Selective Interaction of Local Anesthetics with a Peptide Derived from the Voltage-Gated Na^+ Channel

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Abstract: *Sodium channels are concentrated in axons and muscle and have an overall architecture similar to those of K^+ and Ca^{2+} channels consisting of α and β subunits. The α-subunit (260 kDa) is composed of four homologous domains (I-IV), each containing six transmembrane segments (S1-S6). Recently, many authors, using site-directed mutagenesis, have shown that IV/S4-S5 loop is a good candidate for being involved in Na^+ channel inactivation process, and, therefore, a possible site of local anesthetics (LA) interaction. In this work, we used NMR to study the interaction of two Las , namely, benzocaine (BZC) and lidocaine (LDC), with a fragment of IV/S4-S5 loop, comprising residues 1466-1486, KGIRTLLFALMMSLPALFNIG-NH_2 (Chan1). DOSY experiments provide evidence for a possible formation of a complex between LAs and Chan1. TOCSY and ^{15}N-HSQC experiments have been used to monitor the change of the chemical shift of αCH, NH and ^{15}N of Chan1 upon increasing addition of LAs up to 2 mM. For LDC, no significant changes were observed. By contrast, in the case of BZC, changes of αCH and ^{15}N chemical shift for the residues in the region L^{10}-S^{13} were detected. Overall, our data show that the interaction of the local anesthetics with Na^+ channel seems to be specific for BZC, which is able to perturb some structural features of Chan1 and is non-specific for LDC. This distinct behavior is most likely associated with the physico-chemical properties of BZC, which is neutral and more hydrophobic than LDC.*

The voltage-gated Na^+ channel is an integral membrane protein, which plays a fundamental role in the generation and propagation of action potentials in the majority of multicellular organisms.[1] Sodium channels are concentrated in axons and muscle, and they have an overall architecture similar to that of K^+ and Ca^{2+} channels[1] consisting of α and β subunits. The α-subunit (260 kDa) is composed of four omologous domains (I-IV), each containing six transmembrane segments (S1-S6).[2]

Recently, several authors[3-5], using site-directed mutagenesis, have shown that the loop IV/S4-S5 is a good candidate for controller of the Na^+ channel inactivation process, thus becoming a possible site of local anesthetics interaction (Figure 1). In this work, we used NMR spectroscopy to study the interaction between two local anesthetics (LA) (Figure 2): benzocaine (an aminoester) and lidocaine (an aminoamide) with the fragment of the IV/S4-S5 loop, encompassing residues 1466-1486, GIRTLLFALMMSLPALFNIG-NH_2(Chan1).

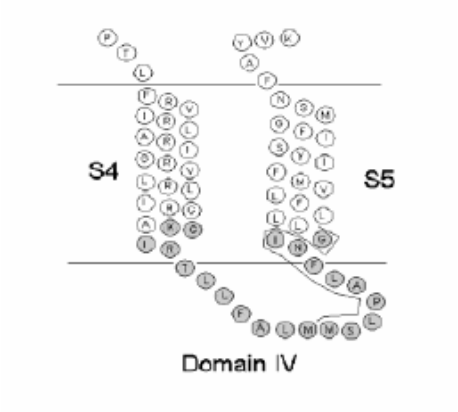

Figure 1. Sequence of the S4-S5 loop from domain IV (gray residues shows the peptide Chan1).

For the NMR measurements, 1 mM of peptide was dissolved in 70% H2O/ 30% TFE-d3 at pH 4.0, a condition that mimics a lipid interface environment. ^{1}H NMR spectra were recorded at 20^{o}C on a Varian Inova 600 MHz NMR spectrometer, operating at 14.1 T. Sequential assignment was achieved by standard procedures[6]. The interaction between the local anesthetics and Chan1 was monitored by DOSY, TOCSY (60 ms) and ^{15}N-HSQC. The resonances were referenced to DSS at 0 ppm. NMR data were processed using the nmrVIEW/nmrPIPE software. Molecular modeling calculations were performed using DYANA software[7]. The resulting structures were energy minimized by the DISCOVER module of INSIGHT II package (Accelrys Inc., San Diego, CA). The NMR solution structure of Chan1 in 30% TFE presents a helical conformation (Figure 3).

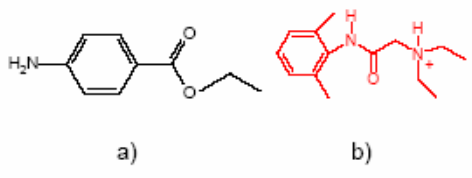

Figure 2. Chemical structure of the local anesthetics; a) benzocaine and b) lidocaine

TOCSY and ^{15}N-HSQC experiments were carried out to investigate the effect of increasing amounts of the local anaesthetics, up to 2 mM. In particular, we analyzed the chemical shift variation of αCH, NH and ^{15}N.

Figure 3. Ensemble of the 20 final structures of Chan1 with superimposition of heavy atoms from 3 to 19, RMSD (0.70 ± 0.02 Å).

Using DOSY experiments[8] we measure the information about the complex between local anesthetic and Chan1. When in the presence of peptide, changes in the diffusion of LDC and BZC were observed. The complexes formed with LA:peptide are 13% and 30% for LDC and BZC respectively. For LDC, no significant changes were observed. For BZC, we detected changes in the ^{15}N chemical shift of Chan 1, indicating that the local anesthetics interact with L10-S13 region (Figure 4).

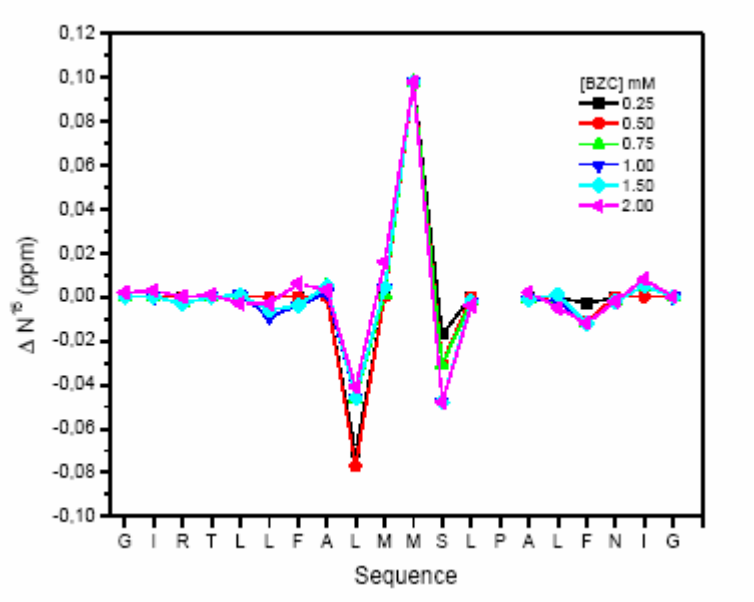

Figure 4. Effect on ^{15}N chemical shif of Chan1 upon addition of BZC.

Overall, our data show that the interaction of the local anesthetics with the Na^+ channel seems to be specific for BZC, able to perturb some structural features of Chan1, and non-specific for LDC. This distinct behavior is most likely associated with the physical-chemical properties of BZC, which is neutral and more hydrophobic than LDC.

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