Volume 311, number 2, 81–84 FEBS 11622 October 1992

Hypothesis

Ligand-activated ion channels may share common gating mechanisms with the *Shaker* potassium channel

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Received 24 August 1992

This paper proposes a detailed gating mechanism for the *N*-methyl-D-aspartate (NMDA) channel. In the NMDAR1 subunit, the signal of agonist binding may be carried from Y456 to W590 through an electron transport chain, including W480 which could be the glycine modulatory site. The channel's opening may arise from repulsion between negatively charged W590s, analogous to W435s of the *Shaker* K^+ channel. The cyclic nucleotide-gated channels may be activated by a similar mechanism, but the opening of nicotinic acetylcholine receptor (nAChR) channels is likely to be initiated by the formation of tyrosine radicals. The role of disulfide-bonded cysteines in the redox modulation can also be explained.

NMDA receptor; Ion channel; Gating mechanism; Electron transfer

1. INTRODUCTION

In our brain, cyclic nucleotide-gated (cNG) channels play a major role in the transduction of visual and olfactory signals into nerve impulses [1,2], while various synaptic channels carry out the signal transmission between nerve cells [3]. During the last decade, significant advances have been made on the molecular structures of these ligand-activated ion channels [3]. However, the physical process between ligand binding and channel's opening remains unclear.

In a previous paper [4], we suggested that tyrosine and tryptophan could be used for signal transduction and biological control, due to their unique long range electron transfer properties. After electron transfer, the acceptor becomes negatively charged, but the donor will quickly deprotonate to become a radical [4]. This feature has been able to explain successfully the gating mechanism of *Shaker* K^+ channels [5]. In this model, the physical gate is located at tyrosine-445 (Y445) and tryptophan-435 (W435). The two regions are too narrow to conduct ions when the channel is in the resting state [6,7]. After the electron jumps from Y445 to W435, the latter is negatively charged while the former becomes a radical. When four subunits are all activated, the modification of hydrogen bonding opens the Y445 region and the electrostatic repulsion widens the W435 region.

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In this paper, we shall show that the ligand-activated ion channels could also be controlled by the YW electron transfer system. The electrostatic repulsion between negatively charged tryptophans may be used to open the cNG and NMDA channels. On the other hand, formation of tyrosine radicals is likely to initiate the conformational change of the nAChR channel. Let us start with cNG channels which are structurally homologous to the *Shaker* K^+ channel [1].

2. CYCLIC NUCLEOTIDE-GATED (cNG) CHANNELS

Like K^+ channels, a cNG channel protein contains six putative helical transmembrane segments, S1–S6. The S4 segment is also dominated by positively charged residues [1]. In the putative pore-forming region between S5 and S6, a catfish olfactory cNG channel (lower line) can be aligned with the *Shaker* K^+ channel (upper line) as follows [1],

$DAFWW_{435}AVVTMTTVGY_{445}GD_{447}MT$

YCFYW STLTLTTIGE MP PP

where W435, Y445 and aspartate-447 (D447) are specifically indicated because they are the major gating components in the YW-gated model for K^+ channels.

The K^+ channel has a unique selectivity that the Na^+ ion is excluded while the larger K^+ ion may pass through. Investigators have been trying to pinpoint the selectivity filter by site-directed mutagenesis. Most residues in the pore-forming region have been mutated, but none of the functional mutants can conduct Na^+ ions

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[8–10]. The YW-gated model suggests that Y445 be the selectivity filter [4]. This idea is difficult to test by site-directed mutagenesis, since mutation of Y445 to any other amino acid would render the channel into non-functional [4].

The cNG channel is a natural 'mutant' which conducts both Na^+ and K^+ ions [2]. It is interesting to note, from the above sequence alignment, that Y445 and D447 are absent from the cNG channel. In the YW-gated model, D447 could be the major voltage sensor [4,5]. The absence of Y445 and D447 may explain why the cNG channel is not K^+-selective and not voltage-sensitive [1,2]. On the other hand, W435 is conserved in the cNG channel. The activation-inactivation coupling in the *Shaker* K^+ channel supports the idea that this residue becomes negatively charged during activation [5]. Repulsion between negatively charged W435s in the four subunits may widen the pore.

We note that the agonist of a cNG channel is negatively charged. In the agonist binding domain, there are a few tyrosine residues [1]. This suggests that the negatively charged agonist may be able to stimulate electron transfer from one of the tyrosines in the agonist binding domain to the 'gating tryptophan' corresponding to W435. If this is the case, the gating mechanisms for cNG and *Shaker* K^+ channels are essentially the same. The agonist of the cNG channel is equivalent to D447 for stimulating electron transfer, and the tyrosine at the agonist binding site is similar to Y445 as the electron donor [4,5]. The major difference is that the tyrosine at the agonist binding site, being away from the pore, is no longer the selectivity filter.

In the cNG channel, the residue at the position of Y445 is a negatively charged glutamate (E). Due to the structural homology to K^+ channels, a cNG channel may also consist of four subunits. Repulsion between the glutamates in four subunits is likely to make this region wider than 3.3 × 3.3 Å, formed by the Y445s in the open state of K^+ channels [4]. This leaves the gating tryptophan to be a major determinant for ionic conductance. From the sizes of permeable organic cations, the pore size of a fully open cNG channel has been deduced to be at least 3.8 × 6 Å [11], which is larger than that of Na^+ and K^+ channels. In this case, repulsion between three charged gating tryptophans in the four subunits may be sufficient to conduct ions, but with different conductance from repulsion between four charged gating tryptophans. This explains the observed multiple conductance states in the cNG channels [12–14]. Our model also agrees with the finding that three cyclic nucleotides are sufficient to open a channel [12–14].

Multiple conductance states are commonly observed in synaptic channels [3,15]. In the next section, we shall present further evidence that the channels whose agonists are negatively charged may be gated by a similar mechanism.

3. SYNAPTIC CHANNELS

The primary structures of major synaptic channels belong to nAChR family, which contains four putative transmembrane segments, M1–M4 [16–20]. The M2 has been demonstrated to be the pore-forming region [3]. In the M2 of glutamate receptors (GluR), only two residues are conserved among all subtypes whose amino acid sequences have been determined. One of them is tryptophan, located near the center of the pore [19,20]. However, tryptophan and tyrosine are totally absent from the M2 of nAChR, glycine receptors (GlyR) and γ-aminobutyric acid type A receptors ($GABA_AR$) [16–18]. After comparing their agonists, we find that all GluR agonists contain two negative charges and one positive charge [21], while acetylcholine, glycine and GABA contain a positive charge which is important for their activities [22]. The structurally simple cations such as Ca^{2+}, Ba^{2+} and Sr^{2+} can also potentiate the nAChR response [23].

Like cyclic nucleotides, the negative charges on the GluR agonist may be able to repel an electron from the agonist binding site to the gating tryptophan in the pore. When two or more subunits are activated, repulsion between negatively charged gating tryptophans can widen the pore. In contrast, the positively charged agonist would induce electron transfer from the pore to the agonist binding site. In the YW electron transfer system, the electron acceptor becomes negatively charged, but the electron donor will quickly deprotonate to become a radical [4,5]. Therefore, the channel's opening for 'positive agonist' channels cannot be controlled by the electrostatic repulsion even if their M2 contained tryptophan or tyrosine.

Involvement of negatively charged tryptophans in the gating of GluR channels is further supported by the binding affinity of open channel blockers in the positive and negative agonist channels. A positively charged drug, MK-801, can block both NMDA (a subtype of GluR) and nAChR channels when they are in the open state [24,25]. However, MK-801 produces a long-lasting block in the NMDA channel while its binding affinity for the nAChR channel is 40–100 times smaller [25]. Since both channels contain no charged residues in the M2 region, the unusually high binding affinity in the NMDA channel could be due to the attraction between the positive charge on MK-801 and the negative charges on gating tryptophans.

To carry the signal from the agonist binding site to the gating tryptophan may require more than one electron transfer step. As pointed out in [4], tyrosine and tryptophan are unique in forming an electron transport chain to carry messages to a distant location. Most of the other amino acids cannot participate in the YW electron transport system. This feature, together with the sequence alignment for GluR subunits, has helped us to identify the residues which could be involved in the

charge relay. We shall use the sequence number in NMDAR1 [20] to label these residues (Fig. 1) In the extracellular domain, Y456 and W480 are the only conserved tyrosine and tryptophan among GluR subunits. They may correspond to the agonist and glycine binding sites. Y456 is identified as the agonist binding site because its neighboring residues are similar in the NMDAR1 and ε1 subunits [20], both responding to the same agonists. On the other hand, NMDAR1, ε1 and non-NMDA subunits have different responses to glycine, which may be explained by their distinct primary structures in the region preceding W480 [20].

The gating tryptophan W590 is located near the center of the pore. The distance between W480 and W590 is likely to be longer than the limit (~15 Å) of a single electron jump. W545, located at the extracellular end of M1, could be an intermediate site between W480 and W590. Experiments have indicated that M1 is close to M2 [16]. Moreover, W545 is the only conserved residue in the whole M1 segment [20].

Recalling that the negative charges on GluR agonists are important for channel's activation and glycine contains a positive charge at its nitrogen atom. Therefore, binding of the agonist at Y456 tends to repel the electron from Y456 to W480 and binding of glycine at W480 may also attract the electron from Y456 to W480. In either case, the probability of electron transfer toward the gating tryptophan is increased. This explains the potentiation effect of glycine. Fig. 1b shows the possible energy levels of the transferring electron at different electron transport sites. The dashed lines represent the modification by the agonist and glycine. In the NMDAR1, the agonist and glycine may change the energy levels only moderately so that each alone is not sufficient to induce electron transfer from Y456 to W480. In the ε1 subunit, the positive charge on glycine may be able to get closer to W480 than in the NMDAR1 so that the potential energy at W480 can be lowered even more. This explains why glycine alone can activate the heteromeric ε1/NMDAR1 channel. Glycine has no effect on non-NMDA subtypes, indicating that its positive charge may not be able to reach W480 in the non-NMDA receptors.

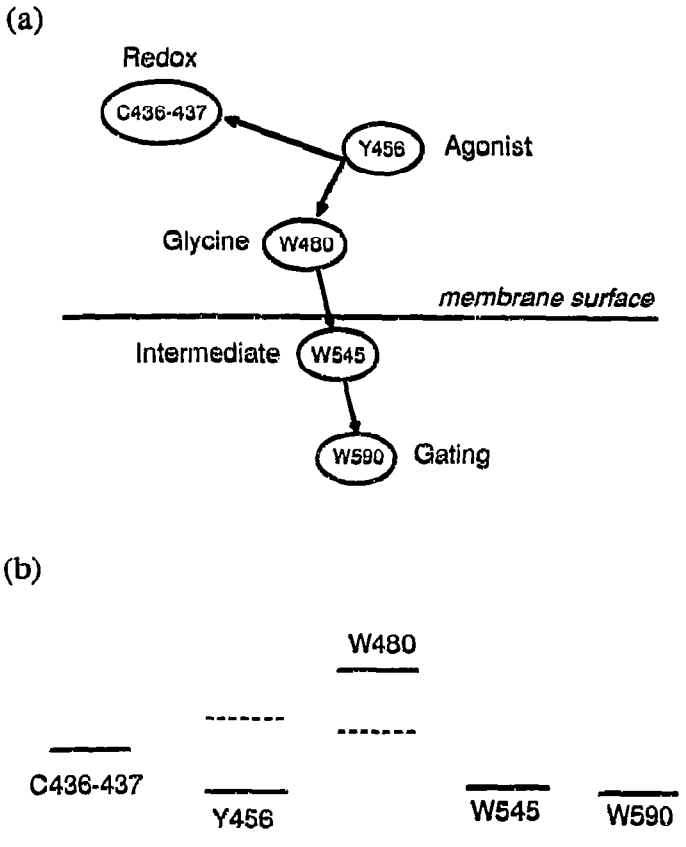

Fig. 1. The electron transport chain in the NMDA receptor channel. (a) The residues involved in carrying the signal from the agonist binding site to the gating tryptophan. The sequence numbering is based on the NMDAR1 subunit [20]. (b) The energy level diagram for the transferring electron at different electron transport sites. The dashed lines at Y456 and W480 represent the modification by the agonist and glycine, respectively. The energy level at C436–437 is for its oxidized state (with a disulfide bond). Its reduced state (without a disulfide bond) cannot participate in the electron transfer process.

The NMDA receptor plays a major role in memory and learning. It is not surprising that the receptor is much more versatile than the non-NMDA subtypes. In addition to the glycine modulatory site, the NMDA receptor also contains a redox modulatory site [26,27]. By acting on this site, the disulfide reducing agent, dithiothreitol (DTT), potentiates the NMDA-induced responses and the sulfhydryl oxidizing agent, 5,5-dithiobis-2-nitrobenzoic acid (DTNB) reverses the effect of DTT. It has been suggested that the redox site is a pair of cysteine residues. In the native NMDA receptor, the cysteine pair can be either disulfide-bonded (oxidized state) or not (reduced state). DTT increases the probability of being in the reduced state and DTNB has the opposite effect [26,27]. In the NMDAR1, the adjacent C436 and C437 are likely to be the redox modulatory site. This cysteine pair is not found in the non-NMDA glutamate receptors [20], consistent with the observation that the redox modulatory site is unique for the NMDA subtype [26]. In the nAChR, an adjacent cysteine pair (located at C192 and C193 of the *Torpedo* α subunit) has been demonstrated to be disulfide-bonded [28].

We mentioned that most amino acids cannot participate in the YW electron transfer system. Interestingly, the disulfide-bonded cysteine pair is a notable exception. The electron transfer between tyrosine and a disulfide-bonded cysteine pair has been demonstrated [29]. Thus, when the redox site is in the oxidized state, the electron may jump from Y456 to either W480 or C436–437 upon agonist binding. The presence of a disulfide-bonded C436–437 makes the electron less likely to reach

the gating tryptophan through normal electron transport chain. This explains the inhibitory effect of DTNB. Reduction of the disulfide bond by DTT will increase the channel's opening probability, since the electron cannot jump from Y456 to C436–437 when the disulfide bond is broken.

4. DISCUSSION

We have been able to propose a detailed model for the gating mechanisms of cNG and GluR channels. The model can further be tested by site-directed mutagenesis and biochemical methods. For the cNG channel, the 'gating tryptophan' corresponding to W435 of the *Shaker* K^+ channel is an essential residue. In the NMDAR1, Y456, W480, W545 and W590 are all essential. Mutations of any of these residues to other amino acids (except tyrosine or tryptophan) are expected to result in non-functional channels.

The gating mechanisms for 'positive agonist' channels are less clear. However, photoaffinity labeling has indicated that the agonist of nAChR binds at or near W149, Y151, Y190, C192–193 and Y198 in the *Torpedo* α subunit [30,31]. One or more of these residues could be involved in carrying the electron from the channel pore to the agonist binding site. In the GlyR channel, tyrosine and a cysteine pair have also been shown to be located near the agonist binding site [32].

If nAChR and GlyR channels are controlled by the YW electron transfer system, then the tyrosines near the extracellular end of M1 or M3 could be responsible for inducing conformational changes after they donate electrons to the agonist binding sites. Experiments have shown that the open pore of a nAChR channel narrows from the extracellular side to the cytoplasmic side [16,33], suggesting that the physical gate be located at the extracellular end. In the closed state, the M2 helices in the five subunits of a channel may be linked by hydrogen bonds or other forces. During channel's opening, the formation of tyrosine radicals near the outer membrane surface may be able to change the binding forces so that the extracellular ends of M2 helices move away from each other, resulting in the observed cone-like shape.

Acknowledgements: I thank Prof. Stuart A. Lipton for bringing the redox modulation in NMDA receptors to my attention, and Prof. Edward Hawrot for helpful discussion.

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