JGP Journal of General Physiology

COMMENTARY

ASIC3, a proton-gated ion channel with preference for polyunsaturated lipids with specific headgroup and tail properties

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Polyunsaturated fatty acids (PUFAs) are increasingly being recognized as regulators of ion channel function, e.g., acting on vascular potassium channels to induce vasodilation (Bercea et al., 2021) and voltage-gated neuronal and cardiac sodium, calcium, and potassium channels to tune cellular excitability (Elinder and Liin, 2017; Moreno et al., 2012). Although it has been known for some time that the PUFA arachidonic acid (AA), one of the archetypic PUFAs, can regulate acid-sensing ion channels (ASICs; Allen and Attwell, 2002; Smith et al., 2007), in the present issue of *JGP*, Klipp and Bankston (2022) have revealed a more detailed description of the regulation of ASICs by PUFAs, along with an evaluation of the structural determinants of PUFAs that contribute to ASIC regulation.

The comprehensive and carefully designed study by Klipp and Bankston (2022) focuses on ASIC3, which is a member of the evolutionarily conserved (Lynagh et al., 2018) ASIC family of voltage-insensitive cation channels gated by protons (Deval et al., 2010). Because of their activation in response to extracellular acidification, ASICs are put forward as important chemo-sensing transducer molecules reporting on tissue acidosis. This important function is exemplified by ASIC3, which is expressed in dorsal root ganglion neurons of the peripheral nervous system (Deval et al., 2010, 2008; Waldmann et al., 1997). In these neurons, the activation of ASIC3 generates a transient inward cationic current with a small, sustained component, contributing to cellular depolarization and increased excitability (Deval et al., 2008; Waldmann et al., 1997). Hence, increased activity of ASIC3 induced by tissue acidosis during inflammatory conditions likely plays a role in the perception of inflammatory pain (Deval et al., 2010).

Functional ASICs are formed by assembly of three ASIC subunits, where each subunit contains two transmembrane helices, intracellular N and C termini, and an extensive extracellular loop (Jasti et al., 2007). Interestingly, the pH sensitivity of ASICs can be tuned by endogenous factors whose tissue abundance is increased during inflammatory conditions. For instance, lipid factors such as AA and lysophosphatidyl cholines can potentiate the inward cationic ASIC3 current by shifting the pH-dependence of channel activation towards less acidic pH values and increasing the sustained current component (Allen and Attwell, 2002; Marra et al., 2016; Smith et al., 2007). This is of physiological and pathophysiological interest in the context of pain sensing, as the increased ASICs activity could make specific neurons more active at less acidic pH.

Unesterified PUFAs circulate in the plasma bound to transport proteins such as albumin, readily available to dissociate and interact with ion channels, and can also be locally released as unesterified PUFAs through enzymatic hydrolysis of membrane phospholipids (Elinder and Liin, 2017). Naturally occurring PUFAs share a general structure with a carboxylic acid headgroup linked to an unbranched acyl tail with two or more carbon–carbon double bonds. AA, which is one of the most abundant PUFAs in the human body (Di Marzo et al., 2004), has a 20-carbon-long tail with 4 double bonds. Although AA has been suggested as an important inflammatory agent involved in enhanced pain sensing, there has been limited understanding of PUFA properties required to induce ASIC3 effects. This is of great interest given the rich flora of endogenous PUFAs and structurally related factors, which may or may not induce effects similar to those of AA. Moreover, although previous studies have shown that AA acts directly on ASICs (Allen and Attwell, 2002; Smith et al., 2007), little has been known about putative AA binding sites and underlying mechanisms of action.

The work by Klipp and Bankston (2022) addresses these critical open questions by studying the effect of AA and other PUFAs on rat ASIC3 expressed in Chinese hamster ovary cells, using electrophysiology. By stepping from a holding pH of 8 in the extracellular solution (at which ASIC3 is closed) to more acidic pH values (to activate ASIC3), the pH dependence of ASIC3 in the absence and presence of PUFAs was assessed. In

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line with previous studies, the authors found that extracellularly applied AA shifts the pH dependence of channel activation towards less acidic pH values, resulting in a larger fraction of channels already being open at less acidic pH. This effect nicely increased with rising AA concentrations, with a maximal shift of 0.23 pH units at AA concentrations exceeding 50 µM and ∼12 µM of AA required to induce half the maximal effect. In addition to the AA-induced shift in channel pH dependence, AA increased the current amplitude at saturating proton concentrations and slowed down the rate of desensitization. All of these effects contribute to augmentation of ASIC3 activity.

To identify lipid properties important for inducing augmenting effects, the authors took advantage of the plethora of commercially available PUFAs/PUFA derivatives with different structural properties. Through laborious systematic experimental work, they assessed the response of ASIC3 to >30 different PUFA compounds, with focus on their ability to shift the pH dependence of channel activation. This systematic comparison under similar experimental conditions is a great strength of this study, as it allows for conclusions about critical lipid properties without the caveat of comparing data collected in different experimental models and by different research groups. The results revealed that several PUFAs induce comparable effects to those of AA and that the ability of PUFAs to modulate the activity of ASIC3 depends on both headgroup and acyl tail properties. The most striking finding was that a negatively charged headgroup is required for the effect, with larger effects induced by headgroups with a lower calculated pKa, which favors headgroup ionization at physiological pH. This is best exemplified by comparing the effect of AA derivatives with different headgroups. AA derivatives with uncharged headgroups (i.e., ester or amide headgroups) had no effect on the pH dependence of ASIC3. In contrast, AA derivatives with negatively charged headgroups with lower calculated pKa than AA (i.e., alanine, glycine, or serine headgroups) induced larger effects than AA did. However, interestingly, the authors also revealed a fine balance between headgroup charge and geometry. Bulkier head groups (i.e., taurine or ethanolamide phosphate headgroups) induced smaller effects than anticipated based on their calculated pKa values, possibly because headgroups that are too large sterically prevent PUFA interaction with ASIC3. How these PUFA requirements for ASIC3 enhancement compare to PUFA selectivity among other ion channels is considered below.

In addition to carrying a negative charge, the authors clearly demonstrate that the headgroup needs to be attached to an acyl tail with specific properties to allow for ASIC3 effects. A general observation was that longer tails (20 or 22 carbons long) are more favorable than a shorter tail (18 carbons long). Moreover, the number and position of double bonds are of great importance as PUFAs of equal tail length but different double bond profiles could either be ineffective or among the most prominent ASIC3 modulators. However, the pattern of ideal tail properties remains elusive, as just adding or moving one double bond could make an immense difference in the PUFA effect in an apparently unpredictable manner. Despite challenges in understanding why some tails work better than others, the authors nicely display examples of good and bad matchmaking between tails and headgroups. As an example, the authors showed that a good augmenter, with a 22-carbon-long tail with 4 double bonds attached to a carboxyl head group, could be converted to a poor augmenter by substituting the headgroup for an uncharged methyl ester. Conversely, a poor augmenter, with an 18-carbon-long tail with two double bonds attached to a carboxyl headgroup, could be converted to a good augmenter by substituting the headgroup for a glycine headgroup.

This impressive, extensive assessment clearly identifies PUFA properties important for ASIC3 effects. Next, the authors went one step further to find residues on ASIC3 important for the effect. The decision of which PUFA-interacting residues to test was guided by the findings that PUFA derivatives act only from the extracellular side, that PUFAs commonly interact with arginine, lysine, or tyrosine residues on other ion channels, and that ASIC3 and ASIC1a (which are both modulated by PUFAs) have specific conserved residues. Notably, neutralizing R64 (by the R64Q mutation) at the extracellular end of transmembrane helix 1 (Fig. 1 A) eliminated the ability of the PUFA docosahexaenoic acid to shift the pH dependence of ASIC3 activation. This is an initially valuable indication that R64 may be part of a PUFA binding site in the outer leaflet of the cell membrane, although the authors acknowledge that further studies are needed to establish this. Of note, however, is that mutating R64 did not abolish the effect of the PUFA-derivative arachidonoyl glycine.

Altogether, the findings presented by Klipp and Bankston (2022) highlight several fascinating aspects of lipid signaling via ion channels. First, this study reveals that many endogenous PUFAs and PUFA derivatives induce comparable or even larger effects on ASIC3 than those produced by AA. This raises the intriguing possibility that some of these lipid factors (i.e., arachidonoyl glycine) play a more prominent role than AA in tuning pain signaling, and that many PUFAs/PUFA derivatives may act in concert to augment ASIC3 currents. Hence, this study sets the stage for future work to assess the importance of more efficient endogenous PUFAs/PUFA derivatives in a physiological setting. One challenging aspect in such future studies is the difficulty in establishing the physiological range of concentrations of these lipid factors near their target proteins. The plasma concentration of unesterified PUFAs has been reported to be roughly 10–50 µM and to increase during consumption of certain diets (Fraser et al., 2003; Siddiqui et al., 2008). Hence, the micromolar PUFA concentrations used in the present study to induce ASIC3 effects may well be within an anticipated physiological concentration range. However, less is known about the local PUFA and PUFA-derivative concentrations in specific tissues and how they change during inflammatory conditions. Therefore, establishing the local concentration of the most efficient PUFAs/PUFA derivatives would greatly help interpretation of the both the present and future studies.

Second, this study beautifully defines specific elements of PUFAs that are essential for PUFA modulation of ASIC3. Despite shared general properties such as acyl tail polyunsaturation among tested PUFAs, some PUFAs are efficient ASIC3 modulators whereas others largely fail to modulate the channel. That specific physico-chemical properties of different PUFAs

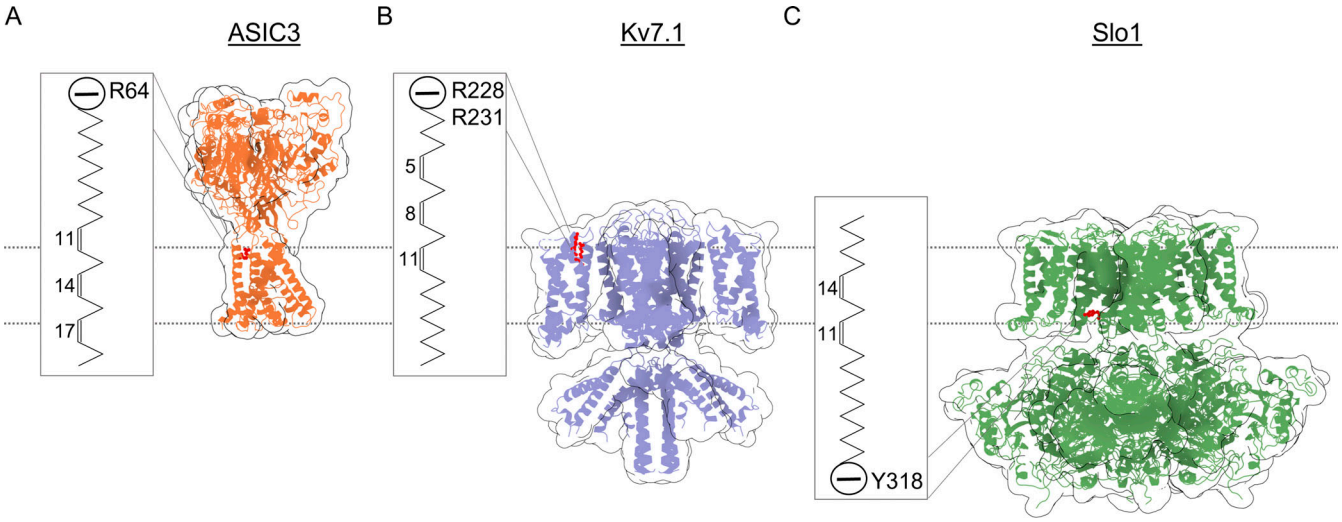

Figure 1. **Schematic illustration of similarities and differences in PUFA modulation of indicated ion channels. (A)** Klipp and Bankston (2022) propose that the negatively charged PUFA headgroup interacts with a positively charged arginine (R64) in transmembrane helix 1 of rat ASIC3 to shift the pH dependence of channel activation. The corresponding residue is indicated here in the structure of chicken ASIC1 (PDB accession no. 6VTK; Yoder and Gouaux, 2020). The inset highlights the need of a negatively charged PUFA headgroup and shows one of the most efficient tails for ASIC3 modulation (20-carbons long with double bonds at positions 11, 14, and 17). **(B)** Previous studies propose that the negatively charged PUFA headgroup interacts with the outermost positively charged arginines (R228 and R231) in transmembrane helix 4 of the voltage-sensing domain of human Kv7.1 to shift the voltage dependence of channel activation. These residues are indicated in the structure of human Kv7.1 (PDB accession no. 6UZZ; Sun and MacKinnon, 2020). The inset highlights the need of a negatively charged headgroup and shows a variant of a 20-carbon long tail (with double bonds at positions 5, 8, and 11) able to modulate Kv7.1/KCNE1. **(C)** Previous studies propose that the negatively charged PUFA headgroup interacts with a tyrosine (Y318) in transmembrane helix 6 of the pore domain of human Slo1 to shift the voltage dependence of channel activation. This residue is indicated in the structure of human Slo1 (PDB accession no. 6V3G; Tao and MacKinnon, 2019). The inset highlights the need of a negatively charged headgroup and shows a variant of a 20-carbon-long tail (with double bonds at positions 11 and 14) able to modulate Slo1. Structures are visualized using 3D Protein Imaging.

influence their ability to modulate ASIC3 supports the authors' suggestion of direct PUFA interaction with ASIC3.

Third, given that ASIC3 exhibits a certain selectivity profile for effective PUFAs, if other ion channels sensitive to PUFAs exhibit different selectivity profiles, there may be differential effects of PUFAs among different ion channels. This idea can be highlighted by comparing the PUFA effect on ASIC3 with those on the Kv7.1/KCNE1 and Slo1 BK channels. There are many similarities in PUFA modulation of these three channels, despite Kv7.1 and Slo1 belonging to the superfamily of voltage-gated ion channels with a completely different channel topology to that of ASICs (Fig. 1). Just as for ASIC3, PUFAs augment the activity of Kv7.1/KCNE1 and Slo1 (Bohannon et al., 2019, 2020; Hoshi et al., 2013; Tian et al., 2016). This is primarily seen as a shifted voltage dependence of channel activation towards more negative voltages (and increased overall currents for Kv7.1/KCNE1). Also, similar to ASIC3, a negatively charged headgroup linked to a polyunsaturated tail is required for augmenting effects. However, there are also important differences. Whereas PUFAs have been suggested to shift the voltage-dependence of Kv7.1/KCNE1 through electrostatic interactions with arginines in the extracellular end of transmembrane helix 4 (Fig. 1 B; Liin et al., 2018; Yazdi et al., 2021), quite like the type of interaction proposed in this study for ASIC3, PUFAs are suggested to interact with a tyrosine in the intracellular end of transmembrane helix 6 of Slo1 (Fig. 1 C; Tian et al., 2016). For ASIC3, the bulky taurine and ethanolamide phosphate headgroups impaired or abolished effects, whereas for Kv7.1/KCNE1 PUFA-taurines are among the best modulators (Bohannon et al., 2020) while PUFA-ethanolamide phosphates work well on Slo1 (Tian et al., 2016). Moreover, despite limited understanding of why certain acyl tails work better than others, the channels display different preference for different tails. For ASIC3, one of the best tails was 20 carbons long and had three double bonds, at positions 11, 14, and 17 counting from the headgroup (shown in Fig. 1 A). In contrast, a similar tail with only two double bonds, at positions 11 and 14, was ineffective on ASIC3. This is in striking contrast to Slo1, for which the 20-carbon-long tail with double bonds at positions 11 and 14 induced large effects (shown in Fig. 1 C; Tian et al., 2016). This is also in striking contrast to Kv7.1/KCNE1, for which the 20-carbon-long tail with double bonds at positions 11, 14, and 17 failed to induce prominent effects, whereas similar tails with double bonds closer to the headgroup induced large effects (shown in Fig. 1 B; Bohannon et al., 2019).

The topics highlighted above point to several open questions regarding regulation of ion channels by PUFAs. For instance, are PUFAs interacting directly with R64 of ASIC3 and, if so, how is this interaction tuning channel pH dependence? What is the mechanistic basis by which PUFAs with specific tail properties induce effects on ASIC3 and other PUFA sensitive ion channels? Are there PUFAs/PUFA derivatives that are even better at differentiating among different ion channels? As the mechanistic underpinnings that define effective PUFAs for a given channel are determined, they may open a pathway for design of more selective pharmacological ion channel modulators utilizing PUFA binding sites and mechanisms.

Acknowledgments

Christopher J. Lingle served as editor.

The assistance of Michael Pökl in preparing Fig. 1 is gratefully acknowledged.

This paper was supported by a grant from the Swedish Research Council (#2021-01885).

The author declares no competing financial interests.

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