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COMMENTARY

A loosely coordinated interaction site for arachidonic acid on ASICs

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There is an increasing body of research that suggests that polyunsaturated fatty acids (PUFAs) have a significant impact on the way that ion channels function in cells. These fatty acids have long carbon chains and multiple double bonds and can become esterified in processes such as inflammation. These properties allow them to interact with voltage-gated sodium, potassium, and calcium channels as well as a number of ligand-gated channels including acid-sensing ion channels (ASICs; Elinder and Liin, 2017). It has been long known that the PUFA arachidonic acid (AA) can regulate ASICs (Allen and Attwell, 2002; Smith et al., 2007), but interest in the potential of lipids as novel activators of ASICs has grown over the last few years, with more than a dozen different lipids having been shown to potentiate ASIC currents including PUFAs, lysophosphatidylcholines (LPCs), and N-acyl amino acids (Klipp and Bankston, 2022; Marra et al., 2016; Jacquot et al., 2022). To date, the bulk of knowledge on how lipids alter ASIC function has come from patch-clamp experiments, but little is known about how and where they bind. The only hint about where on the channel these lipids interact comes from functional measurements of mutated channels showing that these lipids likely act on the outer leaflet and that mutation of R64 in rat ASIC3 (R63 in human) eliminates regulation of the channel by the PUFA docosahexaenoic acid (DHA; Klipp and Bankston, 2022). However, mutagenesis may alter channel function and eliminate the AA effect for a number of unintended reasons, so a clearer picture of how lipids interact with this family of channels has been critically lacking. In the present issue of *JGP*, Ananchenko and Musgaard (2023) have provided the first atomistic look of lipid interaction with ASICs using molecular dynamics simulations, a critical first step in understanding lipids as a potential regulator of this class of channels.

ASICs are members of the DEG/ENaC family of channels and have long been known to act as sensors of extracellular acidification (Boscardin et al., 2016). This pH sensing function allows these channels to participate in a wide array of physiological functions. For instance, ASIC3 is important in the perception of inflammatory pain in the peripheral nervous system (Deval et al., 2008). Protons are among the first signals released from damaged cells during injury and inflammation. However, a number of other molecules associated with inflammation, including lipids, have been shown to increase the sensitivity of ASIC3 to protons or even activate the channel at neutral pH (Boscardin et al., 2016; Marra et al., 2016; Abdelhamid and Sluka, 2015; Voilley et al., 2001; Deval et al., 2008).

Of particular interest to the work here, research has shown that exudates from patients with joint inflammation contain sufficient amounts of the lipids LPC and AA to activate cells expressing human ASIC3 at neutral pH (Marra et al., 2016). In addition, injection of this combination of lipids into mice was able to elicit an ASIC3-dependent pain response. More recent work revealed that arachidonoyl glycine (AG), an N-acyl amino acid, was also sufficient to activate rat ASIC3 at neutral pH (Klipp and Bankston, 2022). Given our growing understanding of the importance of lipids as regulators of ASICs, it is crucial to have deeper knowledge of how lipids with different structural properties bind and affect ASICs.

The work by Ananchenko and Musgaard (2023) takes the first important step toward filling in this gap in our understanding. They used the MARTINI forcefield and coarse-grained molecular dynamics simulations to examine the binding of AA to the transmembrane segments of human ASIC1a and ASIC3. The study confirmed several hypotheses that had been suggested by previous functional studies, including that PUFAs act on ASICs through direct interaction with the channel rather than changing the membrane structure (Klipp and Bankston, 2022; Smith et al., 2007). 30-µs simulations in membranes containing 80% palmitoyl-oleoyl-phosphatidylcholine, 10% LPC, and 10% AA revealed that AA accumulates around both ASIC3 and ASIC1a, which is consistent with an interaction-dependent regulation. Shorter 500-ns simulations in membranes containing the same large percentage of AA (10%) showed no change in the curvature of the membrane around ASIC3, suggesting that the potentiation of the channel by lipids is not due to non-specific change in membrane shape. To understand where AA interacts with ASICs, the average occupancy and interaction duration of the carboxyl head group of AA on various amino acids in TM1 and TM2 was determined, which uncovered several residues in TM1 of

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both ASIC3 and ASIC1a that had an increased occupancy. Interestingly, while the overall region of interaction was similar between the two isoforms, the specific residues that showed the highest occupancies were different. Furthermore, mutating the most occupied residues in ASIC3 to their counterpart residues in ASIC1a changed the occupancy pattern to resemble that of ASIC1a while mutation of the same ASIC1a residues to their equivalent residues in ASIC3 changed the occupancy pattern to resemble that of ASIC3.

The results of the study offer valuable insights into how PUFAs bind to ASICs. However, when considered in light of existing functional knowledge, it suggests that a reevaluation of how these lipids, which act as low-affinity ligands, interact with and influence ion channels may be necessary. Both ASIC1a and ASIC3 are known to be regulated by AA (Klipp and Bankston, 2022; Smith et al., 2007). In both cases, the pH dependence of activation is shifted toward the basic side, and the rate of desensitization is slowed. The concentration range in which AA affects each isoform is also similar. However, the study's results found a completely different occupancy pattern for each isoform. Additionally, the details of the interaction seem to be different as well. The authors found a stable salt bridge formed between the arginine at position 68 of ASIC3 and the carboxyl head group of AA, which is not present in ASIC1a, where this residue is a phenylalanine. Despite this difference, the head group still occupies the same general area at the top of TM1.

We often assume that ligands bind tightly to a coordinated array of interactions and that if two isoforms bind the same ligand at the same position then the interactions are most likely conserved. However, it seems possible that the interaction between the carboxyl head of AA and the top of TM1 is more loosely coordinated. The negative charge of the lipid's head group, previously shown to be critical, may interact with the positively charged top of TM1. Together with interactions formed by the tail, shown to be important both here and previously, these interactions provide enough energy for the lipid to bind in this general region (Klipp and Bankston, 2022).

So then where does the potentiation of the channel arise from? Interestingly, even though binding of the lipids favors channel opening, simulations of both the resting and open structures showed no state-dependent differences in the occupancy or average interaction duration for any of the residues posited to be involved in the binding interface. Although it does not have high occupancy, there is a notable state-dependent variation in the interaction duration for R63 (R64 in rat). This residue was previously mutated in a study that eliminated the effect of DHA in both rat ASIC3 and rat ASIC1a (Klipp and Bankston, 2022). It is possible that the binding interfaces identified in this work secure the lipid in place, allowing for a series of state-dependent contacts that are the primary drivers of the change in channel gating. This would align with the previous observation that AG potentiated ASIC3 in the rat R64Q mutant background (Klipp and Bankston, 2022). It seems plausible that AG is loosely coordinated in the same region but may make different state-dependent contacts that alter channel function.

Most excitingly, this work provides a number of important and testable hypotheses and again shows the potential synergy between atomistic simulations and patch-clamp experiments. One would predict that mutation of these high-occupancy sites might reduce AA binding and thus the AA effect on the channel. It would also be critical to use atomistic simulations to look at other types of lipids such as LPC and N-acyl amino acids. A combined approach of simulation and patch-clamp experiments could help determine which residues are involved in binding and which ones may be involved in conformational changes that result in changes in gating.

Patch-clamp experiments cannot directly measure lipid binding, and atomistic simulations cannot simulate events on the time scale of ion channel conformational changes. However, by using these methods together, it is possible to describe both structurally and functionally how lipids affect ASIC function. More generally, thorough understanding of the binding and effects of lipids on an ion channel like ASIC could help us understand the fundamental properties of lipid-channel interactions, discover lipids that can distinguish between different ion channels, and even design new ion channel modulators targeted at these important ligand binding sites.

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