Computational and Experimental Analysis Reveals the Arachidonic Acid Metabolite 20-Hydroxyeicosatetraenoic Acid is a Novel Ligand of the Na/K-ATPase

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Objective: We sought to determine the ability of 20-HETE to bind with the NKA relative to other known NKA ligands using a computational molecular modeling approach. We further sought to test the ability of 20-HETE to stimulate NKA mediated signaling in renal proximal tubule cells.

Methods: Computational molecular modeling to investigate the interaction of 20-HETE and NKA was performed using Maestro software analysis (Schrodinger 2021-2). In vitro experiments of NKA signaling were performed with both 20-HETE and its stable analog, 5,14-20-HEDE, in renal LLC-PK1 proximal tubule cells.

Results: First, we performed induced fit docking to predict the binding free energy of both 20-HETE and its stable analog, 5,14-20-HEDE, in comparison with the well-established cardiotonic steroid NKA ligand telocinobufagin. This docking analysis predicted that 20-HETE and 5,14-20-HEDE interact with the NKA with similar binding free energy as cardiotonic steroids (Predicted binding free energies: telocinobufagin =-9.2; 20-HETE= -8.5 and 5,14-20-HEDE = -8.18). Further this computational modeling demonstrated that all of these molecules interact in the same binding pockets of the NKA. Next, our in-vitro experiments showed that 20-HETE and its analog 5,14-20-HEDE increased MAPK activation in a dose dependent manner from 10 nM to 10 uM in LLC-PK1 cell lines. This MAPK activation was significantly reduced after pretreatment with pNaKtide, a specific inhibitor of the NKA-Src signaling complex (1uM pNaKtide, 30 minutes).

Conclusion: The result of these study suggests that 20-HETE interacts with NKA in similar manner as cardiotonic steroids and is capable of inducing NKA signaling in renal proximal tubules.

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