Abstract

Neuronal excitability is mediated by ion-specific channel proteins through which membrane currents flow. In particular, the rising phase of the action potential is caused by an influx of Na\(^+\) ions through voltage activated Na\(^+\) channels. These channels are large membrane glycoprotein complexes and have been shown to consist of a single major polypeptide, the \(\alpha\) subunit, and other auxiliary subunits. The genes encoding Na\(^+\) channel \(\alpha\) subunits have been cloned from different organisms. All these cloned \(\alpha\) subunits, when expressed in either *Xenopus* oocyte or in cell lines, can form voltage gated Na\(^+\) channels with kinetic and pharmacological properties similar to those of native Na\(^+\) channels, which shows that the \(\alpha\) subunit is necessary and sufficient for channel formation. The work presented here deals with the expression of Na\(^+\) channel in a mammalian cell line and kinetic characterization of the expressed channel by patch-clamp technique.

Towards this end, the cDNA encoding for Rat brain type IIA sodium Channel (RIIA) \(\alpha\) subunit was subcloned into a mammalian expression vector (pCDM8). CHO cells were cotransfected with the recombinant plasmid along with a selection marker pSV2neo. After selection for stable expression in G418 containing medium, one cell line (CNa18) was found to express large Na\(^+\) currents. The primary characterization of the channel properties by whole cell patch clamp was done, where it showed typical fast activation followed by a slower inactivation phase. It also showed Tetrodotoxin induced block - a very characteristic property of voltage gated sodium channels. The northern hybridization analysis showed a 6.5 kb band which is expected from the cells expressing the channel while the control CHO did not express any detectable level of Na\(^+\) channel specific mRNA.

The macroscopic kinetic properties of the expressed sodium channel were analyzed following classical Hodgkin-Huxley formalism and the results are as follows. The inactivation phase of the sodium current relaxation within the physiological range of potentials cannot be explained by a single exponential; it has a slower component as well. This has also been substantiated by studying the development of inactivation through tail current measurements in response to a series of depolarization pulses of different durations. The recovery process from inactivation at a number of potentials is characterized by an initial delay and the total process...
can be well described by a biexponential function. This is again consistent with earlier results. The steady state inactivation process is better fitted with a modified Boltzmann function for a three-state kinetic scheme. These experimental results can be summarized by a modified Hodgkin-Huxley model, assuming a three-state scheme for the inactivation process as follows:

\[ \text{Noninactivated} \leftrightarrow \text{Inactivated} \leftrightarrow \text{Inactivated*} \]

The rate constants for this scheme have also been worked out. The activation phase of these \(Na^+\) currents can be approximated by an \(m^2\) type of kinetics rather than classical \(m^3\) type though the power of \(m\) varies with potential. Instantaneous current-voltage plot shows that the single channel current behavior for these channels is non-ohmic and more accurately described by constant-field equation.

By using a conditioning prepulse, the process of inactivation was studied at lower membrane potentials where sodium currents could not be elicited. The results of these experiments confirmed the biphasic nature of inactivation at lower potentials also. The time constants of inactivation determined by this method is found to be larger than those determined from single pulse experiments at the same potentials which indicates that the process of inactivation from the closed state is slower compared to that from the open state. The biphasic nature of inactivation of these channels was further investigated by separating slow and fast inactivation on the basis of their recovery kinetics. The results indicate that the biexponential behavior of these channels is not arising from two kinetically distinct subpopulations of channels. The inactivation process was also studied at the microscopic level from single channel recordings by cell attached patch clamp. The ensemble averages of single channel currents from a number of patches showed a biphasic inactivation which suggests that the property of bi-phasic inactivation is intrinsic to the Rat brain type IIA sodium channels. Finally, a kinetic model has been proposed to account for all the experimental results.

In addition to the above, the effect of iodate - a known modifier of sodium channel inactivation on the expressed channel has been studied. At 30 mM concentration, it substantially removed fast inactivation without affecting the activation. Both the components of inactivation become slow revealed by increase in the time constant values and in the steady state inactivation experiment, a substantial amount of inward current remains even after a long prepulse to high positive potentials.