

Late sodium current and cardiac electrical and mechanical dysfunction

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Abstract

Late sodium current ($I_{Na,L}$) is a small sustained current existed during repolarization phase of cardiac action potential after peak I_{Na} is complete. Although relatively small in magnitude in normal heart (endogenous late I_{Na}), it represents a functional relevant contributor to cardiomyocyte repolarization and is potentially proarrhythmic in hearts with reduced repolarization reserve. The increased amplitude of late I_{Na} (enhanced late I_{Na}) is recognized in patients with long QT syndrome 3 (LQTS 3), as well as plays an important role in the arrhythmogenesis and mechanical dysfunction under various cardiovascular pathological and pharmacological conditions, including slow heart rate, myocardial ischemia and reperfusion injury and heart failure. Basic and clinical research indicates that inhibition of late I_{Na} is effective in preventing and treating cardiac arrhythmias with different mechanisms, and improving ventricular pump (?) function. Selective inhibitor of late sodium current has no or minimal effect on peak sodium current and/or I_{Kr} , which may have no or minimal risk of proarrhythmia in comparing with classic class I or III antiarrhythmic drugs (AADs), especially in patients with ischemic heart diseases. With the increase in understanding of the late sodium current, it may be helpful for clinicians to use late sodium current inhibitors to treat cardiac arrhythmias in patients with the enhancement of late sodium current, such as LQT 3, etc. In this review, we will discuss the mechanistic role of endogenous and enhanced late sodium current in arrhythmogenesis and mechanical dysfunction, and the late sodium current targeted applications.

This review illustrates a wide range of situations that may accentuate I_{Na-L} . These include (1) overlaps between steady-state activation and inactivation increasing *window current*, (2) kinetic deficiencies in Na^+ channel inactivation leading to *bursting phenomena* associated with repetitive channel openings and (3) *non-equilibrium gating* processes causing channel re-opening due to more rapid recoveries from inactivation. All these biophysical possibilities were identified in a selection of abnormal human *SCN5A* genotypes. The latter presented as a broad range of clinical arrhythmic phenotypes, for which effective therapeutic intervention

would require specific identification and targeting of the diverse electrophysiological abnormalities underlying their increased $I_{\text{Na-L}}$.

Introduction

. The normal function of cardiomyocytes relies in part on the coordinate work of various ion channels acrossing sarcoplasmic membrane of these cells including voltage gated Na, potassium and Ca^{2+} ion channels. The voltage-gated sodium channel Nav1.5, encoded by gene of SCN5A, is primarily expressed in cardiaomyocytes, where it mediates the influx of Na^+ across cell membrane. Depolarization causes transient (1~3 ms) opening of sodium channels, followed by a large amount of Na^+ , following the concentration gradient, rushing into cells to further depolarize them and generating the upstroke of action potentials (APs). The transient but large sodium current (I_{Na}) during this period is termed as peak I_{Na} . While the majority of these channels inactivate quickly and remain inactivated until the repolarization of cell membrane is complete, however, there are still some channels remaining opened or reopen after inactivation, allowing influx of Na^+ during repolarization period and resulting in a tiny late component of sodium current (late I_{Na}) that may persist for several hundred of milliseconds throughout the repolarization of an AP. The magnitude of late I_{Na} is small (<0.5 % of peak I_{Na})[1-3] which cause no significant changes in the duration and configuration of an AP without any change in mechanical function of the cells (Fig. 1). Therefore, late I_{Na} was seen just as the sustained tail of sodium current for many years until the identification of mutations in the gene SCN5A that caused the congenital long-QT syndrome type 3[4]. In recent years, it attracts increasing interests because enhanced late I_{Na} is associated with many pathological, pharmacological and toxicological conditions and may cause an increase in the duration of an AP (APD) with proarrhythmic activities, i.e., EAD and DAD, (Fig. 1) [5, 6]. In the last decade, increasing evidence indicates that late I_{Na} could be a new and promising therapeutic target for the treatment of cardiac ischemia and cardiac arrhythmias [7-10]. Clinical evidence indicates that class I antiarrhythmic drugs inhibit peak I_{Na} to slow myocardial conduction and increase the mortality in patients with ischemic heart disease. Because selective late I_{Na} inhibitors affects

neither peak I_{Na} nor I_K at or slightly above therapeutic concentrations, they may have low or no chance to cause proarrhythmic activities (Fig. 2).

The mechanism of endogenous and enhanced late I_{Na}

Although it is small under normal circumstances, endogenous late I_{Na} may be relatively greater in cardiac cells with slow rate and Purkinje cells. The enhanced magnitude of late I_{Na} is seen under many conditions, including gene mutations[4, 11-14], or pharmacological agents, myocardial ischemia and reperfusion[15, 16], heart failure[17, 18], etc. Table 1 in a recent review [19] lists conditions associated with increased late I_{Na} .

Sodium channel is a macro-molecular complex consisting of a primary α subunit and other regulatory subunits including β_1 , β_2 , β_3 and β_4 [20]. It also assembles with a variety of other proteins, including Snat1[14], Cav3[21] and CaMK-II[22], to form a complex with sodium channels and influence its function/expression. Thus, mutations of any gene encoding these proteins might cause abnormalities of sodium current. For example, gain-of-function mutations in SCN5A disrupt Na^+ inactivation and consequently result in increased I_{Na} presented as LQT3[4]. In addition, gene mutations of CAV3, SCN4B and SNTA1 are responsible for LQT9, LQT10 and LQT12, respectively[13, 14, 23]. Rett syndrome is a neuro developmental disorder caused by mutations in methyl-CpG-binding protein 2 (MECP2). McCauley et al. found that the prolongation of the QTc interval was common in patients with Rett syndrome, and late I_{Na} was greater in cardiomyocytes isolated from both Mecp2Null/Y and nervous system-specific conditional Mecp2 knockout mice[24].

Late I_{Na} is also regulated by endogenous agents such as Ca^{2+} -CaM dependent protein kinase II (CaMK II), protein kinase C (PKC), nitric oxide (NO) and carbon monoxide (CO), via post-translational modification of the channel protein. These agents modulate sodium channel function individually and/or cooperatively. Ca^{2+} /calmodulin-dependent protein kinase II δ (CaMK II δ), the predominant isoform of CaMK II expressed in the heart, is the most well-known signaling pathway for the regulation of Nav1.5. CaMKII facilitates late I_{Na} through phosphorylating Thr-594

and Ser-516 of Nav1.5 to enhance accumulation of sodium channel into an intermediate inactivated state[22]. Ser571 is also an important regulatory locus for CaMK II, and its phosphorylation is increased in vitro and human failure hearts [25-27].

Protein kinase C (PKC) is a group of serine–threonine protein kinases, and there are at least seven members identified in mammalian myocardium[28]. Some of them are calcium-dependent and others that are insensitive to Ca^{2+} could be activated by diacylglycerol (DAG) or lipid-derived second messengers[29]. The phosphorylation of Nav1.5 at S1503 is involved in the inactivation of sodium channels. Therefore, inhibition of PKC causes an increase in late I_{Na} [30-33] .

Late I_{Na} is also affected by other endogenous agents or cellular metabolites. PKA is found to increase late I_{Na} in disease-linked sodium channel mutant (D1790G)[34]. H_2O_2 and free radicals were found to stimulate late I_{Na} , and inhibition of late I_{Na} by ranolazine or TTX attenuated the AP lengthening by H_2O_2 [35]. This effect may come from the free radicals' directly activation on CaMK II[36]. Lysophosphatidylcholine (LPC) accumulates under ischemic conditions, and it may increase cardiac late I_{Na} through peroxynitrite formation[15]. Nitric oxide (NO) could reduce the inactivation of Na^+ channels, thus increase late I_{Na} [37].

In addition, sodium current is modulated under pathological conditions which ectopically active signaling pathways or accumulate cellular metabolites. Increased late I_{Na} in ischemia may be caused by acidosis and ischemic metabolites such as LPC as indicated above. Calcium overloading occurs in heart failure, which excessively activates CaMK II resulting in augmentation of late I_{Na} .

In summary, these pathological conditions and agents augment late I_{Na} by disrupting the inactivation of sodium channels, turning sodium channel into an intermediate inactivated state that is susceptible to be re-opened or fast recovery of channels from inactivation during non-equilibrium conditions[10].

Late I_{Na} associated arrhythmias and mechanical dysfunction

Late I_{Na} associated arrhythmias

For its small magnitude, the effects of late I_{Na} on AP and arrhythmia are under debate for a long time. It should be noticed that the net current flowing during AP plateau is relative small and the algebraic sum of the currents for repolarization is close to zero during this phase. Therefore, even subtle changes in any inward or outward current can have a relative larger impact on AP morphology.

It has been observed that TTX shortened the APD and an increase in late I_{Na} lengthened APD[3, 38]. In our previous studies, inhibition of outward potassium current would lengthen APD of rabbit cardiomyocytes, increase transmural dispersion of repolarization, and result in torsade de pointes ventricular tachycardia. These effects were more obvious at low pacing rate, i.e., reverse rate dependent. While application of inhibitor that decreases endogenous late I_{Na} could attenuate these effects [39, 40]. As mentioned above, late I_{Na} is increased under many pathological or pharmacological conditions. Enhanced late I_{Na} may initiate and maintain arrhythmia through inducing after-polarization (early and delayed), and reentry[10].

During the AP plateau, either an increase in inward currents (late I_{Na} , I_{Ca-L}) or a decrease in outward repolarizing currents (I_{Kr} , I_{Ks}) prolongs the duration of AP plateau, corresponding with a lengthened QT interval in ECG recording. The prolonged AP plateau facilitates the occurrence of early after-depolarizations (EADs), a primary arrhythmic activity. Inward currents, including I_{Ca-L} [41], sodium current, and forward mode of the sodium/calcium exchange current (I_{NCX}) are responsible for the upstroke of an EAD[38]. Increased late I_{Na} is associated with AP prolongation, which provide adequate time for L-type Ca^{2+} channels to recover from inactivation. In addition, increased intracellular Ca^{2+} drives a reverse mode of I_{NCX} , generating a depolarizing current[42]. All these currents contribute to the formation of an EAD.

Delayed after-depolarizations (DADs) are transient depolarizations of the cell membrane following repolarization of a previous AP, and the transient inward current (I_{Ti}), which is observed under condition of overloaded intracellular Ca^{2+} , is responsible for a DAD[19]. Increasing evidence indicates that enhanced late I_{Na} is associated with DADs, and inhibition of late I_{Na} suppresses DADs and other triggered activities[43, 44]. The underlying mechanisms may be that increased intracellular Na^+

following enhanced late I_{Na} decreases the Na^+ gradient to extrude Ca^{2+} through Na–Ca exchange, and even promotes Na–Ca exchanger to work in a reverse mode with Ca^{2+} actually entering the cell[45].

Normal transmural heterogeneity of an AP in the heart may act as substrate for reentrant arrhythmia in certain conditions. Transmural differences in ionic currents densities underlie these heterogeneities. Differences in the density of late I_{Na} are also observed in canine and murine hearts [46, 47]. In the ventricles, late I_{Na} was found to be larger in M cells and Purkinje fiber than in the epicardial or endocardial cells[48]. Mutations causing facilitation of late I_{Na} are found to be associated with increased QT dispersion, this may partly be explained by the difference in the dispersion of distribution of the late I_{Na} [49]. Enhanced late I_{Na} increases transmural dispersion of repolarization time and this effect could be suppressed by inhibiting late I_{Na} [50].

LQT3 results from the “gain-of-function” mutations of SCN5A encoding the α subunit of sodium current. The first reported LQT3 mutation in patient was Δ KPQ, a deletion of amino acids 1505–1507 in NaV1.5[4]. Since then, more than 80 SCN5A mutations associated with an increase in late I_{Na} have been reported in patients with LQT3. Most of these mutations increase sodium current by delaying Na^+ channel to inactivate or facilitating it recover from the inactivation during the plateau of an AP and reopen, thereby rendering the inactivation process to be unstable or slow or incomplete in association with increasing number of open channels.

Dysfunction of other proteins assembled in sodium channel complex may also enhance late I_{Na} , and thus lead to LQT. Caveolin 3, an integral membrane protein encoded by CAV3, plays important roles in signal transduction and vesicular transportation. In 2006, Vatta, et al, found two kinds mutation of CAV3 (F97C and S141R) in LQT patients which resulted in a 2- to 3- fold increase in late I_{Na} [23]. Cheng, et al, demonstrated that the F97C CAV3 mutation relieved the repressive effect of caveolin on NO synthase 1, which increased S-nitrosylation of SCN5A and late I_{Na} [21]. Sodium channel has a main pore-performing α subunit and is associate with one or more auxiliary β subunits. In human, four different β subunits have been identified and perturbation of any of these components may affect the normal function

of sodium channel. Medeiros-Domingo, et al, reported a LQT pedigree with L179F-SCN4B mutation[13]. Heterologous expression of this mutated subunit caused an 8-fold increase in late I_{Na} , and this new type of LQT was classified as LQT10. Another type of LQT associated with increased late I_{Na} is LQT12, which was induced by mutation of α -1-syntrophin. Encoded by SNTA1, α -1-syntrophin interacts with SCN5A, nNOS, and PMCA4b, while A390V-SNTA1 disrupted interaction of PMCA4b with this complex and increases nitrosylation of SCN5A, leads to an increase in both peak and late I_{Na} [14].

Late I_{Na} associated mechanical dysfunction

Na^+ enters the cell through I_{Na} , Na–Ca exchange and Na–H exchange, and leaves the cell through Na–K ATPase. The amount of Na^+ enters the cell is called “ Na^+ loading”. Na^+ loading is determined by the amplitude and duration of sodium current [51]. Although the amplitude of late I_{Na} is much lower than peak I_{Na} (<0.5 %) under normal circumstances, it contributes the majority of Na^+ loading because the duration is much longer, especially under pathological conditions, e.g. myocardial ischemia, heart failure[19]. The increase in the amplitude of late I_{Na} results in an extension of the duration of phase 2 of an AP. The increase of the amplitude of late I_{Na} and the duration of phase 2 of an AP leads to increases in Na^+ loading and intracellular Na^+ concentration. Increased intracellular Na^+ concentration will cause an increase in intracellular Ca^{2+} concentration by reverse mode of NCX [45]. The direct effect of increased Ca^{2+} concentration on mechanical function is impaired relaxation or decreased diastolic function. The increase of late I_{Na} and subsequent Ca^{2+} overloading cause an elevation of ventricular end-diastolic pressure[45]. With increased diastolic tone, it also increases the resistance to blood flow in the microcirculation resulting in reduced oxygen supply. A vicious cycle is thus created where diastolic dysfunction after ischemia and heart failure increases oxygen and energy consumption and aggravates deficiency of oxygen in ischemic myocardial tissue and failing myocardium [52] (Fig. 3).

Therapeutic applications of late I_{Na} inhibitors

Because increased late I_{Na} causes arrhythmias and mechanical dysfunction, it is an attractive target for the treatment. There have been some studies demonstrated that inhibition of late I_{Na} play a therapeutic role on arrhythmias, myocardial ischemia and mechanical dysfunction.

Therapeutic applications in cardiac arrhythmias

Basic studies have demonstrated that late I_{Na} inhibitors suppress ventricular arrhythmias (e.g. torsade de pointes VT and VF)[39, 40, 53-57]. In hearts with ventricular arrhythmias, the inhibition of late I_{Na} reduces repolarization heterogeneity due to the preferential abbreviation of an AP of M cell and suppresses EAD- and DAD- triggered activity[58-61]. Inhibition of endogenous late I_{Na} attenuated MAPD prolongation, beat-to-beat variability of repolarization (BVR), reverse rate dependence (RRD) and abolished arrhythmic activity caused by I_{Kr} inhibitors[39, 40]. When late I_{Na} was increased, inhibitions of either endogenous or augmented late I_{Na} were responsible for the elimination of the proarrhythmic effects of some antiarrhythmic drugs (e.g., amiodarone, quinidine and moxifloxacin, etc.) [55-57]. In addition, Kumar, et al, reported that ranolazine (9.2 +/- 2.1 microM in plasma level) increased ERP and raised repetitive extrasystole threshold, and VF threshold in porcine model[53]. Nieminen, et al, also found that ranolazine increased VF threshold and suppressed ischemia-induced increase in T-wave alternans significantly in severe coronary stenosed porcine hearts[54]. Some class Ia and Ic antiarrhythmic drugs also have the inhibitory effects on late I_{Na} with no or minimal selectivity on peak I_{Na} . In contrast, selective late I_{Na} inhibitors (e.g., ranolazine) have no significant effect on peak I_{Na} at therapeutic concentration range. So they may not induce reentry and proarrhythmic activities when they are used in clinic. Recently, Bacic et al reported that a novel selective late I_{Na} inhibitor eleclazine was effective in treating catecholamine-induced ventricular tachycardias and T-wave alternans in an intact porcine model and the effect was better than flecainide[62].

In clinical studies, inhibition of late I_{Na} is also reported to attenuate ventricular arrhythmias in patients with LQTS. Compared to β blockers in the treatment of LQTS, late I_{Na} inhibitor may be potentially used as a specific, at least in some types of

mutations, the treatment of LQT 3. Moss, et al, reported that ranolazine shortened a prolonged QTc interval in patients with the LQT3-ΔKPQ mutation at therapeutic concentrations[63]. Recently, Mazzanti et al found that another late sodium current inhibitor, mexiletine, caused a major reduction of life-threatening arrhythmic events in LQT3 patients besides shortening QTc interval in a retrospective cohort[64]. Besides LQT3, late sodium current inhibitors also have effect on other LQTS. Gao, et al, treated a LQT8 patient with mexiletine and found that mexiletine shortened QTc, attenuated QT–RR slope, abolished 2:1 AV block and T wave alternans. In MERLIN (metabolic efficiency with ranolazine for less ischemia in non-ST-elevation acute coronary syndrome)-TIMI36 trial, ranolazine was found to reduce ventricular (non sustained ventricular tachycardia) and atrial (new atrial fibrillation) arrhythmias in patients with non ST-segment elevated acute coronary syndrome[65].

In animals and humans with atrial fibrillation, APD and ERP are shortened. Ranolazine blocked I_{Kr} and peak I_{Na} to prolong APD and ERP to increase post-repolarization refractoriness and decrease conduction velocity[61]. Because selective late I_{Na} block does not prolong ERP, reduction of late I_{Na} only is not supposed to be significantly effective for the suppression of AF. However, recent studies indicated that inhibition of late I_{Na} by GS-458967 could suppressed atrial fibrillation caused by augmented late I_{Na} in animal models[66, 67].

Therapeutic applications in mechanical dysfunction

Late I_{Na} inhibitors have been reported to decrease sodium and calcium loading, reduce oxidative stress and improve ventricular function [35, 68-73]. Rocchetti, et al, reported that monocrotaline (MCT) induced pulmonary arterial hypertension (PAH), increased right ventricular (RV) systolic pressure, and caused RV hypertrophy. In the RV, collagen was increased, and myocytes were enlarged with T-tubule disarray and displayed myosin heavy chain isoform switch. Late I_{Na} was also markedly enhanced. Ranolazine completely prevented the increase in late I_{Na} and limited PAH-induced remodeling in RV[74]. Rastogi, et al, reported that in dogs with moderate heart failure, compared with the placebo group, ranolazine alone or in

combined with enalapril or metoprolol prevented the increase in end-diastolic volume (EDV) and end-systolic volume (ESV) with a significant increase in ejection fraction (EF), which was associated with beneficial effects at the cellular level on histomorphometric parameters, including hypertrophy, fibrosis, and capillary density as well as the expression for pathological hypertrophy and Ca^{2+} cycling genes[75]. In Dahl salt-sensitive (DSS) rats model, compared with a normal salt diet, 8 weeks high-salt diet caused increases in LV mass and diastolic dysfunction [isovolumic relaxation time (IVRT), early transmitral flow velocity/early mitral annulus velocity (E/E') ratio. Late I_{Na} in LV myocytes from high-salt rats was significantly increased. Selective late I_{Na} inhibitor GS-967 decreased the enhanced late I_{Na} . Chronic treatment with GS-967 reduced the LV mass, increased the E/E' ratio, and prolonged IVRT without affecting blood pressure or LV systolic function[73].

In clinical studies, the MARISA (monotherapy assessment of ranolazine in stable angina) study was a randomized, multicenter, double-blind, dose-finding study utilizing ranolazine as monotherapy for angina. The result indicated that ranolazine increased the exercise duration, exercise time to angina, and exercise time to 1-mm ST segment depression in dose dependent ways after 4 weeks of treatment[76]. In contrast, the CARISA (combination assessment of ranolazine in stable angina) and ERICA (efficacy of ranolazine in chronic angina) trial were performed to evaluate the efficacy of ranolazine on the basis of standard antianginal drugs. In CARISA study, ranolazine significantly increased the time of exercise, the time to onset of angina during exercise and ST-segment depression after 12 weeks of therapy. Angina frequency was also reduced compared to placebo[77]. In ERICA trial, ranolazine significantly reduced the number of weekly angina episodes compared to placebo[78]. Moreover, The results of the TERISA (type 2 diabetes evaluation of ranolazine in subjects with chronic stable angina) trial showed that the average weekly number of angina episodes among diabetic patients was significantly reduced in the ranolazine group compared with placebo[79].

To evaluate the effect of ranolazine on unstable angina, The MERLIN -TIMI36 trial was conducted. Although there was no difference in the rate of cardiovascular death,

myocardial infarction or recurrent ischemia between ranolazine and placebo group. However, in patients with brain natriuretic peptide (BNP) >80 pg/ml, ranolazine reduced the rate of cardiovascular death, MI, and recurrent ischemia. In addition, the safety of ranolazine in ischemic patients was confirmed in the MERLIN-TIMI 36 trial because of the sample size. The most frequent adverse events were dizziness, nausea and constipation[80]. Khan et al performed a pilot study to investigate the effect of ranolazine on exercise capacity, right ventricular indices, and hemodynamic characteristics in patients with PAH. After 3 months treatment, ranolazine reduced RV size, improved RV function (improvement in RV strain during exercise at 3 months), and made a trend toward improved exercise time and exercise watts on bicycle echocardiography, but was not associated with improvement in invasive hemodynamic parameters[81].

5 Conclusion

Increasing evidence suggests that both endogenous and enhanced late I_{Na} play an important role in the genesis of cardiac arrhythmias and mechanical dysfunction. Compared with other drugs, selective inhibitors of late I_{Na} has no or minimal effect on electrophysiological properties, blood pressure and heart rate. Therefore, late I_{Na} may become a promising therapeutic target for arrhythmia and mechanical dysfunction.

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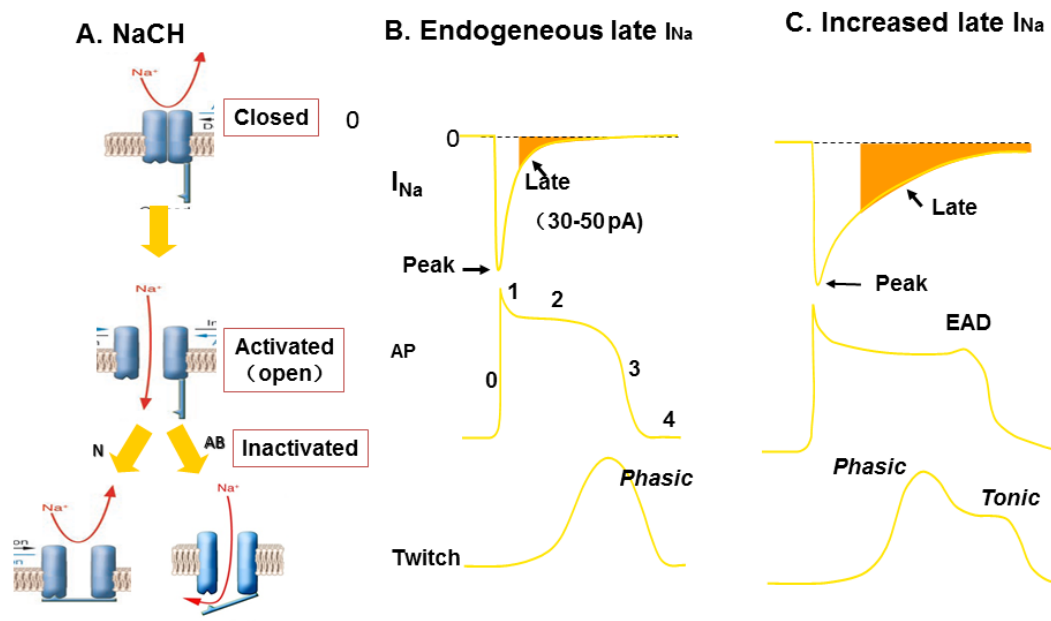


Figure 2

