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Interaction of local anesthetics with a peptide encompassing the IV/S4–S5 linker of the Na^+ channel

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Abstract

The peptide *p*IV/S4–S5 encompasses the cytoplasmic linker between helices S4–S5 in domain IV of the voltage-gated Na^+ channel, residues 1644–1664. [2] The interaction of two local anesthetics (LA), lidocaine and benzocaine, with *p*IV/S4–S5 has been studied by DOSY, heteronuclear NMR ^1H–^{15}N-HSQC spectroscopy and computational methods. DOSY indicates that benzocaine, a neutral ester, exhibits stronger interaction with *p*IV/S4–S5 than lidocaine, a charged amine-amide. Weighted average chemical shifts, $\Delta\delta(^1H–^{15}N)$, show that benzocaine affects residues L^{1653}, M^{1655} and S^{1656} while lidocaine slightly perturbs residues I^{1646}, L^{1649} and A^{1659}, L^{1660}, near the N- and C-terminus, respectively. Computational methods confirmed the stability of the benzocaine binding and the existence of two binding sites for lidocaine. Even considering that the approach of studying the peptide in the presence of a co-solvent (TFE/H_2O, 30%/70% v/v) has an inherently limited implication, our data strongly support the existence of multiple LA binding sites in the IV/S4–S5 linker, as suggested in the literature. In addition, we consider that LA can bind to the S4–S5 linker with diverse binding modes and strength since this linker is part of the receptor for the "inactivation gate particle". Conditions for devising new functional studies, aiming to better understand Na^+ channel functionality as well as the various facets of LA pharmacological activity are proposed in this work.

Keywords: Voltage-gated sodium channel; Peptide; Local anesthetics; Benzocaine; Lidocaine; Nuclear magnetic resonance

1. Introduction

Voltage-gated Na^+ channels are responsible for the initiation and propagation of action potentials in a variety of excitable cells [1–3]. In response to membrane depolarization, the Na^+ channels open and allow the influx of sodium ions into the cells. Sodium channels are concentrated in axons and muscle cells and they have a molecular architecture consisting of α and β subunits. The α-subunit (260 kDa) is composed of four homologous domains (I–IV), each containing six transmembrane helices (S1–S6) [1–3].

During maintained depolarization, the channels switch, in a millisecond time scale, to an inactivated non-conducting state and re-polarization of the membrane is required for its recovery [4]. The fast inactivation gating of the Na^+ channel has been only partly deciphered. The sequence Ile–Phe–Met, the IFM

Abbreviations: BZC, benzocaine; CD, circular dichroism; COSY, correlation spectroscopy; DOSY, diffusion ordered spectroscopy; DSS, 4,4-dimethyl-4-silapentane-1-sulfonate; HSQC, heteronuclear single quantum coherence; LA, local anesthetics; LDC, lidocaine; NMR, nuclear magnetic resonance; NOESY, nuclear Overhauser effect spectroscopy; SDS, sodium dodecyl-sulfate; TFE, trifluoroethanol; TOCSY, total correlation spectroscopy.

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[2] Residues numbering correspond to the primary sequence of the voltage-gated human brain Na^+ channel [1].

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motif, located in the intracellular linker between domains III and IV, has been identified as the "inactivation gate particle" [5,6] since it participates in the channel pore blocking. However, the residues at the channel intracellular mouth required for the binding of the "inactivation gate particle" and for channel fast inactivation have not yet been positively identified.

The short intracellular loops connecting the S4 and S5 helices, both at domains III [7,8] and IV [9,10], appear to be good candidates for this role. For instance, McPhee and co-workers [10] have shown that residues F^{1651}, L^{1660}, and N^{1662} in the S4–S5 linker of domain IV (IV/S4–S5 linker) establish hydrophobic interactions with the IFM motif during the inactivation of human brain Na^+ channels. In addition, site-directed mutagenesis studies revealed that, in rat skeletal muscle Na^+ channel, substitution of the linker non-polar residues before P^{1473} gives rise to non-functional channels [6,8]; similar results have been observed by Tang and co-workers in the human heart Na^+ channel subtype 1 [9]. Overall, these results point to a direct involvement of the IV/S4–S5 linker in channel inactivation.

Filatov and co-workers [6] also suggested that the non-polar surface of the S4–S5 helices of rat skeletal muscle sodium channel interacts with the non-polar surface of helices of adjacent domains in response to conformational changes associated with channel activation [11,12] and this movement would contribute to the formation of a binding site for the "inactivation gate particle", i.e., the so-called "inactivation gate receptor" [6]. Specific residues of the S6 helix of domains I and II [13] and IV [14] have also been found to interact with the "inactivation gate particle". In an interesting manner, these helices, in conjunction with S5 helices of each segment, line the inner part of the channel pore [14]. Taken together, these results suggest that the formation of the "inactivation gate receptor" is the result of a conformational re-arrangement involving at least the S6 helices and the IV/S4–S5 linker [10,14,15].

LA act by binding to the Na^+ channel, inhibiting Na^+ uptake and blocking the nervous impulse. Many LA are ionizable amines and both the charged and uncharged forms are now considered relevant for the mechanism of anesthesia. In the past, it has been proposed that while the uncharged form is ideal to cross the cell membrane, the binding to a specific site on the channel would be achieved by the protonated species [16,17] only in the intracellular side. Nevertheless, this hypothesis has always been questioned since benzocaine (BZC), the only clinically used local anesthetic that is neutral at physiological pH, produces tonic inhibition of Na^+ channels, with little dose-dependent blockade during repetitive depolarization. Interestingly, other hydrophobic analogues of BZC elicit a dose-dependent blockade of the channel [18,19]. In addition, BZC binding, as well as the binding of charged tertiary and quaternary amine LA, seems to be channel-state-dependent, being favored when the channel is in its open or inactivated state [14,20].

Along the years, much work has been devoted to describe the molecular mechanism associated to LA pharmacological activity [13,21,22,23]. The first mapping of LA binding sites in Na^+ channels revealed that three residues (I^{1760}, F^{1764}, Y^{1771} of rat skeletal muscle μ1), in the middle of helix IV/S6, are critical for the uncharged anesthetic species binding [23,24]. Further studies suggested that, particularly when Na^+ channels are in their inactivated state, one additional residue (I^{409}) in the middle of helix I/S6 is aligned in close proximity to the tertiary amine moiety of amino-amide LA [25,26]. Recently, Godwin and co-workers [19] constructed, in silico, a structural model for the binding of BZC to the IV/S6 helix of the same Na^+ channels of rat skeletal muscle. The model proposes that four hydrophobic residues (V^{1582}, M^{1585}, I^{1581} and I^{1589}) form an ideal binding cavity for neutral LA where, in fact, the benzenoid ring of BZC can be docked. Interestingly, since bulkier BZC analogues (ethyl-4-ethoxy and ethyl-4-diethylamino benzoates) are characterized by a higher association constant and best fit that binding site, the authors suggest this might be the explanation for the dose-dependent blockade observed for those analogues and not for BZC [18,19]. Although the authors have analyzed only the IV/S6 helix, a critical and well-known LA binding site [23,27,28], they hypothesize, based on the ideas described in the classical review by Hille [20], that other regions in the Na^+ channel α-subunit ought to be considered as LA potential binding sites. Those regions could include hydrophobic residues of the III–IV linker, the "inactivation gate particle" [23], residues close to a proline in the S5–S6 linker of domains I and IV, which control the ion selectivity of Na^+ channels [29] and the S4–S5 intracellular linker of domain IV that is part of the "inactivating gate receptor" [6,8–10,14,22].

Miyamoto and co-workers [30] have determined the structure, in SDS micelles, of a peptide encompassing a portion of the IV/S4–S5 linker of the human brain Na^+ channel: Ac-TLLFALMMSLPALFNIGLL-NH_2, residues 1648–1666. In partial agreement with a previous secondary structure prediction [6], the study shows that the peptide presents a hydrophobic α-

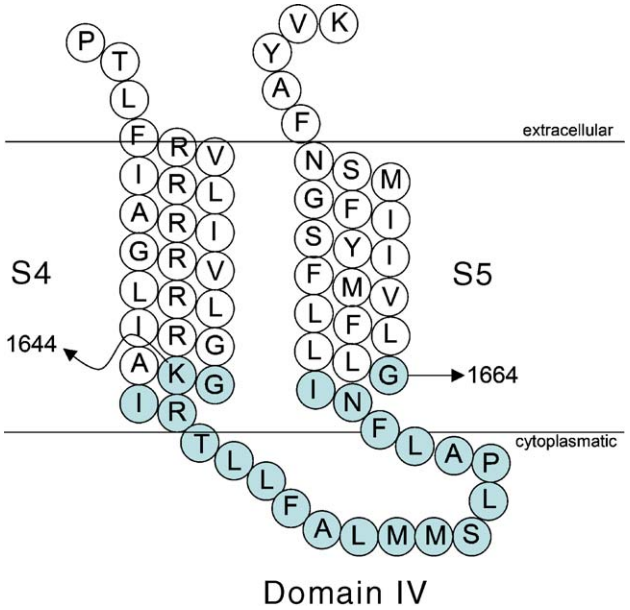

Fig. 1. Model of the cytoplasmic linker connecting helices S4–S5 of domain IV of the α-subunit of adult human brain Na^+ channel. The sequence of the peptide *p*IV/S4–S5 studied in this work is colored in gray.