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Linoleic acid inhibits TRP channels with intrinsic voltage sensitivity: Implications on the mechanism of linoleic acid action

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Abstract

Open channel block (OCB) is a process by which ions bind to the inside of a channel pore and block the flow of ions through that channel. Repulsion of the blocking ions by membrane depolarization is a known mechanism for open channel block removal. For the N-methyl-Daspartate (NMDA) channel, this mechanism is necessary for channel activation and is involved in neuronal plasticity. Several types of Transient Receptor Potential (TRP) channels, including the Drosophila TRP and TRP-Like (TRPL) channels, also exhibit open channel block. For the Drosophila TRP and TRPL channels, removal of open channel block is necessary for the production of the physiological response to light. Recently, we have shown that lipids such as polyunsaturated fatty acids (PUFAs), represented by linoleic acid (LA), alleviate OCB under physiological conditions, from the Drosophila TRP and TRPL channels and from the mammalian NMDA channel. Here we show that OCB removal by LA is not confined to the Drosophila TRPs but also applies to mammalian TRPs such as the heat activated TRPV3 channel. TRPV3 shows OCB alleviation by LA, although it shares little amino acid sequence homology with the Drosophila TRPs. Strikingly, LA inhibits the heat-activated TRPV1 and the cold temperatureactivated TRPM8 channels, which are intrinsic voltage sensitive channels and do not show OCB. Together, our findings further support the notion that lipids do not act as second messengers by direct binding to a specific site of the channels but rather act indirectly by affecting the channelplasma membrane interface.

Keywords

TRP channels; open channel block; divalent cations; lipids; membrane

Open channel block (OCB) is a process by which ions have access to intra-channel binding sites inside the pore of an ion channel and block the flow of ions through that channel. The known mechanism of OCB removal is repulsion of the blocking ion by depolarization. Activation of the NMDA channel is a notable example for OCB and its removal in brain neurons. In the case of the ligand-gated NMDA channel, ligand binding by itself is not sufficient to allow cationic influx through the channel's pore at resting membrane potential because of the OCB by Mg^{2+} . Removal of this Mg^{2+} OCB by depolarization¹ allows activation of the NMDA channel, and serves as a coincident detector of pre and post synaptic activities in the brain². The *Drosophila* transient receptor potential (TRP) and TRP-like (TRPL) channels are also subject to OCB. However, puzzlingly, these channels mediate

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light-induced current in the absence of previous membrane depolarization. Recently we have reported on a novel mechanism of OCB alleviation by lipids. OCB causes an outwardly rectifying current voltage relationship (I–V curve) typical for the TRPL and NMDA channels. Application of lipids such as the polyunsaturated fatty acid linoleic acid (LA), results in a linearization of the I–V curve for both TRPL and NMDA channels in the presence of blocking cations. We have shown that lipids alleviate OCB from both NMDA and TRPL channels by facilitating the passage rate of the blocking cations in the channels' pore. Furthermore, we have suggested that the effect of lipids is indirect and operates by modulating the interactions between the membrane lipids and the channels. Thus, lipids do not affect the TRPL channel as second messengers but rather as modifiers of membrane lipid-channel interactions.

The TRP superfamily is evolutionary conserved and plays important roles in signal transduction of many cells types ^{3–9}. Experimental evidence has suggested that several mammalian members of the TRP family that show outward rectification (e.g. TRPC2 ¹⁰, TRPC6 11, TRPV3 12, TRPM6 13 and TRPM7 14) undergo OCB. However, the physiological mechanism underlying the alleviation of OCB in these channels is still unknown. In contrast, some other TRP channels reveal outward rectification which is not due to OCB. Rather, these TRP channels are thought to have intrinsic voltage sensitivity. Examples for extensively studied TRP channels in which their voltage dependence does not arise from OCB are the heat-activated TRPV1 and the cold temperature-activated TRPM8 channels ⁹.

If alleviation of OCB by lipids is a general phenomenon, it is expected that lipid application will cause linearization of the I-V curve of mammalian TRP channels that reveal OCB but that have not been examined in our previous study 15. To test this possibility we examined in the present study the effect of LA on I-V curve of the mammalian TRPV3 channel, which shows divalent open channel block16. TRPV3 expressed in S2 cells was slightly activated by application of 2-aminoethoxydiphenylborate (2-APB12), which produced an outwardly rectifying current as measured by whole-cell patch recordings (Fig 1, green curve, 2). Application of LA greatly enhanced both inward and outward currents (Fig 1, purple curve, 4) resulting in a virtually linear I–V curve (Fig 1, black curve, 5). Our previous study on the TRPL and NMDA channels revealed that lipids have a faster effect at positive than at negative membrane potentials. Figure 1 shows, in a similar manner, that application of LA to S2 cell expressing TRPV3 enhanced the outward currents, faster than the inward currents. In the previous study we further showed that an increased concentration of the blocking cation (Ca²⁺ for the TRPL and Mg²⁺ for the NMDA channels) in the presence of LA restored the outwardly rectifying I-V curve ¹⁵. Similarly, application of Ca²⁺ (not shown) or La³⁺ (Fig. 1 violet curve, 6) restored the outwardly rectifying I–V curve of the TRPV3 channel in the presence of LA.

If some TRP channels show outwardly rectifying I–V curves because of intrinsic voltage sensitivity and not due to OCB, we expect that application of LA will not cause linearization of their I–V curve. To test this notion we applied LA to the mammalian TRPM8 and TRPV1 channels, which do not exhibit OCB but rather exhibit an intrinsic voltage sensitivity ^{9, 17}. Application of menthol¹⁸ and capsaicin¹⁹, (under low Ca²⁺ conditions to prevent Ca²⁺ dependent inactivation of the channels) activated continuously, without desensitization, the TRPM8 and TRPV1 channels, respectively, during ~ 1min of whole cell recordings (Fig. 2, A, B, respectively). Indeed, in contrast to channels that exhibit OCB, the menthol and capsaicin-induced currents were inhibited rather than enhanced by LA (Fig. 2). Similar inhibition by PUFAs of capsaicin activated TRPV1 channels expressed in HEK 293 cells was previously reported ²⁰.

TRPV3 shares little amino acid sequence homology with the TRPL channel, and this is obviously true for the NMDA channel. It is therefore unlikely that LA acts as a second messenger and affects these channels by a direct binding to a specific site, in all of these channel proteins. Rather, it is more likely that alteration of the channel-membrane lipid interface underlies the effect of LA ^{15, 21}. The common denominator of all these channels, with regard to the effect of LA is OCB alleviation and hence current enhancement. In contrast, the intrinsically voltage sensitive, TRPV1 and TRPM8 do not exhibit OCB and are inhibited by LA. These channels also share little amino acid sequence homology with the channels that exhibit OCB or with each other, further supporting an indirect effect of LA. The fact that LA inhibits rather than enhances the activity of TRPV1 and TRPM8 further supports the notion that the enhanced TRPL, NMDA and TRPV3 currents due to LA is not a general effect on all channels, but is related to the removal of the OCB. Together, the present findings further support our notion that lipids do not act as second messengers but rather act indirectly by affecting the channel-plasma membrane interface.

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Figure 2. LA inhibits the voltage sensitive TRPM8 and TRPV1 channels

A. LA inhibited rather than activated the mammalian TRPM8, which is considered to be an intrinsic voltage sensitive channel. The paradigm of Fig. 1 was repeated. Application of 40μ M LA to S2 cells expressing the TRPM8 channel, which were initially activated by menthol (100μ M) decreased current at both negative and positive membrane potentials (n=4). In control experiments when no LA was applied, no decline of the current was observed as there was no desensitization due to Ca²⁺ cations ²².

B. The mammalian TRPV1 channel is also considered to be an intrinsic voltage sensitive channel. The paradigm of Fig. 1 was repeated. Application of 40μ M LA to S2 cells expressing the TRPV1 channel, which were initially activated by capsaicin (1 μ M),

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decreased channel activity, at both negative and positive membrane potentials (n=4). In control experiments when no LA was applied, no decline of the current was observed as there was no desensitization due to Ca^{2+} cations ²³ (see above)