Channel-Opening Mechanism of a Kainate-Activated Glutamate Receptor: Kinetic Investigations Using a Laser-Pulse Photolysis Technique[†]

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ABSTRACT: Kainate is an excitatory neurotransmitter that binds to the kainate and AMPA receptor subtypes of the glutamate receptor and triggers the formation of cation permeable transmembrane channels in these receptors. In the present report the channel-opening mechanism of the AMPA receptors by kainate has been determined in rat hippocampal neurons using two different kinetic methods, namely, the rapid-flow method (cell-flow) with a 10 ms time resolution and a laser-pulse photolysis technique with a ∼65 μs time resolution. The whole-cell currents induced by kainate, using the cell-flow method, are nondesensitizing and inhibited significantly by CNQX and hence pertain to activation of the AMPA receptors and not the kainate receptors. The cell-flow measurements were used to evaluate the constants pertaining to the minimum mechanism that could account for the concentration of the receptor in the open-channel form over a 500-fold range of kainate concentration. These constants, namely, the intrinsic dissociation constant of kainate from the AMPA receptor and the channel-opening equilibrium constant, were determined to be 140 ± 30 μM and 8 ± 2, respectively. On the other hand, the kinetics of the steps leading to channel opening was evaluated using the laser-pulse photolysis techniques. In this technique whole-cell currents were obtained by releasing kainate in the submillisecond time scale near the cell by photolysis of *N*-(α-carboxy-2-nitrobenzyl) kainate. The concentration of the released kainate was calculated by comparing the whole-cell currents obtained from the laser-pulse photolysis experiments with the whole currents obtained with 100 μM kainate on the same cell using cell-flow measurements. The rate constants for channel opening and closing were then determined from the observed rate constants for the current rise obtained as a function of kainate concentration. These rates were 5000 ± 2000 and 640 ± 30 s^{-1}, respectively. The rate and equilibrium constants obtained in the present report allow an evaluation of the fraction of the receptors in the open-channel form as a function of time and kainate concentration, hence providing insight into the role of kainate in neuronal signal transmission.

Kainate is a neurotoxin that induces seizures and neuropathological symptoms similar to that of temporal lobe epilepsy in humans, when injected in animals (*1*−*3*). These animals have been used extensively as a useful model in the study of epilepsy (*1*−*3*).

At a molecular level, kainate is an excitatory ligand that binds to two subtypes of the glutamate receptor, namely, kainate and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA[1]) receptors, and induces the formation of excitatory cation transmembrane channels in these receptors (*4*−*6*). The movement of ions across these transmembrane channels leads to a change in transmembrane voltage, which results in signal transmission between neurons (*7*). Since the formation of the transmembrane channels is the basis of signal transmission mediated by ligands such as kainate, the mechanism and kinetics of the steps that lead to the formation of these channels in the receptors have been studied extensively using conventional single-channel recording and chemical kinetic techniques (flow measurements) (*8*−*11*). These studies have allowed a determination of the conductance (*8*, *9*) and the dependence of the receptor-mediated current as a function of the ligand concentration under equilibrium conditions (*10*, *11*) for the various types of kainate-activated glutamate receptors. However, since the formation of the transmembrane channel (channel opening) in neurotransmitter receptors occurs in the submillisecond to millisecond time scale (*12*, *13*), the dependence of the receptor-mediated current as a function of time (kinetics) can only be determined using a technique with at least a submillisecond time resolution (*12*−*17*). In the flow measurements the limitation in the time resolution to study membrane-bound receptors is imposed by the slow diffusion and mixing of reactants at the cell surface. This has been overcome by equilibrating a precursor of the desired compound (a "caged" compound) with the cell and photolytically releasing the compound from the inert precursor (*12*, *13*).

The use of this technique (laser-pulse photolysis) for determining the channel-opening kinetics in receptors is best illustrated by the studies on the muscle type nicotinic acetylcholine receptor (*18*). In these studies a 2-nitrobenzyl derivative of carbamoylcholine (*19*), a specific ligand for

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[1] Abbreviations: NMDA, *N*-methyl D-aspartic acid; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione.

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the acetylcholine receptor, was photolyzed to liberate carbamoylcholine in the microsecond time scale (*18*, *19*). This allowed the determination of the rate of formation of the open-channel receptors and hence the determination of the rate constants for channel opening and closing (*18*), which could previously not be determined accurately by using quasi-equilibrium techniques. The evaluation of the rate constants for channel opening and closing is of interest not only because of the importance of these constants in determining the rate of signal transmission between cells but also because it makes it possible to study the effects of drugs that modulate the signal of these receptors (*20*−*21*). This is of importance in the case of the AMPA and kainate receptors, since some of the anticonvulsants currently used are modulators of these receptors (*22*).

In addition to the physical techniques, molecular biological techniques have also been used to characterize the kainate and AMPA receptors (*23*−*26*). These have shown that these receptors are assembled from a family of subunits consisting of several members, GluR1 through GluR7, KA1, and KA2 (*23*−*25*). In situ hybridization studies have indicated that, among these receptor subunits, the GluR2 and GluR4 are the predominant subunits expressed in the CA1 hippocampal cells in rats, and these subunits are AMPA selective (*26*). The high percentage of AMPA receptor subunits in the hippocampal cells is also reflected in the electrophysiological studies by Jonas and Sakmann (*10*), wherein only the nondesensitizing current was observed for the kainate activation of the glutamate receptors. This nondesensitizing current has been assigned to kainate activation of the AMPA receptors through a low-affinity-binding site for this ligand (*27*).

In the present study fast kinetic techniques, such as, the rapid-flow method (cell-flow) with a 10 ms time resolution (*28*) and the laser-pulse photolysis techniques (*18*) with a time resolution of ∼65 μs (*29*), have been used to determine the intrinsic dissociation constant of kainate, the channel-opening equilibrium constant, and the rate constants for channel opening and closing of the kainate-activated glutamate receptor in rat hippocampal neurons. The kainate-activated currents are nondesensitizing and hence have been assigned to the activation of the AMPA receptor subtype of the glutamate receptors.

MATERIALS AND METHODS

Neurons were mechanically dissociated from the hippocampi of 1 day old Sprague−Dawley rats and cultured on dishes coated with 0.5 mg/mL rat tail collagen (*30*). They were maintained in 7.5% carbon dioxide, in the presence of minimal Eagle's medium supplemented with 10 μM glucose, 2.7 μM glutamine, 5% fetal bovine serum, and 5% horse serum (*30*). On the third day, the dishes were treated with 41 μM 5-fluoro-2′dioxyuridine and 102 μM uridine for a day. The cells used in these experiments were 7−14 days in culture. Kainate was purchased from Sigma, and caged kainate, *N*-(α-carboxy-2-nitrobenzyl) kainate, (*29*) was purchased from Molecular Probes.

Krishtal and Pidoplichko (*31*) have described the flow device used for the rapid solution exchange around a single cell, and Udgaonkar and Hess (*28*) have described its use in a cell-flow method. In brief, the flow device is a U-tube with a 100 μm aperture. When a flow rate of 5 cm/s is used and when the cell is placed 50 μm from the aperture, the equilibration of the cell surface receptors with the ligand solution occurs within tens of milliseconds.

In the laser-pulse photolysis experiments, the same flow device as used for the cell-flow experiments was used to deliver the caged kainate to the cell. Once the cell was equilibrated with the caged kainate, the photocleavage of the caged kainate was initiated with a pulse of laser light generated by a Nd:YAG laser ($\lambda = 355$ nm). The output of the laser was coupled into an optical fiber (∼200 μm in diameter), which delivered the light near the cell. The concentration of caged kainate used was 1 mM, and the laser energy at the end of the fiber optic was in the range 100−500 μJ. Before and after each laser-pulse photolysis measurement, whole-cell currents were obtained with 100 μM kainate using the cell-flow method on the same cell; this ensured that there was no cell damage due to the laser beam. In addition, the whole cell currents induced by 100 μM kainate were used to normalize the whole-cell currents obtained by laser-pulse measurements to the values in the dose response curve (this accounts for the variability in the number of receptors from cell to cell). After normalization, on the basis of the whole-cell currents in the laser-pulse photolysis measurements, the value of the fraction of the receptors in the ligated form was obtained using eq 4 as discussed in the results section.

For both the cell-flow and the laser-pulse photolysis experiments the electrode solution contained 140 mM CsCl, 2 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM EGTA, 2 mM Na_2ATP, and 10 mM HEPES (pH 7.4); the extracellular bath solution contained 145 mM NaCl, 1.8 mM $MgCl_2$, 1 mM $CaCl_2$, 3 mM KCl, 10 mM glucose, and 10 mM HEPES (pH 7.4). Whole-cell currents (*32*, *33*) were amplified with an Axon 200B amplifier and low pass filtered at 1 kHz for the cell-flow experiments and at 10 kHz for the laser-pulse photolysis experiments. The filtered signal was digitized using a Labmaster DMA digitizing board controlled by Axon PClamp software. The time constants for the rising and decaying phases for the whole-cell current were obtained by using a nonlinear least-squares fitting program. All of the experiments were performed at room temperature, at pH 7.4, and at a membrane potential of −60 mV.

RESULTS

Cell-Flow Experiments. The whole-cell currents obtained from rat hippocampal neurons upon application of kainate are shown in Figure 1. The rise in the current indicates the formation of the open-channel form of the receptor, which allows the passage of ions across the membrane. This current remains constant and does not decay even when recorded over tens of seconds (data not shown). Typically, whole-cell currents due to neurotransmitter receptors, such as, those mediated by acetylcholine, γ-aminobuytric acid, and glycine, decay within a few seconds after the current rise (*28*, *30*, *34*). This decay, commonly referred to as desensitization, arises due to the formation of a transiently inactivated form of the receptor in the presence of the ligand (*28*, *30*, *34*).

In the case of the kainate activating the glutamate receptor, two types of currents have been reported previously: a fast desensitizing and a nondesensitizing current (*10*, *11*, *27*).

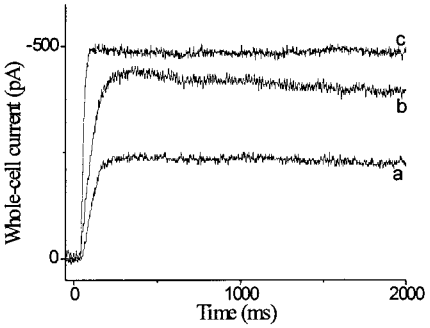

FIGURE 1: Whole-cell currents measured at pH 7.4 and at a membrane potential of −60 mV from rat hippocampal neurons due to the application of (a) 50 μM, (b) 100 μM, and (c) 250 μM kainate. The delay in the current rise from time 0 represents the delay between the trigger and the valve controlling the flow of the kainate solution.

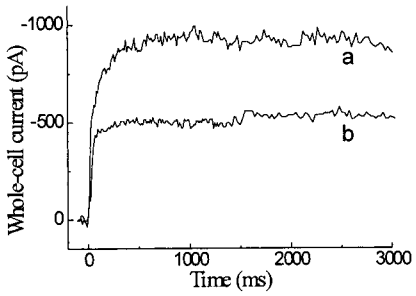

FIGURE 2: Whole-cell current measured at pH 7.4 and at a membrane potential of −60 mV from a rat hippocampal neuron due to the application of 100 μM kainate in the (a) absence and (b) presence of 500 nM CNQX. The cell was preincubated with CNQX for five seconds, prior to the application of 100 μM kainate and 500 nM CNQX. The first five seconds of the current trace has been removed since no changes were observed during this time.

Lerma et al. (*27*) have shown that the nondesensitizing current is nearly completely inhibited by 10 μM 6-cyano-7nitroquinoxaline-2,3-dione (CNQX), while on the other hand 10 μM CNQX has a very small inhibitory effect on the fast desensitizing current. On the basis of the extent of inhibition, they concluded that the fast desensitizing type of current arises from kainate activating the kainate receptor subtypes of the glutamate receptor and the nondesensitizing current arises due to the kainate activating the AMPA receptors (*27*).

In the present study no desensitization was observed in the whole-cell currents obtained upon application of kainate to rat hippocampal neurons. Furthermore, this nondesensitizing current (induced by 100 μM kainate) was inhibited to half of its value in the presence of 500 nM CNQX (Figure 2). On the basis of the absence of desensitization and the large inhibitory effect of CNQX, it is concluded that the kinetics determined in these experiments pertain to the kainate activation of the AMPA receptors.

Equilbrium Constants for Channel Opening. The whole-cell currents arise due to the movement of ions through the open-channel form of the receptor (Figure 3). Therefore the maximum current observed is directly proportional to the fraction of the receptors in the open-channel form. On the basis of the mechanism shown in Figure 3, where "*n*" ligand molecules bind before the channel opens, the relationship between the maximum current (I_A) and the constants pertaining to the mechanism of channel opening is given by the following (*28*):

$$A + L \underset{}{\overset{K_1}{\rightleftharpoons}} AL + L \overset{K_1}{\rightleftharpoons} AL_2 \underset{k_{cl}}{\overset{k_{op}}{\rightleftharpoons}} \overline{AL_2}$$

FIGURE 3: A minimum reaction scheme for the kainate-induced channel opening of the AMPA receptors in hippocampal neurons based on cell-flow experiments. A represents the active form of the receptor, L is the ligand (kainate), $\underline{AL}$ and AL_2 are the ligand-bound closed forms of the receptor, and $\overline{AL_2}$ represents the channel-open form of the receptor. K_1 represents the intrinsic dissociation constant of the ligand and k_{cl} and k_{op} the channel-closing and channel-opening rate constants.

$$I_A = I_M R_M \text{ [fraction of receptors in the open-channel form]}$$

$$= I_M R_M \left[\frac{L^n}{\Phi(L + K_1)^n + L^n}\right] \quad (1)$$

This can be rewritten in a linear form as

$$\left(\frac{I_M R_M}{I_A} - 1\right)^{1/n} = \Phi^{1/n} + \Phi^{1/n}\left(\frac{K_1}{L}\right) \quad (2)$$

where L represents the molar concentration of the ligand, namely kainate, I_M the current due to one mole of receptors, and R_M the moles of receptors in the membrane. Φ^{-1} is the channel-opening equilibrium constant, and K_1 is the intrinsic dissociation constant of kainate. Since the present experiments cannot unambiguously distinguish each of the ligand-binding steps, the intrinsic dissociation constants are assumed to be the same for all of the ligand-binding steps. Thus it should be noted that K_1, Φ^{-1}, and $I_M R_M$ are the "minimum" number of parameters required to satisfy the dependence of the maximum current on the kainate concentration (Figure 4).

The minimum number of kainate molecules that are required to bind to the receptor prior to channel opening can be evaluated by using Hill plots (*35*) (Figure 5). The Hill coefficient evaluated from the slope of the Hill plots was 1.9, which is in good agreement with the value of 1.7 determined by Jonas and Sakmann (*10*). This indicates that a minimum of two ligand molecules is required to bind to the receptor prior to the formation of the open-channel form of the receptor.

Using eq 1, $n = 2$, and the constraint shown in eq 3, the

$$I_A = \frac{I_M R_M}{1 + \Phi} \quad \text{when } L \gg K_1 \quad (3)$$

constants K_1, $I_M R_M$, and Φ^{-1} were evaluated (shown in Table 1) from the dependence of the maximum current as a function of kainate concentration (Figure 4).

The observed value for the concentration of kainate at half of the saturation current (EC_{50}) was 80 ± 30 μM (the theoretical value calculated using eq 1 is 70 ± 30 μM), which is in good agreement with the range of values, 56−150 μM, reported for the kainate-activated currents in rat hippocampal neurons (*8*, *9*, *11*).

The cell-flow measurements as described above allow the determination of the equilibrium constants of the steps that lead to the formation of the transmembrane channel, namely K_1 and Φ^{-1} (Table 1). However, the time resolution of tens of milliseconds of the cell-flow method is insufficient to determine the rate constants of channel opening and closing.

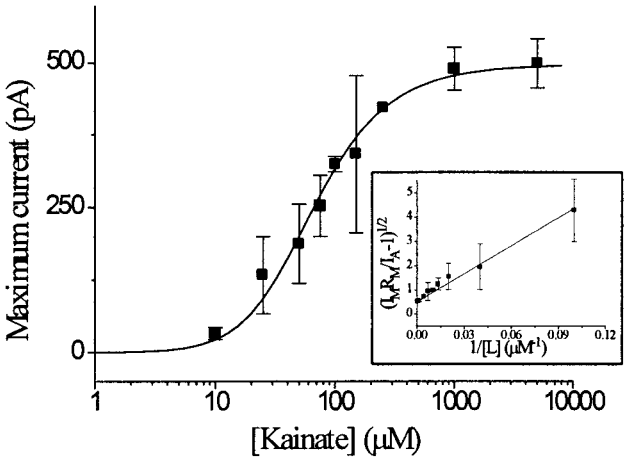

FIGURE 4: Maximum whole-cell current from rat hippocampal cells, using cell-flow measurements, obtained at various concentrations of kainate. Each data point is an average of at least three measurements, with at least two or more cells. The experiments were performed at a membrane potential of −60 mV, at pH 7.4, and at room temperature. The whole-cell currents of the various cells were normalized to the current obtained with 250 μM kainate. Table 1 lists the parameters used for computing the solid line ($I_M R_M$, K_1, Φ) using eq 1 with $n = 2$. The inset shows the same data replotted according to eq 2, with $n = 2$.

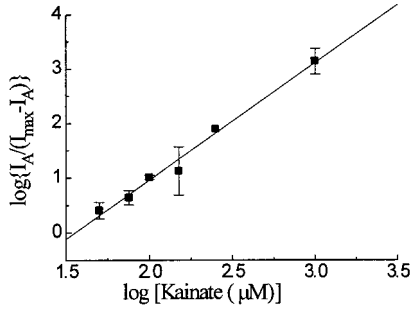

FIGURE 5: Hill plot of the whole-cell current I_A (data shown in Figure 2). I_{max} was calculated using $I_{max} = I_M R_M(1+\Phi)^{-1}$ (*34*).

Table 1: Kinetic and Equilibrium Constants Pertaining to the Various Steps that Lead to the Formation of Open Channels in the AMPA Receptor due to Activation by Kainate in Rat Hippocampal Neurons at a Membrane Potential of −60 mV, pH 7.4, and at Room Temperature

K_1 (μM)	140 ± 30	cell-flow method
EC_{50} (μM)	80 ± 30 (observed)	cell-flow method
	70 ± 30 (calculated)	based on K_1 and Φ^{-1} values
	56−150	cell-flow method (*8*, *9*, *11*)
Φ^{-1}	8 ± 2	cell-flow method
	8 ± 2	laser-pulse photolysis method
$I_M R_M$ (pA)	550 ± 250	cell-flow method
k_{cl} (s^{-1})	640 ± 30	laser-pulse photolysis method
	330−2000	single-channel recording (*35*−*37*)
k_{op} (s^{-1})	5000 ± 2000	laser-pulse photolysis method

Hence, we have used the laser-pulse photolysis experiments which, in the case of caged kainate, has a time resolution of 65 μs (*29*). These experiments are described below.

Laser-Pulse Photolysis Experiments. In the laser-pulse photolysis experiments (Figure 6, inset) 1 mM caged kainate was allowed to equilibrate with the receptors on the surface of a hippocampal cell before photolysis was induced by a laser pulse at time 0. The whole-cell current induced by 100 μM kainate on the same cell before and after the laser-pulse experiments was used to rescale the maximum currents induced by the photoreleased kainate to the measurements

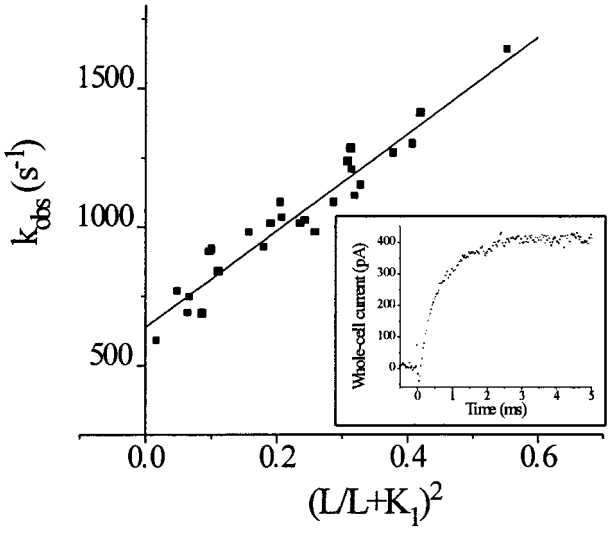

FIGURE 6: Channel-opening rates measured at pH 7.4 and at a membrane potential of −60 mV using laser-pulse photolysis experiments at various concentrations of kainate (L). The value of $(L/L + K_1)^2$ was obtained from the maximum current obtained from the laser-pulse photolysis experiments using eq 4. The procedure is explained in detail in the text. The observed rate constant (k_{obs}) was obtained by using eq 5 for the current rise. The inset shows the whole-cell current induced due to the photolysis of 1 mM caged kainate. The photolysis was initiated at time 0. The rise in the current occurs within a few milliseconds and can be well-represented by a single-exponential rise (eq 5).

indicated in the dose response curve (Figure 4). This rescaling accounts for the cell to cell variability in the number of receptors. The rescaled maximum current (I_A), along with the values of 8 ± 2 and 550 ± 250 nA for Φ^{-1} and $I_M R_M$, respectively (obtained from the dose response curve), was used to determine the fraction of the receptors in the doubly ligated form, $(L/L + K_1)^2$, as shown in eq 4. It must be

$$\left(\frac{L + K_1}{L}\right)^2 = \frac{1}{\Phi}\left[\frac{I_M R_M}{I_A} - 1\right] \quad (4)$$

noted that the assumption made in the cell-flow experiments that the intrinsic dissociation constants are the same for the two ligand-binding steps does not affect the determination of the rate constants, since the fraction of the receptors in the doubly ligated form are calculated from the left-hand side of the eq 4 and the values of Φ and $I_M R_M$ are independent of the model as shown in eq 3.

The current rise that occurs in a few milliseconds (Figure 6, inset) represents the rate of formation of the open-channel form of the receptor. The rise could be well-represented by a single-exponential rate equation:

$$I_t = I_A(1 - e^{-k_{obs}t}) \quad (5)$$

I_t is the current observed at time t. A single-exponential rise of the whole-cell current at all concentrations of kainate would arise if either the ligand-binding or the channel-opening step is rate-limiting. At any given concentration of kainate, if the rates for the ligand-binding steps were in the same time scale as the rates for channel opening, then two exponentials would be required. Furthermore, in the case where the channel-opening step is rate-limiting, the first-order rate coefficient (k_{obs}) shown in eq 5 is related to the constants of the steps pertaining to the channel-opening

process (k_{cl}, k_{op}) by the following relationship (*18*).

$$k_{obs} = k_{cl} + k_{op}\left(\frac{L}{L + K_1}\right)^2 \quad (6)$$

On the other hand, if the ligand-binding steps are rate-limiting, the concentration dependence of k_{obs} as shown in eq 6 will not be satisfied. The plot in Figure 6 shows that the observed rate constants for channel opening obtained at various kainate concentrations can be well-represented by the relationship in eq 6, hence indicating that the ligand-binding steps are fast in comparison to the channel-opening step. On the basis of eq 6, the intercept for the plot in Figure 6 represents the rate constant for channel closing (k_{cl}) and the slope represents k_{op}. The values for the rate constants obtained from this plot are reported in Table 1. The value of k_{cl} is in good agreement with the lifetime of the open channel (equal to $1/k_{cl}$) of 0.5−3 ms determined using single-channel measurements (*36*−*38*). Furthermore, the channel-opening equilibrium constant obtained from the ratio of the rate constants for channel opening and closing is 8 ± 2, in good agreement with the value of 8 ± 2 obtained from cell-flow experiments.

DISCUSSION

Kainate-Activating AMPA Receptor Subtype of the Glutamate Receptor. Two different types of kainate-activated currents have been detected in hippocampal neurons. Jonas and Sakmann (*10*) observed the nondesensitizing kainate-activated response in adult hippocampal neurons, and Lerma et al. (*27*) detected only the fast desensitizing type of kainate-activated currents in embryonic hippocampal neurons. Patneau et al. (*11*) questioned the presence of two different forms, suggesting that the nondesensitizing currents could arise due to a lower time resolution of the measurements, wherein the fast desensitization was not detected. However, in the present study no desensitization was observed for the kainate-activated current in adult rat hippocampal neurons, even with the laser-pulse photolysis technique with tens of microseconds as the time resolution, indicating that the nondesensitizing response corresponds to the activation of a specific type of glutamate receptor. The two types of kainate-activated responses have been assigned on the basis of inhibitor studies (*27*). The low-affinity nondesensitizing form of the kainate-activated current has been attributed to the activation of the AMPA receptor while the high-affinity, fast desensitizing kainate-activating current is believed to be due to the activation of the kainate receptor (*27*). On the basis of these studies, the constants determined in the present study have been assigned to that of the kainate-activated AMPA receptor. The absence of the fast desensitizing form in the present measurements, as well as in those of Jonas and Sakmann (*10*), indicates possible functional differences between postnatal and embryonic neurons.

Kinetics of the Channel-Opening Steps. In the present study chemical kinetic techniques with a millisecond to submillisecond time resolution have been used to evaluate the various equilibrium and rate constants of the steps (Figure 2) that lead to channel opening for the kainate-activated glutamate receptor. Previously the receptor-mediated reactions were studied either under quasi-equilibrium conditions where the equilibration of neurotransmitter with the receptor was the rate-limiting step, or under equilibrium conditions using single-channel current recording (*8*−*11*). These techniques have been useful in determining the conductance and the k_{cl} values of the kainate-activated receptors. However, the k_{op} values cannot be determined using these techniques. This is due to the difficulty in the assignment of the measured rate constant to a particular step in the mechanism (since there is more than one closed form), a recognized and difficult problem in kinetic studies of complex reactions in quasi-equilibrium (*15*, *39*). This has been overcome in the present study by using the laser-pulse photolysis technique. In this technique the time resolution is in the submillisecond time scale; hence the kinetics of the individual steps leading to channel opening can be studied before the reaction has reached an equilibrium.

The channel-opening equilibrium constant for this receptor is much lower than that observed in the other ligand-gated receptors. The muscle type nicotinic acetylcholine receptor expressed in BC_3H1 cells has a channel-opening equilibrium constant of ∼17 (*18*), and for the slowly desensitizing type of $GABA_A$ receptor, the value is 20 (*40*). This indicates that the channel-opening step for kainate-activated AMPA receptors is much less efficient relative to the other ligand-gated channels. The k_{op} value of 5000 ± 2000 s^{-1} for the kainate-activated AMPA receptor is significantly lower than the 9400 s^{-1} value for the nicotinic acetylcholine receptor expressed in BC_3H1 cells (*18*) and marginally lower than the value of 6700 s^{-1} for the slowly desensitizing subtype of the $GABA_A$ receptor in rat hippocampal neurons (*40*). On the other hand the k_{cl} value of 640 ± 30 s^{-1} for the kainate-activated AMPA receptor is marginally higher than the 580 s^{-1} value for the nicotinic acetylcholine receptor expressed in BC_3H1 cells (*18*) and significantly larger than the value of 100 s^{-1} for the slowly desensitizing type of the $GABA_A$ receptor in rat hippocampal neurons (*40*).

The rate and equilibrium constants determined in the present and the previous studies are extremely important since these along with the conductance and receptor concentration determine the change in transmembrane voltage as a function of time and ligand concentration. The integrated change in transmembrane voltage due to the different receptors determines whether a signal will be transmitted to the next neuron. Changes in one or more of these parameters would lead to an imbalance in the signal, which could result in seizures. Furthermore, some of the currently used anticonvulsant drugs are inhibitors of the AMPA receptor. Determining the changes in the kinetic parameters of this receptor in the presence of these drugs would make it possible to determine the value of the intrinsic dissociation constant of the drugs from the open and closed forms of the receptor and hence provide insight into the mechanism of action of these drugs, which would be the first step toward designing better drugs.

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