channels (Schwartz and Yu, 1992; Hamano et al., 1996), as well as NMDA (Miller et al., 1992) and kainate (Wilding et al., 1998) receptors, which form ion channels gated by the excitatory transmitter \( \alpha \)-glutamate. For NMDA receptors (Miller et al., 1992; Nishikawa et al., 1994; Casado and Ascher, 1998) and two-pore domain channels (Patel et al., 2001), exposure to AA or DHA potentiates channel activity. In contrast, cis-unsaturated fatty acids inhibit kainate receptors (Wilding et al., 1998) and produce a variety of effects on voltage-gated \( K^+ \) channels, including inhibition (Poling et al., 1996; Villarroel and Schwarz, 1996) and kinetic modulation (Honore et al., 1994; Keros and McBain, 1997; Oliver et al., 2004). The mechanisms that underlie these varied effects on channel gating are not fully understood, but they are thought to involve either a direct interaction of fatty acids with the channel protein or a local change in the lipid environment immediately surrounding the channel.

RNA editing regulates the ionic selectivity and pharmacology of ion channels formed by non-NMDA receptor subunits (Seeburg and Hartner, 2003). mRNAs for the glutamate receptor 2 (GluR2) subunit of AMPA receptors, and the GluR5 and GluR6 subunits of kainate receptors, undergo editing at a site within the channel pore that encodes a glutamine (Q) residue in the genomic sequence, but which can be edited to encode for an arginine (R) in the mature polypeptide (Sommer et al., 1991). All of the other AMPA and kainate receptor subunits encode for a Q at this location and are not susceptible to editing (Dingledine et al., 1999). The change from Q to R at the Q/R site regulates channel properties (Seeburg and Hartner, 2003). Channels composed only of subunits that contain a Q at this site are permeable to sodium, potassium, and calcium and are subject to voltage-dependent block by internal polyamines, which produces strong inward rectification of the current–voltage relationship (Dingledine et al., 1999). In contrast, the inclusion of one or more subunits that contain an R at the Q/R site reduces the single-channel conductance (Howe, 1996; Swanson et al., 1996), eliminates block by polyamines (Bowie and Mayer, 1995; Kamboj et al., 1995; Bähring et al., 1997; Mott et al., 2003), and reduces channel...
permeability to calcium (Egebjerg and Heinemann, 1993; Köhler et al., 1993; Burnashev et al., 1995, 1996).

In a previous study of kainate receptor modulation by AA and DHA (Wilding et al., 1998), we demonstrated strong inhibition of native receptors expressed by cultured postnatal rat hippocampal neurons and by freshly dissociated sensory neurons from early postnatal rat dorsal root ganglia. In addition, we observed a potent block of homomeric recombinant receptors formed by transient transfection of human embryonic kidney 293 (HEK 293) cells with cDNA for the GluR6 subunit. The present study extends this analysis to additional kainate receptor subunits and demonstrates that susceptibility to modulation by fatty acids exhibits a dramatic dependence on editing at the Q/R site within the channel pore. Fully edited channels are strongly inhibited by low concentrations of fatty acid, whereas channels that include unedited subunits resist inhibition, even by the maximal tolerated dose of fatty acid (50 μM).

Materials and Methods

Cell culture and transfection. HEK 293 cells were maintained in MEM (Invitrogen, Carlsbad, CA) supplemented with 10% fetal calf serum (Sigma, St. Louis, MO), as described previously (Wilding et al., 1998). Cells were cotransfected with channel subunit cDNAs and with the mouse L3T4 surface antigen for identification. Subunit cDNA (1–3 μg) and L3T4 cDNA (0.5 μg) were incubated in 80 μl of OptiMEM (Invitrogen) with Superfect reagent (15 μg; Qiagen, Santa Clarita, CA) for 30 min and then diluted onto HEK 293 cells at 50–70% confluence in 600 μl of Tyrode’s solution containing the following (in mM): 150 NaCl, 4 KCl, 2 MgCl2, 2 CaCl2, 10 glucose, and 10 HEPES, pH adjusted to 7.4 with CsOH. Whole-cell currents were recorded with an Axopatch 200A amplifier (Molecular ATP, 1 GTP, and 10 HEPES, pH adjusted to 7.4 with CsOH. Whole-cell currents were recorded with an Axopatch 200A amplifier (Molecular ATP, 1 GTP, and 10 HEPES, pH adjusted to 7.4 with CsOH. Whole-cell currents were recorded with an Axopatch 200A amplifier (Molecular ATP, 1 GTP, and 10 HEPES, pH adjusted to 7.4 with CsOH. Whole-cell currents were recorded with an Axopatch 200A amplifier (Molecular ATP, 1 GTP, and 10 HEPES, pH adjusted to 7.4 with CsOH. Whole-cell currents were recorded with an Axopatch 200A amplifier (Molecular ATP, 1 GTP, and 10 HEPES, pH adjusted to 7.4 with CsOH.

Electrophysiology. Culture dishes were perfused at 1–2 ml/min with Tyrode’s solution containing the following (in ms): 150 NaCl, 4 KCl, 2 MgCl2, 2 CaCl2, 10 glucose, and 10 HEPES, pH adjusted to 7.4 with NaOH. Boralex glass recording electrodes were pulled to an open tip resistance of 1–5 MΩ when filled with internal solution containing the following (in ms): 140 Cs gluconate, 10 EGTA, 5 CaCl2, 5 MgCl2, 5 ATP, 1 GTP, and 10 HEPES, pH adjusted to 7.4 with CsOH. Whole-cell currents were recorded with an Axopatch 200A amplifier (Molecular Devices, Union City, CA), filtered at 1 kHz (~3 dB, four-pole Bessel), and digitized at 5–10 kHz. Agonist solutions were applied by local perfusion from an eight-barreled pipette as described previously (Wilding et al., 1998). For rapid solution exchange, the drug reservoirs were maintained under static air pressure (~10 psi), and flow was controlled by computer-gated electronic valves. Fatty acid stock solutions (50 ms) were prepared in DMSO and stored in aliquots under argon gas.

Data were fit to modified Hill equations by nonlinear regression (SigmaPlot; Systat Software, Point Richmond, CA). Concentration–inhibition relationships were fit with Equation 1 as follows:

\[
I/I_0 = 1 + (1 + ([DHA]/IC_{50})^n) / ((1 + ([DHA]/IC_{50})^n) + n), \tag{1}
\]

where IC50 is the DHA concentration producing half-maximal inhibition, and n is the slope factor. Equation 1 provides an empirical description of antagonist potency, without implying a specific inhibition mechanism. Concentration–response relationships in the presence of DHA were fit with Equation 2 as follows:

\[
I/I_0 = 1 + ([DHA]/EC_{50})^b / (1 + (EC_{50}/[A])^n), \tag{2}
\]

where [A] and [DHA] are the agonist and DHA concentrations, respectively; EC50 and IC50 are the agonist and DHA concentrations producing half-maximal activation and inhibition, respectively; and n and b are the Hill coefficients for activation and inhibition. Measured parameters are reported as mean ± SEM, and statistical significance was assigned at p < 0.05.

Results

Differential block of GluR5 and GluR6

Our previous study of kainate receptor modulation by fatty acids (Wilding et al., 1998) demonstrated potent inhibition of recombinant homomeric GluR6(R) channels expressed in transfected HEK 293 cells. DHA concentrations as low as 0.5–1 μM produced maximal inhibition, blocking ~80–90% of peak whole-cell current evoked by rapid applications of kainate (Fig. 1A). To facilitate analysis of fatty acid inhibition, we blocked kainate receptor desensitization by exposing transfected cells to concanavalin A (Con A) (Huettert, 1990; Wong and Mayer, 1993). As shown in Figure 1A, DHA inhibited homomeric GluR6(R) channels that had been treated with Con A, albeit with lower potency (Fig. 1C) (IC50 ~ 2.6 μM) than that observed in our previous study without Con A treatment. To extend our analysis to additional kainate receptor subunits, we transfected HEK 293 cells with cDNA encoding the GluR5-2a(Q) subunit, which was kindly provided by Peter Seeburg (Max-Planck-Institute for Medical Research, Heidelberg, Germany) (Sommer et al., 1992). Surprisingly, DHA caused significantly less inhibition of homomeric channels formed by transfection of GluR5(Q) (Fig. 1B) than occurred in cells transfected with GluR6(R). In Con A-treated cells tested with 10 μM kainate, exposure to 15 μM DHA produced 17 ± 4% inhibition of homomeric GluR5-2a(Q) compared with 75 ± 2% block of homomeric GluR6(R).

The GluR5 and GluR6 subunits display ~75% amino acid identity, with the exact percentage depending on which alternately spliced version of GluR5 is used for comparison (Hollmann and Heinemann, 1994). This high degree of homology has facilitated efforts to identify key amino acid residues that underlie differences in physiology and/or pharmacology between these two subunits (Swanson et al., 1997). As an initial step toward defining the region, or regions, that allow for potent fatty acid inhibition of GluR6 channels, we tested four different chimeric subunits, generated by Swanson et al. (1997), that combined N- and C-terminal segments from GluR5 and GluR6. All four of these chimeric subunits code for a glutamine residue at the Q/R site, and all four of them, when expressed as homomeric receptors, displayed <50% inhibition by 15 μM DHA (Fig. 2D).

Homomeric GluR6(Q) resists inhibition

The failure of DHA to block the GluR5/GluR6 hybrid channel subunits suggested that susceptibility to inhibition might depend on the presence of an R at the Q/R site. Previous work has shown that GluR5(R) subunits function poorly as homomeric channels when transfected into heterologous cells (Sommer et al., 1992; Partin et al., 1993; Swanson et al., 1996), whereas homomeric GluR6 receptors produce functional channels in either the Q or R form (Egebjerg and Heinemann, 1993; Köhler et al., 1993; Partin et al., 1993). Therefore, we prepared GluR6(Q) cDNA and tested for inhibition by DHA. As shown in Figure 2, A and D, exposure to 15 μM DHA produced only minimal inhibition of homomeric GluR6(Q) receptors, compared with the strong block of GluR6(R). The effect of DHA on GluR6(Q) was weak either with (Fig. 2A) or without (Fig. 2C) previous exposure to ConA. These results demonstrate the requirement for arginine at the Q/R site of GluR6 in order for strong inhibition to occur, and they suggest that the presence of a glutamine residue at this location in GluR5-
2a(Q), and in the four hybrid channel subunits, likely explains their reduced susceptibility to inhibition. To confirm this point, we generated a Q to R point mutation of the R5-TM1-R6 hybrid channel subunit. This hybrid links the N-terminal domain of GluR5-2, up to the first transmembrane domain (TM1), with the channel core and C-terminal domain of GluR6(Q) (Swanson et al., 1997). Homomeric R5-TM1-R6(R) channels were strongly blocked by DHA (Fig. 2A). We also observed strong inhibition of homomeric R5-1-TM1-R6(R) channels (Fig. 2D), which include the GluR5-1 N-terminal domain inserted of 15 amino acids encoded by an alternately spliced exon (Bettler et al., 1990).

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To test whether strong inhibition by fatty acids was restricted to homomeric GluR6(R) receptors, we transfected HEK 293 cells with cDNA for both the GluR5(R) and GluR6(R) subunits. These two subunits form heteromeric receptors (Bortolotto et al., 1999; Cui and Mayer, 1999; Paternain et al., 2000) that are sensitive to the GluR5-selective agonist (RS)-2-amino-3-(3-hydroxy-5-tertbutylisoxazol-4-yl)propanoic acid (ATPA). Homomeric GluR6 receptors...
The stoichiometry of heteromeric GluR5(R) plus GluR6(R) receptors is not known, their strong block by DHA (Fig. 3) demonstrates that simple inclusion of GluR5 subunits in a heteromeric assembly does not preclude fatty acid inhibition.

Weak inhibition of Q/R heteromeric receptors

Among kainate receptor subunits, only GluR5 and GluR6 can undergo editing at the Q/R site. The remaining subunits, GluR7, kainate receptor subunit 1 (KA1), and KA2, all encode for a glutamine at the homologous location with in the pore loop. To test for inhibition of receptors that included both edited and unedited subunits, we cotransfected HEK 293 cells with several different combinations of subunit cDNAs (Fig. 4). In cells that received GluR6(Q) plus GluR5(R) or GluR6(R) plus GluR5(Q), exposure to 15 μM DHA produced only 9 and 11% inhibition, respectively. In both of these cases, the currents evoked by ATPA were similarly resistant to blocking by DHA (data not shown). Moreover, currents elicited by either kainate or ATPA displayed relatively linear current–voltage relationships (Fig. 4C), suggesting that there was little contribution by homomeric unedited GluR6(Q) or GluR5(Q) channels, which exhibit inward rectification (Fig. 2B).

In addition to GluR5 plus GluR6 heteromeric channels, we also examined cells that received the KA2 subunit together with either GluR5(R) or GluR6(R). KA2 does not form functional homomeric channels but can combine with GluR5 or GluR6 into functional heteromeric receptors (Herb et al., 1992; Sakimura et al., 1992). Cells cotransfected with either GluR5(R) plus KA2 or GluR6(R) plus KA2 displayed linear current–voltage relationships and only weak inhibition by 15 μM DHA. Figure 4D shows concentration–inhibition relationships for seven different homomeric or heteromeric subunit combinations. For comparison, data points for DHA inhibition of kainate receptor-mediated current in cultured hippocampal neurons taken from our previous study (Wilding et al., 1998) have also been plotted. These graphs highlight the difference in potency observed at receptors in which all subunits are edited versus receptors that include one or more unedited subunits. Notably, our previous results in hippocampal neurons (Wilding et al., 1998) agree best with the potency of inhibition at fully edited receptors.

We also examined the effect of 15 μM DHA on cells that received equal cDNA mixtures of GluR6(Q) plus GluR6(R) and GluR5(Q) plus GluR5(R) (Fig. 5). In this case, similar to the GluR5/GluR6 heteromeric receptors just described, exposure to DHA produced only weak inhibition of whole-cell currents. It is more difficult to rule out the formation of subpopulations of channels that included only Q or only R subunits; however, we note that the linear whole-cell current–voltage relationships for both the GluR6(Q) plus GluR6(R) and GluR5(Q) plus GluR5(R) transfected cells argue against a significant contribution by fully unedited channels (Fig. 5C). Furthermore, the weakness of block in cells that received the GluR5(Q) plus GluR5(R) or GluR6(Q) plus GluR6(R) mixtures (Fig. 5D) suggests that few fully edited channels were produced, because these should have been strongly inhibited (Fig. 1).

Voltage-independent, noncompetitive inhibition

Our previous work on kainate receptor modulation by fatty acids used rapid agonist applications to test cells that had not been exposed to Con A. In those experiments, fatty acid inhibition of peak kainate-evoked current was not surmounted by increasing the agonist concentration or by depolarizing cell membrane potentials to +40 mV. Because our results in the present study implicated the Q/R site within the channel pore as a critical de-
terminant of inhibitory potency, we examined the effect of voltage and agonist concentration more rigorously on homomeric GluR6(R) channels in cells that had been exposed to Con A. In most cells (Fig. 6A), the onset of block was well fit by the sum of two exponential decay functions, whereas a single exponential was sufficient to describe recovery from inhibition. As shown in Figure 6, there were no significant differences in the kinetics or the steady-state level of block at holding potentials of \(-70\) and \(-40\) mV. Thus, in contrast to block of GluR6(Q) channels by polyamines, which displays modest voltage dependence for inhibition from the extracellular solution (Bähring et al., 1997) and much stronger voltage dependence for cytoplasmic exposure (Bowie and Mayer, 1995; Kamboj et al., 1995), we observe essen-

Figure 4. Weak inhibition of Q/R heteromeric receptors. A, B, Current evoked by 10 \(\mu\)M kainate applications indicated by open bars, before and after exposure to 15 \(\mu\)M DHA, as indicated by the solid bar. C, Normalized currents as a function of holding potential for cells transfected with R6(R) (\(n = 8\) cells), R6(R) plus R5(Q) (\(n = 4\) cells), R5(Q) (\(n = 4\) cells), R6(R) plus KA2 (\(n = 5\) cells), R5(R) plus KA2 (\(n = 6\) cells), R6(R) plus R5(Q) (\(n = 8\) cells), or R6(Q) plus R5(R) (\(n = 6\) cells). D, Inhibition as a function of DHA concentration. Current evoked by 10 \(\mu\)M kainate (\(n = 3–9\) cells per point) immediately after exposure to DHA is plotted as a fraction of the current before DHA. Smooth curves are the best fit of the following: \(I/I_{\text{control}} = 0.1 + (0.9)/(1 + ([\text{DHA}]/IC_{50})^n)\), where \(IC_{50}\) is the DHA concentration producing half-maximal inhibition, and \(n\) is the slope factor. R6(R) plus R5(Q), \(IC_{50} = 2.6\ \mu\)M, \(n = 1.02\); R6(R) plus KA2, \(IC_{50} = 36.4\ \mu\)M, \(n = 2.73\); R5(Q) plus KA2, \(IC_{50} = 51.5\ \mu\)M, \(n = 2.24\); R6(R) plus R5(Q), \(IC_{50} = 86.1\ \mu\)M, \(n = 1.01\); R6(Q) plus R5(R), \(IC_{50} = 192.3\ \mu\)M, \(n = 0.74\). Data for R6(R) and R5(Q) are replotted from Figure 1C. Data from cultured rat hippocampal neurons (hippocampus, dark gray circles) were adapted from Wilding et al. (1998).

Figure 5. Weak inhibition of GluR6(Q/R) and GluR5(Q/R) receptors. A, B, Current evoked by 10 \(\mu\)M kainate applications indicated by open bars, before and after exposure to 15 \(\mu\)M DHA, as indicated by the solid bar. C, Currents plotted as a function of holding potential for the two cells shown in A and B. D, Current evoked by 10 \(\mu\)M kainate immediately after exposure to 15 \(\mu\)M DHA as a fraction of control current before DHA (\(n = 6\) cells for each subunit combination).
tially no voltage dependence to inhibition by extracellularly applied DHA. Figure 7 illustrates the dependence of inhibition on both agonist and antagonist concentration, confirming the non-competitive nature of block. Smooth curves in this figure represent the best simultaneous fit of all data points to Equation 2 (see Materials and Methods), a modified form of the Hill equation that describes fatty acid inhibition as a monotonic reduction in maximal current.

Discussion

Our results show that inhibition of kainate receptors by DHA depends on the editing status at the Q/R site within the channel pore. In contrast to the potent block of unedited channels by internal and external polyamines (Bowie and Mayer, 1995; Kamboj et al., 1995; Bähring et al., 1997; Mott et al., 2003), we find that extracellular fatty acid applications only produce strong inhibition of recombinant kainate receptor channels that are composed of fully edited subunits. Heteromeric GluR5(Q)/GluR6(R) or GluR5(R)/GluR6(Q) channels were inhibited much less than were fully edited GluR5(R)/GluR6(R) heteromeric receptors or homomeric GluR6(R) receptors. In addition, heteromeric combination of the KA2 subunit, which encodes a glutamine at the location homologous to the Q/R site, with either GluR5(R) or GluR6(R) produced channels that displayed only weak inhibition by DHA. Importantly, the potency of inhibition is nearly identical at native receptors in cultured neurons and at fully edited recombinant receptors that were exposed to Con A.

Although we only tested combinations that include three of the five known kainate receptor subunits, our results collectively provide strong evidence that inclusion of an unedited subunit dramatically weakens the ability of DHA to inhibit the channels. This conclusion is surprising for several reasons. First, our previous study documented relatively strong inhibition of whole-cell currents mediated by native kainate receptors in cultured rat hippocampal cells and in sensory neurons from dorsal root ganglia (Wilding et al., 1998). The maximum level of inhibition (~90%) produced by 50 μM DHA, the highest concentration tolerated, was nearly identical in native receptors as in recombinant GluR6(R) receptors (Wilding et al., 1998), although the potency of block was at least 10-fold greater for GluR6(R) expressed in HEK 293 cells without Con A treatment (IC50 ~ 136 nM) than for native receptors in neurons (IC50 ~ 10 μM). In the present study, the concentration dependence for DHA inhibition of homomeric GluR6(R) or heteromeric GluR5(R)/GluR6(R) channels after exposure to Con A agreed well with the potency of block demonstrated previously at native receptors (Wilding et al., 1998). In contrast, blockade of recombinant channels that included a sub-

Figure 6. Voltage-independent inhibition of R6(R). A. Currents evoked by 1 μM kainate (open bar) at −70 and +40 mV were strongly blocked by 15 μM DHA (solid bar). Superimposed lines show the best fits of the sum of two exponentials plus a constant to block onset or one exponential plus a constant to recovery from block. B. Steady-state block by 15 μM DHA at −70 mV (open bar; n = 18 cells) and +40 mV (shaded bar; n = 14 cells). C. Time constants (left) and relative amplitudes (right) for exponential fits to onset of block and recovery from block. Error bars represent SEM.

Figure 7. Noncompetitive inhibition of R6(R). A. Currents evoked by 10 mM kainate (open bars) in control solution or by 160 nM through 160 μM kainate (shaded bars) during exposure to 15 μM DHA (solid bar). B. Current (mean ± SEM; n = 6–14 cells per point) as a fraction of the control response to 10 mM kainate versus kainate concentration. R6(R) in control solution (○) or during exposure to 0.3 μM (●), 1 μM (■), 5 μM (▲), or 15 μM (○) DHA. Smooth curves are the best fit of all data points to Equation 2. Error bars represent SEM.
Channels lacking an edited subunit exhibit large unitary conductance (Seeburg and Hartner, 2003) and plays a role in subediting at the Q/R site regulates channel conductance and ion selectivity (Sommer et al., 1991; Bernard and Khrestchatisky, 1994; Paschen et al., 1997). GluR6(R) (Sommer et al., 1991; Bernard and Khrestchatisky, 1994) that can extract fatty acids from membranes. In contrast, GluR5 and GluR6 are edited with lower calcium permeability, whereas cells with calcium-activated potassium channels by free DHA or AA at the protein–lipid interface might affect the gating of several different ion channel types (Valiyaveetil et al., 2002), which exhibit homology to glutamate receptor channels. Such specific contacts might be sensitive to membrane curvature, comparable with mechanical deformations that affect the gating of several different ion channel types (Casado and Ascher, 1998; Patel et al., 2001). Direct contact with channels by free DHA or AA at the protein–lipid interface might also alter gating properties. Structural analysis indicates that lipid moieties co-crystallize with and are required for correct refolding and functional operation of purified bacterial KcsA channels (Valiyaveetil et al., 2002), which exhibit homology to glutamate receptor channels. Such specific contacts might be sensitive to free DHA or AA within the membrane and thereby alter channel operation.

DHA is a major component of brain plasma membrane phospholipids (Salem et al., 2001), representing up to 30% of PE and PS fatty acyl chains in synaptosomal (Breckenridge et al., 1972) and synaptic vesicle (Breckenridge et al., 1973) membranes. The high flexibility of unsaturated acyl chains contributes to membrane fluidity and is thought to allow for close apposition between integral membrane proteins and surrounding lipids (El-dho et al., 2003; Feller et al., 2003). Various stimuli elicit mobilization of DHA and AA as free fatty acids (Kim et al., 1999). Interestingly, analysis of synaptosomes and cultured cells suggests that astrocytes release both AA and DHA into the surrounding medium, whereas neurons preferentially release AA but retain DHA (Kim et al., 1999). The effects of cis-unsaturated fatty acids on ion channels have been proposed to involve changes in membrane curvature, comparable with mechanical deformations that affect the gating of several different ion channel types (Casado and Ascher, 1998; Patel et al., 2001). Direct contact with channels by free DHA or AA at the protein–lipid interface might also alter gating properties. Structural analysis indicates that lipid moieties co-crystallize with and are required for correct refolding and functional operation of purified bacterial KcsA channels (Valiyaveetil et al., 2002), which exhibit homology to glutamate receptor channels. Such specific contacts might be sensitive to free DHA or AA within the membrane and thereby alter channel operation.

Dietary depletion of DHA, particularly during early development, is known to change the membrane composition (Salem et al., 2001) and might consequently alter the function of kainate receptors and other sensitive channels. Free DHA and AA have direct effects on a number of different channel types. Exogenously applied AA has recently been reported to produce direct inhibition of presynaptic kainate receptors in hippocampal area CA1 (Cunha et al., 2004). In addition, exposure to exogenous DHA produces a variety of specific changes in hippocampal synaptic transmission and excitability (Itoiz et al., 2000; Young et al., 2000). Extending these studies to evaluate the role of fatty acids released from endogenous sources will require development of better pharmacological reagents to mimic or inhibit the
direct actions of DHA and AA on channel function. Site-directed mutagenesis of channel subunit cDNA has yielded important insights into the structural basis for polyamine regulation of GluR6 (Panchenko et al., 2001; Mott et al., 2003). Our discovery that Q/R site editing also controls fatty acid inhibition of kainate receptors suggests that a similar approach may provide additional understanding of the mechanism of channel regulation by DHA. Such studies could provide new information about the basic operation of glutamate receptor channels (Wollmuth and Sobolevsky, 2004) and aid in efforts to identify more selective reagents to test for channel regulation by endogenous fatty acids.

References


