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Title: Mechanism of interaction of local anesthetics with voltage gated sodium channels.

Abstract in Englisch

Introduction: Voltage gated sodium channels (VGSCs) are responsible for the initiation and propagation of action potentials in the excitable cells. Various isoforms of VGSCs are involved in the multiple physiological and pathophysiological processes. Thus, they are important molecular drug targets in the treatment of various diseases. Local anesthetics (LAs) are well-known inhibitors of VGSCs having complex mechanism of action. Upon depolarization VGSCs undergo multiple inactivated states. LAs bind with high affinity to the fast-inactivated state of VGSCs. However, the studies suggest that LAs also bind to slow inactivated state of VGSCs. Thus, the aim of our study is to understand the relative affinities of LAs to the fast and slow inactivated state of VGSCs.
Methods and Results: Site directed mutagenesis was used to generate a serial cysteine replacement in the middle part (I1575C to Y1586C) of the S6 transmembrane segment of domain IV of the skeletal muscle isoform of VGSCs (rNav1.4). These mutations were tested with and without the application of lidocaine for the time course of recovery from fast and slow inactivation using whole cell patch clamp technique in transiently transfected tsA201 cells. The time course of recovery from short and from long depolarizations had two and three exponential phases, respectively. We refer to these states from which recovery occurs as fast, intermediate and slow inactivated states (IF, IM and IS). The introduced mutations produced alterations of both the amplitudes and the time constants of recovery from these states. Superfusion with lidocaine gave rise to mutation-specific alterations of the respective amplitudes of IF, IM and IS.

Following short depolarizations lidocaine increased the amplitude of a recovery phase similar to native IM suggesting drug-induced stabilization of this state. However, there was no correlation between the mutation-induced alterations in the native IM and the respective lidocaine-induced slow recovery. On the other hand, analysis of lidocaine-induced modification of recovery from long depolarizations revealed a significant correlation between the lidocaine modulated IM state is and the native IM state. Thus, the results suggest that during IF state lidocaine binds to it and slowly dissociates from this state. During slow inactivation lidocaine binds to the IM state and stabilizes the same by reducing the fraction of channels entering into the IS state. An additional mutation (W1531G) resides in the p-loop of the channel and opens a rapid access and egress pathway for lidocaine. This construct was also tested to evaluate the dissociation and stabilization effect of lidocaine on IF and IM states respectively. In W1531G, lidocaine quickly dissociated during IF state, whereas during slow inactivation it stabilized the IM state by significantly reducing the fraction of the channels entering into the IS state.

Conclusion: The study concludes that upon prolonged depolarization, VGSCs recover from minimum three inactivated states. During fast inactivation lidocaine binds to the fast-inactivated state and dissociates slowly from this state. However, during slow inactivation, lidocaine binds to intermediate inactivated state and stabilizes the same. Thus, the binding of lidocaine is mechanistically distinct in various inactivated states of VGSCs.