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A COMPUTATIONAL STUDY ON Ca^{2+} MODULATION OF ASIC 1 PHARMACOLOGIC PROPERTIES

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ABSTRACT. Acid-sensing ion channel (ASIC) is involved in important processes like synaptic plasticity and learning, fear and anxiety, pain sensation. Due to its role in neurodegeneration and neuroinflammation, the channel is a viable pharmacological target. The channel is activated by acid pH pulses and it rapidly desensitizes; therefore the channel can exist in open, closed and desensitized states. Here we performed a molecular docking study of some ASIC1 ligands like amiloride, cocaine, histamine, ibuprofen, sinomenine and Zn^{2+} in the transmembrane region of ASIC1 channel models in different states (closed, open and desensitized). Also, since channel properties are influenced by Ca^{2+}, we performed a set of calculations when Ca^{2+} is present in the channel pore. In addition, we modelled mutant channels in different states with substitutions of residues forming Ca^{2+} binding sites. The interaction of ligands with mutant channel models was investigated in the presence and absence of Ca^{2+}. Our results show an affinity of ASIC1 for ibuprofen, followed by Zn^{2+}, histamine and amiloride. Sinomenine and cocaine do not appear as ASIC1 ligands regardless of channel state. Overall, Ca^{2+} enhances the interactions of ligands with the channels, including the interactions of cocaine that is not recognized as an ASIC1 ligand. The effect of mutations is to reduce the favourable interactions with ligands. The results obtained on the three channel states are consistent, showing that results are not significantly influenced by the choice of model. Our results bring new information on ASIC1 pharmacological modulation by showing that Ca^{2+} presence in the pore enhances channel affinity for ligands.

Keywords: *ASIC1, molecular docking, protein-ligand interaction, pharmacology*

INTRODUCTION

Acid-sensing ion channels (ASICs) are proton-activated ion channels that are part of the Na^+ channel superfamily along with sodium epithelial channel (ENaC), *Caenorhabitis elegans* degenerines (DEG), *Drosophila*

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melanogaster channels, „orphan" bile acid-activated channels BLINaC and INaC [1] and FMRF (Phe-Met-Arg-Phe-NH2) – activated sodium channels (FaNaC) of invertebrates [2].

The expression of ASIC channels in the nervous system is both central (ASIC 1a, -2a, -2b and -4) and peripheral (ASIC1-3), being involved in synaptic plasticity and learning [3], fear and anxiety [4], pain sensation [5], mechanosensation [6], ischemic stroke [7] and axonal degeneration in multiple sclerosis [8].

ASIC channels are highly expressed in the brain (ASIC1-4), especially in the hippocampus, amygdala, cerebellum and pituitary gland [9], but are also present in other regions like the cervix, uterus, endometrium, smooth muscles (ASIC2) [10]. They are mainly localized in the plasma membrane and the Golgi apparatus (ASIC1-4), nucleoplasm and cytosol (ASIC3), centriolar satellites and nucleoplasm (ASIC4) [3].

ASIC1a has a high selectivity for sodium ions, transports lithium ions with high efficiency and potassium ions with low efficiency. It mediates glutamate-independent entry of calcium into neurons under acidic conditions. Ca^{2+} overload is toxic to neurons and may be partly responsible for ischemic brain damage [11]. Injuries, inflammation, or ischemia are associated with a decrease of extracellular pH that has an activating role on ASIC channels [12].

ASIC1a works as a postsynaptic proton receptor that contributes to the postsynaptic excitatory current. In this case, ASIC channels sense the pH changes in the synaptic cleft upon neurotransmission [13]. A sudden drop of extracellular pH will produce a transient activation of ASICs followed by a rapid desensitization. Two types of desensitization were described in the case of ASIC, namely a low pH desensitization in which channels are shut from the open state and a steady-state desensitization that shuts the channel from a pre-open closed state [2]. ASIC1a properties are modulated by Ca^{2+}, the cation being responsible for a reduction in amplitude of ASIC1a currents and for shifting the activation pH to more acidic values [14, 15].

Previous studies have shown that the inhibition of ASIC1a by psalmotoxin 1 (PcTx1) reduced the infarct volume in the experimental stroke model by > 60% [12] and intracerebroventricular administration of PcTx1 up to 5 hours after transient occlusion of the middle cerebral artery reduced the infarct volume by 0.50% [16], thus demonstrating an important contribution of ASIC1a to stroke-induced ischemic neurodegeneration and the beneficial outcome of ASIC1a inhibition in such a situation. Moreover, disruption of ASIC gene or inhibition of ASIC function has shown a protective effect in several neurodegenerative diseases, including multiple sclerosis (MS), Huntington's disease and Parkinson's [17] or a reduced clinical deficit and axonal degeneration in experimental autoimmune encephalomyelitis [8]. Due

to its involvement in before mentioned pathological processes, ASIC represents a viable target in neurodegeneration and neuroinflammation [18].

Also, ASIC1 is considered a target in pain and inflammatory processes [18]. In the inflammation process, histamine potentiates ASIC1, leading to hypersensitivity [18]. ASIC1 can be inhibited by anti-inflammatory drugs like ibuprofen, contributing to the analgesic effect and limiting the inflammation [19].

In the present study we performed molecular docking calculations to investigate the interaction of ASIC1 with six possible ligands: a metal ion, namely Zn^{2+} and five small molecules, namely amiloride, ibuprofen, sinomenine, histamine and cocaine. The selected molecules present different affinities for ASIC1 (Zn^{2+} > sinomenine > amiloride > ibuprofen > histamine > cocaine) and different effects, being inhibitors (Zn^{2+}, sinomenine, amiloride, ibuprofen), activators (histamine) or with unknown effect (cocaine). Zn^{2+} is the single metal ion that we considered in the study. In comparison to other metal ions that inhibit ASIC1, like Cu^{2+}, Pb^{2+}, Cd^{2+} or Ni^{2+} (in μM concentrations or undetermined) [20], Zn^{2+} is the strongest inhibitor, acting on ASIC1a in nM concentrations (IC_{50} = ~0.007 μM) [21]. Sinomenine, a bioactive alkaloid, is a strong inhibitor of ASIC1 (IC_{50} of ~0.3 μM [22]) with a broad spectrum analgesic efficacy [23]. Amiloride, a K^+-sparing diuretic, is a strong blocker of ASIC1a channels, with an IC_{50} value of ~10 μM [24], while ibuprofen, a non-steroid anti-inflammatory drug is a low potency inhibitor of ASIC1a, with a IC_{50} of ~350 μM [25]. Histamine is an endogenous compound that induces a voltage-independent potentiation of ASIC1a homomers [26], with an IC_{50} of ~480 μM [27]. In the case of cocaine, recent data obtained on rats showed an association between the overexpression of ASIC1a in nucleus accumbens and the enhancement of cocaine–seeking behaviour [28]. Even if there is no evidence of a direct interaction between cocaine and ASIC1 [29], the compound was included in the present study since lidocaine, a cocaine derivative, inhibits ASIC1 when found in large concentrations (IC_{50} = ~12 mM) [19].

Since multiple crystal structures of ASIC1 are available in Protein Data Bank (PDB) [30], we performed docking calculations using three ASIC1 structures in different states, namely open [31], closed [32] and desensitized [14]. Considering Ca^{2+} effect on ASIC1 functioning, we performed calculations on native structures and on structures with a Ca^{2+} ion placed in the transmembrane (TM) region of the pore, as performed in a previous study [33]. Paukert et al. [15] reported two mutations that render ASIC1a insensitive to Ca^{2+}, namely D433 and E426. Here we also considered the docking of ligands to mutant channels presenting a single mutation at E426 or two mutations at E426 and D433, with and without Ca^{2+}.

Previous molecular docking studies performed on ASIC1 have focused on two regions: the “acidic pocket” (cluster of acidic residues) found in the extracellular region of the channel that is targeted by compounds that modify channel gating [34, 35] and the TM channel pore that is targeted by pore blocking compounds [36, 37]. Amiloride and ibuprofen appear to bind in the TM pore [38, 39], therefore we explore the binding of all selected compounds in the same region. Histamine binding to ASIC1 was previously investigated by molecular docking, but it was docked in the “acidic pocket” [34]. The binding of amiloride to ASIC1 was determined by X-ray crystallography [38], a comparison between the experiment and our results is found in Results and Discussion section. In the case of the other compounds, their docking to ASIC1 is performed here for the first time. In the present study we systematically investigate the binding of ligands on ASIC1a channels with different TM pores conformations according to their activation state, in the presence and absence of Ca^{2+}, resulting in new information on the binding sites of compounds and on the interference of Ca^{2+} with ASIC1a pharmacologic blockade.

RESULTS AND DISCUSSION

Native and mutant ASIC1 structures in different states

In the present study we considered three ASIC1 structures in different states. Information on the structures is summarized in Table 1. 2QTS represents the channel in a closed, desensitized-like state [19], 4FZ0 represents the channel activated by PcTx1, in an open state [18] and 6CMC represents the structure in a desensitized state [20].

Table 1. Description of ASIC1 structures used in the present study

PDB ID	Description	Ligands	pH	Resolution (Å)	Organism	Function	Reference
4FZO	ASIC1a structure with plasmatoxin 1 at 5.5 pH	PcTx1	5.5	2.8	*Gallus gallus*	Functional, open state	[31]
2QTS	ASIC1a structure at low pH	N/A	5.6	1.9	*Gallus gallus*	Nonfunctional, closed state	[32]
6CMC	Barium sites in the structure of a desensitized acid sensing ion channel	Barium	6.9	3.67	*Gallus gallus*	Functional, desensitized	[14]

The architecture of TM pore regions is particular in each state, as presented in Figure 1. In the case of 2QTS, the TM pore is occluded around kinks in the second TM helices (TM2) of two subunits (A and B), near the selectivity filter represented by the GAS motif. Toward the extracellular, the pore opens in a wide outer vestibule [19]. Due to the interaction with PcTx1 in 4FZ0 structure, the outer vestibule is larger, and the TM pore is stabilized in an open conformation, being selective to Na^+ ions. Residues forming the GAS motif are exposed to the extracellular side of the membrane and TM2 helices of subunits A and B are slightly tilted, a resemblance with 2QTS structure [18]. In 6CMC structure, the TM2 helices of ASIC subunits are discontinuous, being interrupted by the residues in the selectivity filter that form a belt-like structure. After the interruption, the cytoplasmic regions of TM2 helices are swapped between adjacent subunits, these features being described in [18].

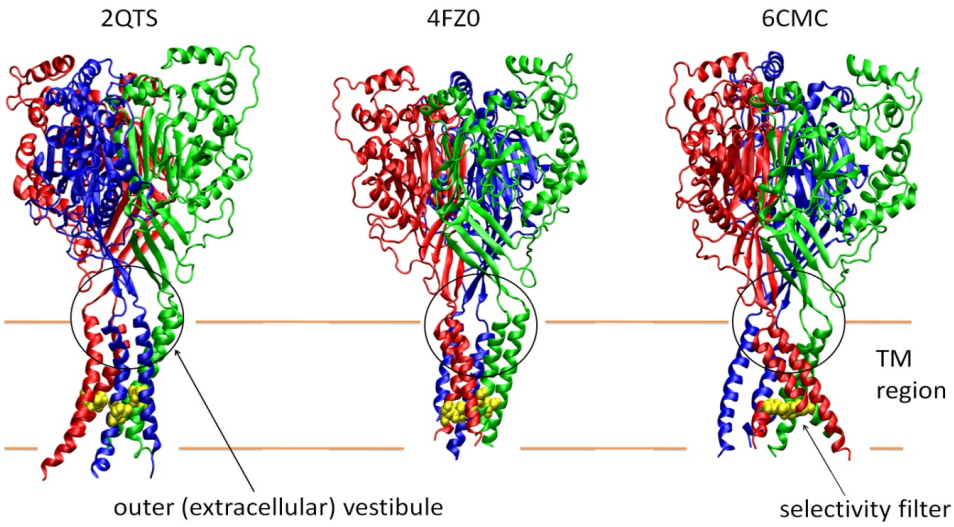


Figure 1. 3D structures of ASIC1 channel in different states: closed, desensitized-like (2QTS structure), open (4FZ0) and desensitized (6CMC). The channels are homotrimers, subunits A are coloured with blue, subunits B are coloured with red and subunits C are coloured in green. The residues from the selectivity filter are represented as van der Waals spheres coloured in yellow. The plasma membrane is schematically represented with orange lines in order to highlight the TM regions of channels. The outer vestibules representing the location where Ca^{2+} and ligands were docked are circled with black.

Mutant channel models were built by changing the residues D433 and E426 that represent Ca^{2+} binding sites [22]. These are located in the TM regions of the channels, in the outer vestibules. The mutations that we modelled are E426G and D433C, meaning that we replaced the negatively charged residues with neutral residues. This has an important impact on the electrostatics of the outer vestibules, as presented in Figure 2. In the case of all three crystal structures, the outer vestibule is electronegative, being coloured with red. The introduction of a single mutation and of two mutations renders the outer vestibule increasingly electropositive, which can be seen as an increase in areas coloured in blue. Even more, we prepared a set of structures with Ca^{2+} in the pore. The ion additionally perturbs the electrostatics of the TM pores. The ion brings two positive charges that on top of the replacement of negative residues leads to a channel pore that is strongly electropositive (in Figure 2 we represented the electrostatics of the double mutant channels with Ca^{2+}). We expect these changes to have an important impact on the interaction between ASIC1 and the six ligands that we considered.

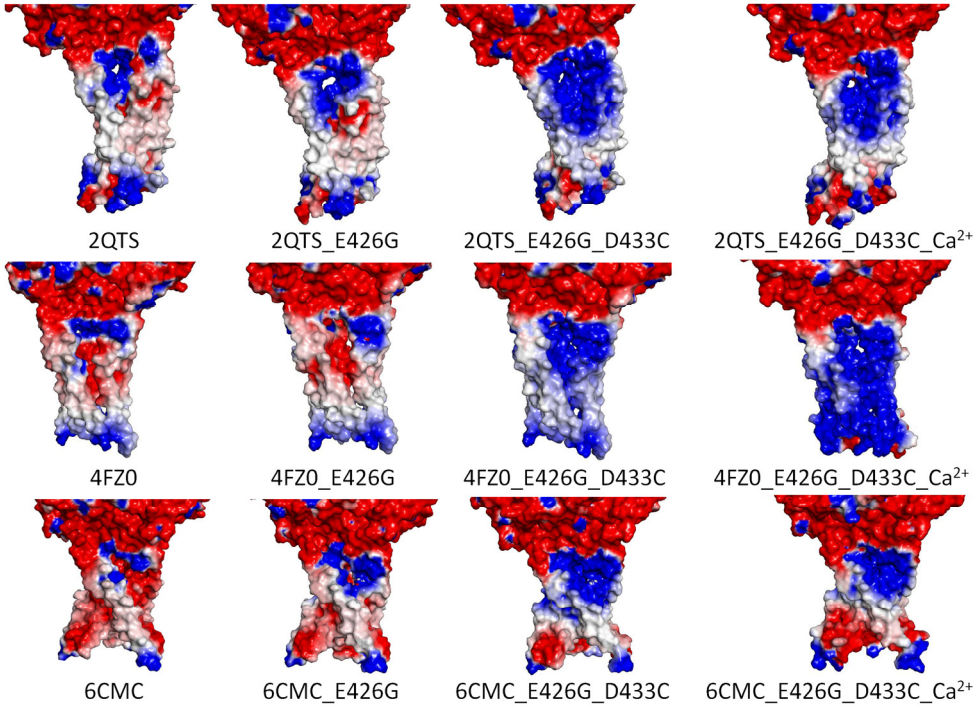


Figure 2. Details on TM regions of native, mutant ASIC1 models and double mutant model with Ca^{2+} represented as surfaces coloured according to the distribution of electrostatic potential. Red is used to highlight electronegative regions and blue is used for electropositive regions.

Physicochemical properties of considered ASIC1 ligands

In our study we considered six ligands: amiloride, cocaine, histamine, ibuprofen, sinomenine and Zn^{2+}. The properties of the ligand molecules that we considered are presented in Table 2. It can be seen that compounds present a variable number of hydrogen bonds donors and acceptors, from a total of 3 in the case of cocaine and ibuprofen, to a total of 12 in the case of amiloride. Amiloride is the most rigid molecule, presenting only 1 rotable bond while cocaine is the most flexible molecule, presenting 5 rotable bonds. Ibuprofen presents the smallest polar surface area (37.3 $Å^2$), followed by cocaine, histamine and sinomenine (~55 $Å^2$) and the largest polar surface area was seen in the case of amiloride (156.79 $Å^2$). According to logP (partition coefficient) values, cocaine, ibuprofen and sinomenine are hydrophobic while amiloride and histamine are hydrophilic. The differences in the physicochemical properties of ligands suggest differences in their interaction with ASIC1 channel models.

Table 2. Physicochemical properties of considered ASIC1 ligands. The data was retrieved from DrugBank [40], except for sinomenine, whose properties were retrieved from PubChem [41].

Ligand	No. of H-bonds donors	No. of H-bonds acceptors	No. of rotable bonds	No of aromatic rings	Polar surface area (PSA, in $Å^2$)	LogP
Amiloride	5	7	1	1	156.79	-0.5
Cocaine	0	3	5	3	55.84	2.28
Histamine	2	2	2	1	54.7	-0.7
Ibuprofen	1	2	4	1	37.3	3.84
Sinomenine	1	5	2	4	59	2.2

Docking of ligands to native ASIC1 structures in the absence of Ca^{2+}

Initially amiloride, cocaine, histamine, ibuprofen, sinomenine and Zn^{2+} were docked at the native channel structures in the three states. CDOCKER algorithm has generated 10 poses for each ligand at each structure, but only the top ranking pose was analysed here. CDOCKER energies of these poses were retrieved and compared in order to estimate which ligands present the strongest interaction. These are represented in Figure 3 and values are reported in Table 3.

In the closed state (2QTS structure), the best binding ligand is ibuprofen (CDOCKER energy = -34.17 kcal/mol), followed by Zn^{2+} (CDOCKER energy = -29.45 kcal/mol), histamine (CDOCKER energy = -11.57 kcal/mol)

and amiloride (CDOCKER energy = -2.35 kcal/mol). Unfavourable interactions were obtained for sinomenine (CDOCKER energy = 5.10 kcal/mol) and cocaine (CDOCKER energy = 10.86 kcal/mol). In the case of ASIC1 in open state (4FZ0 structure), the compounds ordered from the most favourable to the most unfavourable binding are: Zn2+ (CDOCKER energy = -45.28 kcal/mol) > ibuprofen (CDOCKER energy = -31.36 kcal/mol) > histamine (CDOCKER energy = -11.41 kcal/mol) > amiloride (CDOCKER energy = 2.75 kcal/mol) > sinomenine (CDOCKER energy = 13.20 kcal/mol) > cocaine (CDOCKER energy = 16.78 kcal/mol). The order of ligands based on the interaction with ASIC1 in desensitized state (6CMC structure) is: ibuprofen (CDOCKER energy = -31.78 kcal/mol) > histamine (CDOCKER energy = -24.64 kcal/mol) > Zn^{2+} (CDOCKER energy = -18.79 kcal/mol) > amiloride (CDOCKER energy = -18.75 kcal/mol) > sinomenine (CDOCKER energy = -1.30 kcal/mol) > cocaine (CDOCKER energy = 0.94 kcal/mol).

Table 3. CDOCKER Energy determined for the ligands docked at native, simple and double mutation ASIC1 channel models in the three states: closed (2QTS), open (4FZO) and desensitized (6CMC). Results are given for the two datasets: without and with Ca^{2+}.

Ligand name	Receptor structure	CDOCKER energy (kcal/mol)					
		wt		E426G		E426G_D433C	
		no Ca^{2+}	with Ca^{2+}	no Ca^{2+}	with Ca^{2+}	no Ca^{2+}	with Ca^{2+}
Amiloride	2qts	-2.36	-9.40	-2.79	-10.07	-3.83	-8.21
	4fzo	2.75	-16.45	2.77	-9.19	-2.11	-12.77
	6cmc	-18.75	-28.26	-12.00	-13.04	-8.95	-13.09
Cocaine	2qts	10.86	-1.61	9.43	4.24	5.16	4.54
	4fzo	16.78	-3.92	15.75	-0.43	9.98	-1.56
	6cmc	0.94	-4.83	-0.52	4.34	-0.53	-6.48
Histamine	2qts	-11.57	-19.39	-13.59	-18.58	-13.30	-14.76
	4fzo	-11.41	-20.80	-9.25	-18.54	-11.83	-22.77
	6cmc	-24.64	-25.61	-20.58	-16.34	-18.22	-23.43
Ibuprofen	2qts	-34.17	-50.13	-29.14	-48.66	-29.95	-44.89
	4fzo	-31.36	-55.00	-15.65	-47.08	-32.55	-52.63
	6cmc	-31.78	-49.27	-34.89	-45.69	-39.60	-50.84
Sinomenine	2qts	5.10	2.15	4.35	-2.91	4.19	4.35
	4fzo	13.20	5.02	15.41	-4.01	11.39	0.83
	6cmc	-1.30	30.01	-0.04	-4.98	2.59	4.00
Zinc	2qts	-29.45	-16.87	-9.10	-21.75	-13.01	-6.79
	4fzo	-45.28	-14.83	-6.40	-15.95	-8.69	5.37
	6cmc	-18.79	0.98	-45.31	-9.49	-6.51	5.34

By comparing the results we observe that ibuprofen presents the most favourable interaction with ASIC1 independent of channel state. A similar situation can be seen in the case of histamine, but the interaction is less favourable than in the case of ibuprofen and in the case of Zn^{2+} where the interaction with the open channel structure is more favourable than the interactions with the closed or desensitized channels.

Amiloride presents the most favourable interactions with the desensitized channel and even unfavourable interactions with the open channel. The binding of amiloride in ASIC1 TM pore of the desensitized state structure is confirmed by the X-ray diffraction study of Baconguis et al [38] on ASIC1 crystals soaked in amiloride. They identified that the TM pore of ASIC1 is partially occluded by three amiloride molecules bound at superficial locations. The authors suggested that pore blockage should occur when amiloride is bound in a deeper location [38]. In the same study, the authors identified amiloride molecules bound in the extracellular domains, in the “acid pockets” [38], confirming the existence of a second amiloride binding site in the extracellular domain [2]. In the case of closed and open state channels, there is no structural evidence on the binding of amiloride into the TM pores.

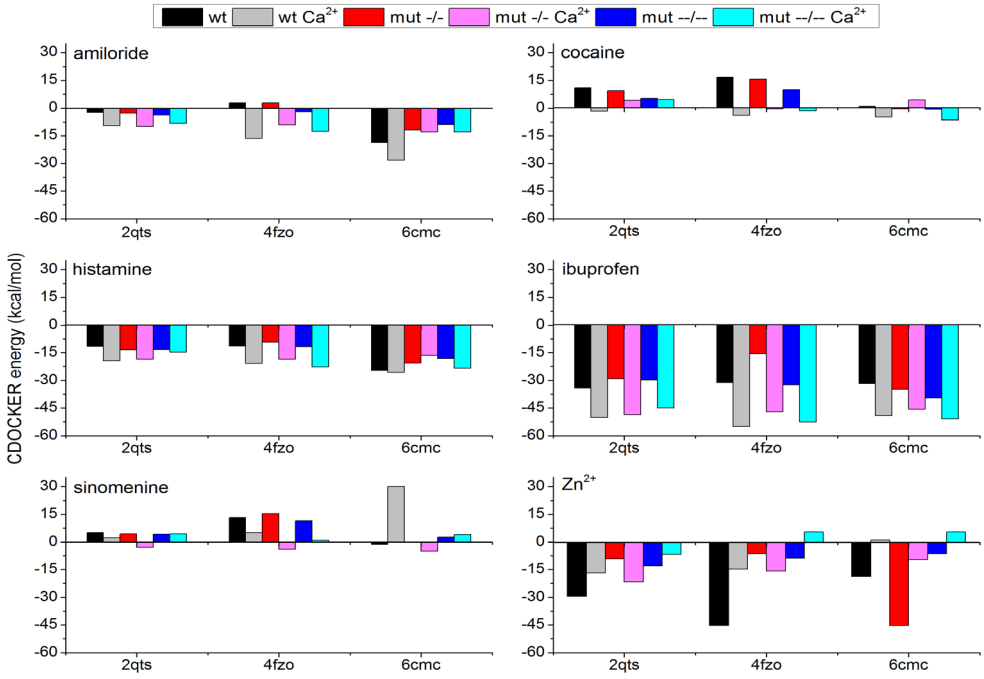


Figure 3. CDOCKER energies associated to the best docking pose of considered ligands at native and mutant ASIC1 channel structures, with and without Ca^{2+}. Docking of ligands to native ASIC1 structures in the presence of Ca^{2+}.

Sinomenine presents unfavourable binding with the closed and open channels and slight favourable binding with the desensitized channel structure. This data suggests that sinomenine does not bind in ASIC1 TM pore. Since data from the literature support the inhibitory effect of sinomenine on ASIC1 [26], we could assume that its binding site was not sampled here and it might be located elsewhere.

In the case of cocaine, it presents unfavourable interactions with all structures, but especially with the closed and open channels. This result was expected, as there are no indications to cocaine being an ASIC1 ligand.

Docking of ligands to native ASIC1 structures in the presence of Ca^{2+}

As can be seen in Figure 3 and according to the data in Table 3, the presence of Ca^{2+} mostly enhances the favourable interactions, with some exceptions that will be discussed below. In the case of the closed channel, we observe that Ca^{2+} facilitates the interaction with ibuprofen (CDOCKER energy = -50.13 kcal/mol), histamine (CDOCKER energy = -19.39 kcal/mol), amiloride (CDOCKER energy = -9.40 kcal/mol), cocaine (CDOCKER energy = -1.61 kcal/mol) and even renders more favourable the interaction with sinomenine (CDOCKER energy = 2.15 kcal/mol). It is worth mentioning that cocaine presents an unfavourable interaction with the channel in the absence of Ca^{2+}, but the presence of Ca^{2+} stabilizes the interaction. Also, as expected due to the positive charge of Ca^{2+}, the interaction with Zn^{2+} becomes less favourable (CDOCKER energy = -16.87 kcal/mol).

The interaction of the open channel model with the ligands (except for Zn^{2+}) becomes more favourable in the presence of Ca^{2+}, in a similar manner as observed in the case of the closed channel. The ligands ordered based on their affinity to the channel bound to Ca^{2+} is: ibuprofen (CDOCKER energy = -55.00 kcal/mol), histamine (CDOCKER energy = -20.80 kcal/mol), amiloride (CDOCKER energy = -16.45 kcal/mol), Zn^{2+} (CDOCKER energy = -14.83 kcal/mol), cocaine (CDOCKER energy = -3.92 kcal/mol). Due to the presence of Ca^{2+} the interactions with amiloride and cocaine have become favourable, while the interaction with Zn^{2+} has become less favourable than in the absence of Ca^{2+}. Less unfavourable interaction energies can be seen in the case of sinomenine (CDOCKER energy = 5.02 kcal/mol).

In the case of the channel in desensitized state, the presence of Ca^{2+} also changes the interactions. These become more favourable for ibuprofen (CDOCKER energy = -49.27 kcal/mol), amiloride (CDOCKER energy = -28.26 kcal/mol), histamine (CDOCKER energy = -25.61 kcal/mol) and cocaine (CDOCKER energy = -4.83 kcal/mol). The interactions with Zn^{2+} (CDOCKER energy = 0.98 kcal/mol) and sinomenine (CDOCKER energy = 30.01 kcal/mol) become unfavourable.

Ca^{2+} is important for ASIC1 activity as it was proved to be an allosteric modulator and channel blocker [42]. Therefore, we considered appropriate to investigate the interaction with ligands in the presence of Ca^{2+}. Overall, regardless of channel state, our results suggest that Ca^{2+} enhance the interactions with ibuprofen, histamine, amiloride and cocaine. This is especially interesting for cocaine that presented unfavourable binding to the channels in the absence of Ca^{2+}. The interactions with sinomenine become more favourable in the presence of Ca^{2+}, except for the structure in desensitized state. Zn^{2+} also presents less favourable interactions with the channels and even unfavourable interactions with the channel in desensitized state with Ca^{2+}.

Docking of ligands to mutant ASIC1 models

According to CDOCKER energies presented in Table 3 and Figure 3, the mutations impact the interactions with ligands, both in the absence and in the presence of Ca^{2+}. The results depend on the state of the channel. For instance, in the case of ibuprofen, in comparison to the native channel, the interaction is slightly less favourable with mutant channel models in closed state in the absence (CDOCKER energy = -29.14 kcal/mol for simple mutation and -29.95 kcal/mol for double mutation) and presence of Ca^{2+} (CDOCKER energy = -48.66 kcal/mol for single mutation and -44.89 kcal/mol for double mutation), Ca^{2+} having the same effect on enhancing CDOCKER energies. In the open state models, relative to the native channel, the single mutation model presents less favourable interaction energies (CDOCKER energy = -15.65 kcal/mol without Ca^{2+} and -47.08 kcal/mol with Ca^{2+}), while the double mutant model presents slightly more favourable interaction energies (CDOCKER energy = -32.55 kcal/mol without Ca^{2+} and -52.63 kcal/mol with Ca^{2+}). In the desensitized state, mutant channels present enhanced favourable interaction energies relative to the native channel (CDOCKER energy for single mutation model = -34.89 kcal/mol without Ca^{2+} and -45.69 kcal/mol with Ca^{2+}; CDOCKER energy for double mutation model = -39.60 kcal/mol without Ca^{2+} and -50.84 kcal/mol with Ca^{2+}).

Amiloride docking at mutant channels in closed states resulted in more favourable GDOCKER energies, especially in the presence of Ca^{2+}. The docking of amiloride to native and single mutation models leads to positive CDOCKER energies (loss of inhibition), only the double mutant channel model in open conformation presents favourable interactions with amiloride. The presence of Ca^{2+} results in favourable interactions between mutant channel models in open state and amiloride. The tendency is inverse in the case of desensitized mutant channel models, where the interactions with amiloride become less favourable, without significant differences in the presence of Ca^{2+}.

The docking of cocaine to mutant channel models results in positive GDOCKER energies (unfavourable) in the absence of Ca^{2+}. Only the mutant channels in open conformation and the double mutant channel in desensitized state, all in the presence of Ca^{2+}, present slightly favourable interactions with cocaine. Histamine presents favourable interactions with mutant channel models regardless of state and the addition of Ca^{2+} enhances the interactions, except for the interaction with single mutation channel in desensitized state. Sinomenine presents unfavourable interactions with ASIC1 mutant channel models, especially with the mutants in open state. It appears that favourable interactions can be seen only in the case of single mutation channels with Ca^{2+}, regardless of channel state. The interaction with Zn^{2+} is less favourable in the case of mutant channel models regardless of state, except for the single mutation channel model in desensitized state, where the interaction with Zn^{2+} is significantly enhanced.

Based on above presented results, it appears that the change in pore electrostatics trough mutations has different effects of the affinity for ligands, increasing the affinity toward some of them, like amiloride or decreasing the affinity for others, like Zn^{2+}. The affinity toward the ligand used as negative control increases in mutant channel models and in the presence of Ca^{2+}.

CONCLUSIONS

Here we performed a systematic investigation on the interaction between six ligands (amiloride, cocaine, histamine, ibuprofen, sinomenine and Zn^{2+}) and ASIC1 channel models in different states (closed, open, desensitized), with and without a Ca^{2+} ion placed in the outer vestibule of the pore. In the study we sampled the outer channels vestibules; sampling other sites in the extracellular domain being a future perspective.

Our results point towards ibuprofen as a high affinity ligand that binds in the TM regions of the channels, regardless of state. Other ligands showing an affinity for this region are Zn^{2+}, histamine and amiloride. Sinomenine and cocaine do not present a favourable binding in TM pores of ASIC1 models. The result was expected in the case of cocaine. In the case of sinomenine, a proved inhibitor of ASIC1, we hypothesize that its binding site is located in a different region than the TM pore.

Obtained results are consistent in the case of channels in the three states for cocaine, histamine, ibuprofen, sinomenine and Zn^{2+} showing that the choice of model doesn’t significantly impact results. Differences were obtained in the case of amiloride, where our docking calculations show a strong favourable interaction with the channel in desensitized state, a weak favourable interaction with the channel in closed state and an unfavourable interaction with the channel in open state.

Adding of Ca^{2+} ion enhances the favourable energies in the case of all ligands except for Zn^{2+} that is also a divalent cation. Changing the electrostatics of the pore by mutations has altered the interaction with ligands, mostly making them less favourable. In the case of some ligands like amiloride, mutant channel models present an increased affinity.

Our study brings new information of the pharmacological modulation of ASIC1, as we showed that the presence of Ca^{2+}, a channel modulator and blocker, enhances the affinity for ligands.

EXPERIMENTAL SECTION

Preparation of protein structures for docking

The crystal structures of ASIC1 under the PDB codes 2QTS, 4FZO and 6CMC were retrieved from Protein Data Bank and were used to generate the coordinates of the receptor. All water molecules and other hetero atoms were removed from the structures. The following operations performed on the structures, including refinement, modelling of mutations, and adding of Ca^{2+} were performed in Biovia Discovery Studio (BIOVIA Inc., https://www.3ds.com).

Initially, the native structures were checked for valence, lack of hydrogen and any structural disturbances of connectivity or connection order. Energy minimization was performed to achieve a stable protein conformation. Mutant channel models starting from each of the three crystal structures were built by considering a single mutation, namely E426G (single mutation models) and two mutations, namely E426G and D433C (double mutations models). Mutant channels were modelled as previously described in [33].

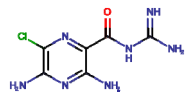
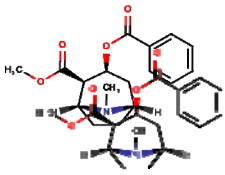
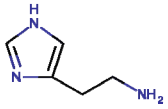
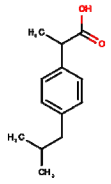
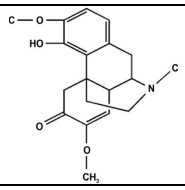
Native and mutant structures with Ca^{2+} were obtained by placing an ion in the transmembrane pore of the channel. As a reference region for placing Ca^{2+} and afterwards the ligands we considered the selectivity filter of the channels represented by the “GAS” motif [32]. The selectivity filters in each structure are marked in Figure 1, as well as the area considered for Ca^{2+} and ligands binding, located above the selectivity filter, in a region called the outer vestibule [18-20]. Ca^{2+} was placed in an optimal position identified by docking calculations using the CDOCKER algorithm implemented in Biovia Discovery Studio.

At the end of structure preparation stage of our study we obtained 18 starting structures representing native and mutant channels modelled according to the three 3D structures (2QTS, 4FZ0 and 6CMC), with and without Ca^{2+} bound in their outer vestibules. The following stage was represented by docking the six ligands to all these models, resulting in a total of 108 docking models.

Molecular docking

Molecular docking is a computational method that predicts the affinity of a ligand for a receptor. The docking of six ligands (amiloride, cocaine, histamine, ibuprofen, sinomenine and Zn^{2+}) in the outer vestibule of ASIC1a native and mutant models was performed using CDOCKER, in Biovia Discovery Studio. The chemical structures of the six ligands are presented in Table 4. Their physicochemical properties were also retrieved from DrugBank. The structures of compounds were retrieved from Drug Bank [30]. Their 3D structures and parameters in CHARMm force field were generated using Biovia Discovery Studio.

Table 4. Details of inhibitors

Molecule name	Chemical structure	Molecular formula	Molecular weight g/mol
Amiloride		$C_6H_8ClN_7O$	229.63
Cocaine		$C_{17}H_{21}NO_4$	303.35
Histamine		$C_5H_9N_3$	111.08
Ibuprofen		$C_{13}H_{18}O_2$	206.28
Sinomenine		$C_{19}H_{23}NO_4$	329.4
Zinc cation	Zn^{++}	Zn^{2+}	65.4
Calcium cation	Ca^{++}	Ca^{2+}	40.08

CDOCKER is a molecular dynamics simulated-annealing-based algorithm, using CHARMm force field (HARvard Macromolecular Mechanical Chemistry) for refining the structure [43]**.** CDOCKER algorithm involved 2000 steps of structure heating up to 700 K followed by 5000 steps of structure cooling to 300 K. The output of CDOCKER was represented by a set of 10 possible docking poses ranked by their CDOCKER energies (interaction energy between receptor and ligand and internal ligand strain energy) and CDOCKER interaction energies (nonbonded energy between receptor and ligand). More negative values are associated with a more favourable interaction. The best ranked pose in all cases was retained for further analysis.

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