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Review

Modulation of Voltage-Gated Na^+ Channel Currents by Small Molecules: Effects on Amplitude and Gating During High-Frequency Stimulation

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Abstract

Cumulative inhibition of voltage-gated Na^+ channel current (I_{Na}) caused by high-frequency depolarization plays a critical role in regulating electrical activity in excitable cells. As discussed in this review paper, exposure to certain small-molecule modulators can perturb I_{Na} during high-frequency stimulation, influencing the extent of cumulative inhibition and electrical excitability in excitable cells. Carbamazepine differentially suppressed transient or peak ($I_{Na(T)}$) and late ($I_{Na(L)}$) components of I_{Na}. Moreover, the cumulative inhibition of $I_{Na(T)}$ during pulse-train stimulation at 40 Hz was enhanced by lacosamide. GV-58 was noted to exert stimulatory effect on $I_{Na(T)}$ and $I_{Na(L)}$. This stimulated I_{Na} was not countered by ω-conotoxin MVIID but was effectively reversed by ranolazine. GV-58′s exposure can slow down I_{Na} inactivation elicited during pulse-train stimulation. Lacosamide directly inhibited I_{Na} magnitude as well as promoted this cumulative inhibition of I_{Na} during pulse-train stimuli. Mirogabalin depressed I_{Na} magnitude as well as modulated frequency dependence of the current. Phenobarbital can directly modulate both the magnitude and frequency dependence of ionic currents, including I_{Na}. Previous investigations have shown that exposure to small-molecule modulators can perturb I_{Na} under conditions of high-frequency stimulation. This ionic mechanism plays a crucial role in modulating membrane excitability, hereby supporting the validity of these findings.

Keywords: voltage-gated Na^+ channel current; late Na^+ current; transient Na^+ current; current kinetics; high frequency stimulation

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1. Introduction

Numerous isoforms of voltage-gated Na^+ (Na_V) channels have been established, namely $Na_V1.1$ to $Na_V1.9$ or *SCN1A* to *SCN5A* and *SCN8A* to *SCN11A* [1]. These Na_V channel proteins consist of a single α subunit, each containing four homologous domains, and each domain comprising six transmembrane segments [1]. Furthermore, the β subunits

of Na_V channels serve as auxiliary proteins that regulate the function and expression of the α subunits, which form the ion-conducting pore for Na^+ ions. These β subunits are essential for the proper operation of Na_V channels and play a critical role in the generation and propagation of action potentials (APs) in excitable tissues, including nerves as well as skeletal and cardiac muscles [1].

During the rapid depolarization phase, the Na_V channels are responsible for providing voltage-gated Na^+ channel currents (I_{Na}), and they undergo rapid transitions from the closed to the open state, followed by a swift shift to the inactivated state. The I_{Na} amplitude, once triggered, propels the cell membrane into depolarization through a positive feedback cycle, initiating the AP upstroke in excitable cells. Their inactivation and recovery properties determine the refractory periods that affect the frequency at which excitable cells can fire APs [1–3].

The transient Na^+ current ($I_{Na(T)}$) represents the fast-activating and rapidly inactivating component responsible for the upstroke of the AP. In addition to this primary current, Na_V channels give rise to late Na^+ currents ($I_{Na(L)}$), which include both persistent and resurgent components. The persistent Na^+ current ($I_{Na(P)}$) consists of a small, non-inactivating inward current that can lower firing thresholds and promote repetitive firing, while the resurgent Na^+ current emerges during repolarization and is thought to support high-frequency firing, particularly in specialized neuronal populations. Moreover, slow inactivation, a distinct gating process that develops over prolonged depolarizations, serves as a protective mechanism by reducing Na_V channel availability during sustained activity and modulating responsiveness to high-frequency input. Together, these current components and gating transitions are critical in defining the cellular response to high-frequency stimulation and are thus essential targets for pharmacological modulation [1,3].

Previous studies have identified a phenomenon where the accumulation of I_{Na} inactivation occurs over successive short depolarizing pulses during high frequency stimulation, leading to cumulative inhibition across diverse cell types [2–4]. It has been demonstrated that the current understanding of slow inactivation occurs as a multistate process influenced by both voltage sensor dynamics and conformational changes in the pore module, supported by evidence from both prokaryotic and eukaryotic sodium channel studies [5]. Figure 1 illustrates this cumulative inhibition of I_{Na} under whole-cell voltage-clamp conditions during high-frequency stimulation using repetitive brief depolarizing pulses. The mechanism underlying the cumulative inhibition of I_{Na} remains unclear. This intrinsic regulation of AP firing is influenced by several confounding factors, including the amplitude and frequency of APs, variable discharge patterns, and even hormonal or neurotransmitter secretion [3].

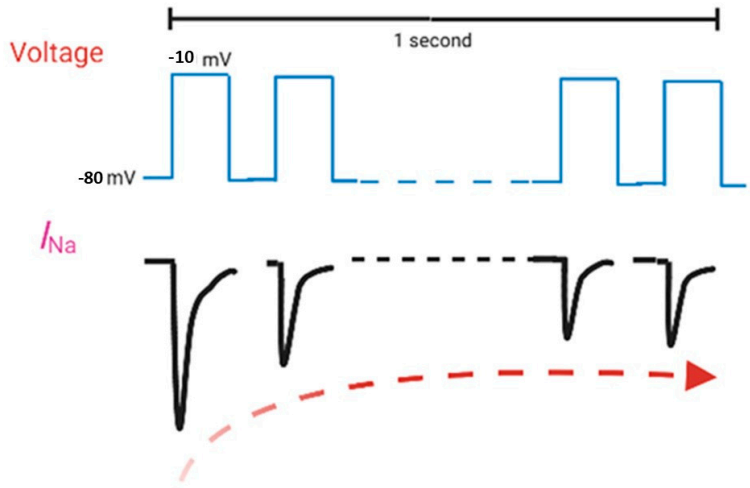


Figure 1. Simplified graph illustrating the elicitation of voltage-gated Na^+ channel currents (I_{Na}) in response to high-frequency stimulation at 20 or 40 Hz. The top graph represents the voltage applied

(light blue) over a total duration of 1 s, while the bottom graph illustrates the corresponding current trace with downward (inward) deflection (black). The current trace exhibits fast activation and inactivation of I_{Na} in response to every brief depolarizing pulse. The dashed red arrow shown below illustrates an exponential decrease (i.e., cumulative inhibition) in the transient or peak I_{Na} ($I_{Na(T)}$) over time, with a deepening color indicating the extent of this decline.

This review synthesizes existing research on the amplitude and gating characteristics of I_{Na}, as well as the cumulative inhibition of I_{Na} during high-frequency stimulation, with particular emphasis on the modulatory effects of various small molecules, as summarized in Table 1. Table 1 also includes representations of the chemical structures of these small-molecule modulators. Although these substances differ in their chemical structures, their regulatory effects on I_{Na} will result in distinct impacts on various excitable tissues.

Table 1. Small-molecule modulators with their abbreviations and chemical structure described in this paper in relation to the inhibition or stimulation of I_{Na} in excitable cells.

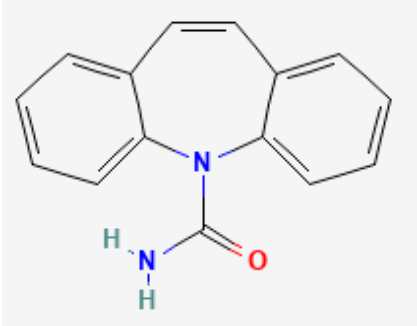
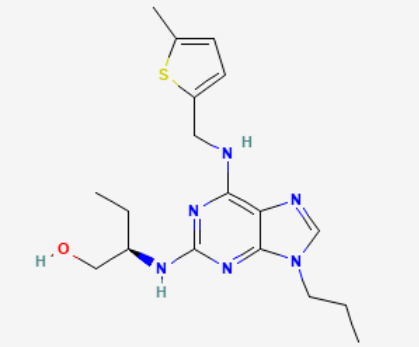
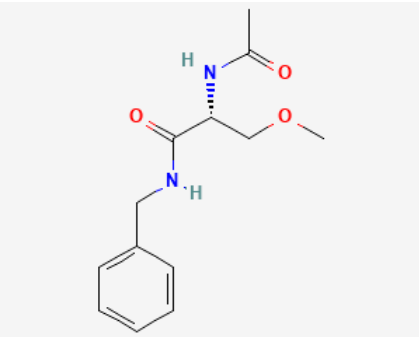
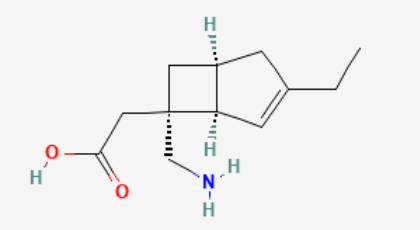
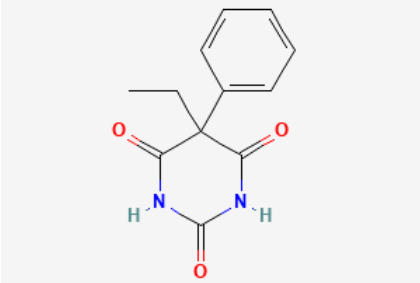
Compound or Drug (Abbreviated Name)	Chemical Structure	Reference
Carbamazepine (CBZ)		[6]
GV-58		[7]
Lacosamide (LCS)		[8]

Table 1. *Cont.*

Compound or Drug (Abbreviated Name)	Chemical Structure	Reference
Mirogabalin (MGB)		[9]
Phenobarbital (PHB)		[10]

2. Effects of Small-Molecule Modulators on I_{Na} During High-Frequency Stimulation (20 or 40 Hz)

2.1. Carbamazepine (CBZ, Tegretol®)

Carbamazepine (CBZ) is an aromatic anticonvulsant commonly used to treat seizures and neuropathic pain, such as trigeminal neuralgia [11]. CBZ stabilizes the electrical activity in the brain, helping to prevent excessive and abnormal firing of neurons. It is also used as an adjunctive treatment for schizophrenia and myotonia and serves as a second-line treatment option for bipolar disorder. Despite its efficacy in anticonvulsant activities, side effects after CBZ treatment include hyponatremia, QT-interval prolongation, hyperprolactinemia, changes in pitch perception, and idiosyncratic reactions. Previous studies have shown that CBZ's presence leads to a concentration-dependent suppression on $I_{Na(T)}$ and $I_{Na(L)}$ in Neuro-2a cells [6]. $I_{Na(T)}$ is the rapid, short-lived influx of Na^+ that occurs right at the beginning of an AP. It activates and then quickly inactivates within a few milliseconds. Persistent $I_{Na(L)}$ is a much smaller but persistent current that continues flowing during the plateau phase of the AP [6]. It results from a small portion of Na_V channels either failing to inactivate completely or reopening intermittently. Mechanistically, the key difference is that $I_{Na(T)}$ is driven by fast activation and rapid inactivation of Na_V channels, whereas persistent $I_{Na(L)}$ arises from slow or incomplete inactivation, or stochastic reopening of these same channels [3]. Resurgent $I_{Na(L)}$ supports high-frequency firing by enabling rapid recovery from inactivation. Whether CBZ can alter resurgent $I_{Na(L)}$ remains to be further determined.

Interestingly, the $I_{Na(L)}$ evoked by short step depolarization is more suppressed than the $I_{Na(T)}$. The estimated IC_{50} values for inhibiting $I_{Na(T)}$ and $I_{Na(L)}$ in Neuro-2a cells are 36 and 18 μM, respectively [6]. IC_{50} (half maximal inhibitory concentration) is the concentration of a compound that is required to reduce a specific biological or biochemical function by 50% under defined conditions. Additionally, CBZ diminishes the amplitude of window Na^+ current in response to short ascending ramp voltage. Window Na^+ current refers to a small, steady, non-inactivating component of sodium current that occurs when the activation and inactivation curves of Na_V channels overlap within a certain voltage range. The concentration-dependent suppression of these currents should be taken into consideration for an understanding of CBZ's mechanisms of action.

CBZ's effect on $I_{Na(T)}$ inactivation, triggered by pulse-train depolarizing stimuli, was reported to exhibit an excessive enhancement. The recovery of $I_{Na(T)}$ inactivation during varying interpulse intervals was slowed in the CBZ presence [6]. Docking studies exploring the interaction between CBZ and Na_V channel predicted that CBZ binds to specific amino-acid residues in Na_V channels in excitable cells like Neuro-2a cells, making them susceptible to modifications by the CBZ molecule [6]. CBZ also suppresses the amplitude of I_{Na} in dorsal root ganglion neurons [11]. We also need to emphasize the importance of the differential inhibition of $I_{Na(T)}$ and $I_{Na(L)}$ by CBZ or other similar compounds. This differential inhibition holds particular significance as it may modulate the electrical behaviors, such as firing discharge patterns, of excitable cells both in culture and in vivo. Comprehending these modulatory effects is crucial, as they hold the potential to have implications for different neurological and neuropsychiatric disorders.

2.2. GV-58 ((2R)-2[[6-[(5-Methylthiophen-2-yl)methylamino]-9-propylpurin-2-yl]amino]butan-1-ol)

GV-58 is an opener of N- and P/Q-type voltage-gated Ca^{2+} (Ca_V) channels. It was hypothesized to impede the closure of the Ca_V channel, leading to a substantial augmentation in overall Ca^{2+} influx (motor nerve activity) during AP. Earlier studies have demonstrated that it is effective for the management of neuromuscular weakness, such as Lambert–Eaton myasthenic syndrome. The immune system of these patients will mistakenly attack the Ca_V channels on the nerve cells, resulting in reduced release of neurotransmitters like acetylcholine.

Recent studies showed that, notwithstanding its effectiveness in stimulating the activity of Ca_V channels, the differential stimulation by GV-58 of $I_{Na(T)}$ and $I_{Na(L)}$ may participate in the regulation of electrical activities of excitable cells (e.g., GH_3 and NSC-34 cells) [7]. The EC_{50} value for GV-58-stimulated $I_{Na(T)}$ or $I_{Na(L)}$ was calculated as 8.9 or 2.6 μM, respectively. EC_{50} (half maximal effective concentration) is a pharmacological term that refers to the concentration of a compound (or ligand) that produces 50% of its maximum possible effect. These values reflect the potency of GV-58 in enhancing each current component, rather than a direct comparison of their absolute magnitudes. Moreover, ω-conotoxin MVIID, a blocker of N- and P/Q-type Ca_V-channel currents, failed to suppress I_{Na} in GH_3 cells, and further addition of ranolazine, an inhibitor of $I_{Na(L)}$ [12], counteracted GV-58-stimulated $I_{Na(L)}$ or AP firing [7]. GV-58 exposure increased recovery of I_{Na} inactivation evoked in response to varying interpulse intervals in a geometrics-based progression. The post-spike I_{Na} contributes to the overall electrical activity of the neuron during its refractory period, and it influences the cell's ability to generate additional APs in response to subsequent stimuli. The GV-58 presence enhances the amplitude of post-spike and steady current, hence raising the occurrence of subthreshold potentials [4,13]. Subthreshold potential refers to the membrane potential of a neuron that has not yet reached the threshold required to trigger an AP, which is the electrical signal that allows neurons to communicate with each other [4].

Taken together, since EC_{50} values in GH_3 cells tend to be lower than those required for its activation of N- and P/Q-type Ca_V channels, the GV-58 actions on I_{Na} are therapeutically or pharmacologically relevant. Whether GV-58-induced amelioration of neuromuscular weakness is pertinent to its stimulatory effect on the amplitude and/or gating of I_{Na} in motor neurons needs to be further delineated.

2.3. Lacosamide (LCS, Vimpat®, (2R)-2-Acetamido-N-benzyl-3-methoxypropanamide)

LCS is a functionalized compound that contains an amino acid-like structure and has been proved to be a valuable antiepileptic and analgesic drug available in both oral and intravenous forms for clinical use. LCS is a synthetic compound designed to modulate Na_V

channels in the brain. It is a medication that is used as an adjunctive therapy in the treatment of epilepsy. Prior investigations have shown LCS to be effective and well-tolerated among patients with various epileptic disorders.

In Neuro-2a cells, the decay of $I_{Na(T)}$ and $I_{Na(L)}$ during a 20 Hz train of depolarizing voltage commands (i.e., 40 ms pulses applied from −80 to −10 mV at a rate of 20 Hz for a total duration of 1 s) was observed to follow an exponential pattern over time. This type of accumulative inhibition displayed a frequency-dependent characteristic in $I_{Na(T)}$ and $I_{Na(L)}$ during rapid repetitive stimuli or high-frequency firing of APs [2,4]. When exposed to LCS, the extent of this accumulative inhibition was enhanced, resulting in a decrease in both current amplitude and the time constant of the time-dependent decaying process in $I_{Na(T)}$ and $I_{Na(L)}$ during pulse-train depolarizing stimuli [8]. Additionally, the LCS-induced increase in the decaying rate was reversed by veratridine, an agonist of Na_V channel [8].

Moreover, LCS presence led to an increase in the values for last and slow time constants of recovery from the I_{Na} block caused by the preceding conditioning pulse-train stimuli. The evidence revealed distinct IC_{50} values required for suppressing $I_{Na(T)}$ and $I_{Na(L)}$ in GH_3 cells, estimated as 78 and 34 μM, respectively, suggesting their differential responses to LCS [8]. These studies indicated that LCS induces a "loss-of-function" change in Na_V channels, maintaining them in the inactivated state (conformations) during repetitive depolarizations, thus preventing excessive excitability.

Furthermore, cell exposure to LCS suppressed the strength of voltage-dependent hysteresis of the persistent Na^+ current ($I_{Na(P)}$) elicited by triangular ramp voltage [8,14]. $I_{Na(P)}$ refers to a non-inactivating or slowly inactivating component of I_{Na} that persists after the initial fast transient current has decayed. Voltage-dependent hysteresis refers to a phenomenon where the behavior of a system or material exhibits a time delay or lag in response to changes in voltage, and this delay is dependent on the voltage itself. This phenomenon can occur in certain biological systems, such change in ionic currents elicited by an isosceles-triangular ramp pulse [15]. By implementing streamlined data acquisition with digital-to-analog conversion, it becomes possible to apply an isosceles-triangular ramp voltage with varying durations to the specified cell, thereby achieving enhanced feasibility. This approach can be utilized for specific objectives, such as inducing hysteresis in different ionic currents [8,9,15].

The response of $I_{Na(P)}$ to a short triangular ramp voltage exhibited a hysteretic behavior, which was marked by a figure-of-eight configuration (also known as an ∝-shaped configuration) within the hysteretic loop [8,14,15]. The $I_{Na(P)}$ induced by using a double ramp voltage was consistently found to exhibit a figure-of-eight configuration. This phenomenon is quite closely related to the chaotic activity produced in neural tissue [16]. Taken together, LCS's presence is effective at inhibiting I_{Na} in a time-, concentration-, hysteresis-, and frequency-dependent manner [8].

Previous electrophysiological studies have demonstrated that LCS selectively enhances the slow inactivation of Na_V channels [5,8], distinguishing it mechanistically from traditional antiepileptic drugs that primarily affect fast inactivation. This enhancement stabilizes hyperexcitable neuronal membranes and reduces repetitive firing without significantly altering fast gating kinetics [5]. While LCS's primary mechanism is well characterized, a detailed comparative analysis of its effects on fast versus slow inactivation has not been comprehensively presented. Therefore, further investigations—particularly using voltage-clamp protocols designed to isolate fast inactivation parameters—would be valuable to delineate any subtle modulatory effect on fast gating.

2.4. Mirogabalin (MGB, Tarlige®, IUPAC Name: 2-[(1R,5S,6S)-6-(Aminomethyl)-3-ethyl-6-bicyclo [3.2.0] hept-3-enyl]acetic Acid)

MGB, an orally administered gabapentinoid, was reported to act as a ligand for the $\alpha_2\delta$-1 subunit of Ca_V channels. This mechanism enables the regulation of pain signal transmission in the nervous system. These included chronic pain resulting from nerve damage or dysfunction, including post-herpetic neuralgia, fibromyalgia, and diabetic neuropathy.

An intriguing report demonstrated that MGB depressed I_{Na} in GH_3 cells, which is concentration-, time-, state-, frequency-, and hysteresis-dependent [9]. MGB treatment resulted in a differential inhibition of two components of I_{Na}, namely $I_{Na(T)}$ and $I_{Na(L)}$, with IC_{50} values of 19.5 and 7.3 μM, respectively, suggesting that MGB's impact on $I_{Na(L)}$ is more significant than on $I_{Na(T)}$. The cumulative inhibition of $I_{Na(T)}$ during a train of depolarizing pulses was enhanced in the presence of MGB. The time-dependent decline of $I_{Na(T)}$ during a 40 Hz pulse-train depolarizing stimulus (lasting 1 s with 20 ms pulses applied from −80 to −10 mV) was more pronounced with MGB exposure. These findings suggest a frequency dependence of $I_{Na(T)}$ during repetitive depolarizations [2,3]. Therefore, MGB exposure resulted in a loss-of-function integrity, attributed to the accentuated modification of I_{Na} inactivation, particularly evident during high-frequency APs.

Earlier studies have indicated that activation of L-type Ca^{2+} channels increased the mRNAs for two specific Na_V-channel α subunits ($Na_V1.2$ and $Na_V1.3$) in GH_3 cells [17]. It is hypothesized that MGB-induced blockage of I_{Na} in GH_3 cells is linked to its inhibitory effects on voltage-gated Ca^{2+} currents, which are functionally present in excitable cells, including GH_3 cells. However, further research has shown that the voltage-activated inward currents were influenced by either tefluthrin (Tef) stimulation or inhibition by tetrodotoxin or ranolazine. Tef and ranolazine have been demonstrated as activators and inhibitors of I_{Na}, respectively [12]. Tef, a type-I pyrethroid insecticide, is well-established to be an activator of I_{Na}, was added [12]. Continued exposure to MGB, followed by the addition of Tef, reversed the suppression of I_{Na} caused by MGB [9]. Although the ionic mechanism underlying MGB's inhibitory action on Na_V channels remains unclear, the MGB molecule may have a greater impact on the open/inactivated state rather than the resting (closed) state of the channel, leading to destabilization of the open conformation. It seems likely that MGB's perturbations on excitable membranes cannot be solely attributed to its action on the $\alpha_2\delta$ subunit of Ca_V channels. Na_V channel activity in excitable cells may render them susceptible to perturbations by MGB or other similar compounds. The interplay between MGB and Na_V channels in excitable cells warrants further investigation to better understand their functional relationship and the potential implications for novel treatment or drug development. Figure 2 illustrates that MGB has an inhibitory effect on both Ca_V and Na_V channels, leading to the alleviation of neuropathic pain.

It should be emphasized that the emergence of many instances of chronic pain sensation is not exclusively linked to Ca_V-channel dysfunction. The intrinsic functionality of I_{Na} plays a role in chronic neuropathic pain [18]. Indeed, ranolazine is a blocker of $I_{Na(L)}$ that has been employed for chronic stable angina pectoris [12,18]. Moreover, as for the reasons behind MGB's attenuation of different painful disorders, the extent to which this is due to the influence on the amplitude and gating of I_{Na} remains to be elucidated.

No studies have yet investigated the direct effect of MGB on Na_V channels in heterologous expression systems such as HEK293 cells or Xenopus oocytes. The present findings in native cells suggest modulation of Na_V currents; however, it is possible that this is an indirect consequence of MGB's action on Ca_V channels via the α2δ-1 subunit. Future studies using heterologous expression of Na_V channels, with or without co-expression of α2δ-1, will be essential to clarify whether MGB exerts a direct modulatory effect on Na_V channel function.

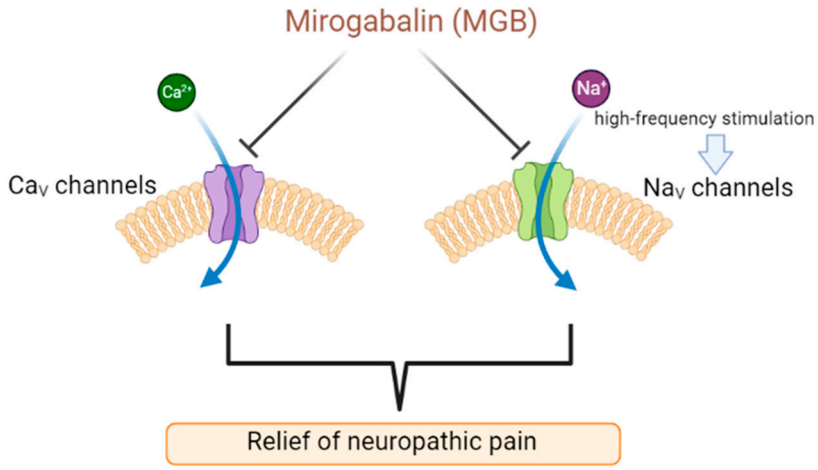


Figure 2. A simplified graph illustrating that mirogabalin (MGB) is capable of inhibiting voltage-gated Ca^{2+} (Ca_V) channels (e.g., $\alpha_2\delta$-1 subunit), as well as voltage-gated Na^+ (Na_V) channels, particularly during high-frequency stimulation. Ca^{2+} and Na^+ ions will flow through specialized Ca_V and Na_V channels, respectively, following their individual electrochemical gradients (as indicated by the blue curved arrow). The alleviation of neuropathic pain is attributed to the combined inhibitory effects of these interventions.

2.5. Phenobarbital (PHB, Luminal Sodium®, Phenobarbitone)

PHB is a barbiturate that depresses the central nervous system, slowing down brain activities and producing both anticonvulsant and hypnotic effects. Its mechanism involves enhancing synaptic inhibitory effects of γ-aminobutyric acid (GABA) in the brain through GABA type A ($GABA_A$) receptors [19]. PHB has a potential for tolerance, dependence, and addiction, making it a controlled drug in recent years.

PHB concentration-dependently suppresses $I_{Na(T)}$ in Neuro-2a neuroblastoma cells, with an IC_{50} of 83 μM [10]. However, the addition of either flumazenil or chlorotoxin, which are agents known to act on $GABA_A$ receptors or Cl^- channels, did not reverse $I_{Na(T)}$ blockages caused by PHB [10]. Alternatively, the regulatory effect of PHB on $I_{Na(T)}$ decay during repetitive stimulation was reversed when Tef was introduced, but not by that of chlorotoxin, a blocker of Cl^- channels. PHB concentration required to achieve half-maximal inhibition of I_{Na} in Neuro-2a cells was 83 μM, a value that falls within the range of clinically applied doses [10]. The results reflect that the perturbations by PHB or other structurally similar compounds on the amplitude, gating, and frequency dependence of $I_{Na(T)}$ in Neuro-2a cells are a direct and fast process, independent of their agonistic actions on $GABA_A$-mediated Cl^- currents [10,20]. These findings shed light on the complex mechanisms of PHB's actions in the central nervous system and its potential implications for the treatment of neurological disorders.

It is also essential to underline the significance of the M-type K^+ current ($I_{K(M)}$), also known as *KCNQx*-encoded currents, which can gradually increase during repetitive APs due to its slow activation and deactivation kinetics [21]. In this context, KCNQx primarily refers to *KCNQ2*, *KCNQ3*, and *KCNQ5*. *KCNQ1* is predominantly expressed in the heart, while *KCNQ4* is mainly found in the inner ear and auditory system. The $I_{K(M)}$ is a slow-activating and non-inactivating K^+ current that plays a role in controlling the resting membrane potential of neurons and regulating their firing patterns. Furthermore, under conditions of high-frequency stimulation, the accumulation of $I_{K(M)}$ can hyperpolarize the after-potential, thereby expediting the recovery process of Na_V channels from inactivation and enhancing Na_V-channel availability [21]. In this context, "after-potential" refers to the membrane potential following long-lasting high-frequency stimulation. During such

stimulation, $I_{K(M)}$ may accumulate progressively. As a result, when the high-frequency stimulation ceases, the enhanced $I_{K(M)}$ leads to a more hyperpolarized after-potential. This membrane hyperpolarization facilitates faster recovery of Na_V channels from inactivation, thereby increasing their availability [21].

Recent findings demonstrated the role of PHB in facilitating the inhibition of $I_{K(M)}$ during pulse train stimulation. This suggests that the presence of PHB may disrupt the waveform of neuronal APs occurring at high frequency [10]. Moreover, the influence of PHB likely extends beyond the inhibition of $I_{K(M)}$, as it also synergistically affects multiple ionic currents, including I_{Na}, the *erg*-mediated K^+ current ($I_{K(erg)}$), $I_{K(M)}$, and the delayed-rectifier K^+ current ($I_{K(DR)}$). The term "*erg*" stands for *ether-è-go-go*-related gene, which is a family of genes that encode these voltage-gated K^+ channels. Moreover, midazolam, which is a benzodiazepine hypnotic, has the ability to reduce the amplitude of $I_{K(DR)}$, while simultaneously increasing the rate at which the $I_{K(DR)}$ undergoes inactivation. These effects are thought to be not dependent on midazolam's binding to benzodiazepine or nuclear receptors [22–26]. Figure 3 illustrates the effects of PHB on the cell membrane, which produce sedative/hypnotic, anesthetic, and anticonvulsant effects. PHB's presence induces other unpredictable but crucial electrophysiological responses, despite its ability to activate $GABA_A$ receptors and enhance Cl^- currents [18,27–29].

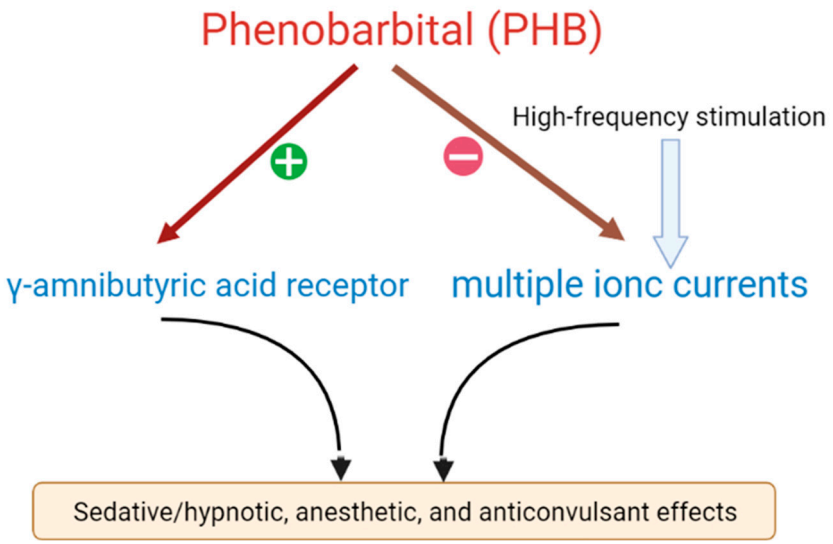


Figure 3. A simplified graph demonstrating cellular responses induced by the action of phenobarbital (PHB). The drug stimulates γ-aminobutyric acid type A ($GABA_A$) receptors and concurrently exerts inhibitory effects on various ion currents across the cell membrane. This inhibitory effect on ionic currents becomes more prominent, especially under conditions of high-frequency stimulations. The currents involved comprise I_{Na}, as well as $I_{K(M)}$, $I_{K(DR)}$, and $I_{K(erg)}$. Due to these effects, the drug produces sedative/hypnotic, anesthetic, and anticonvulsant effects. In the diagram, the symbols "+" and "−" denote stimulatory and inhibitory effects, respectively.

It is important to note the complexity of interpreting modulator (e.g., PHB) effect in native cells due to the presence of multiple ion channels and interacting proteins. Heterologous systems such *Xenopus* oocytes or HEK293 cells offer a reductionist approach that can help isolate the effects of a compound on a specific ion channel subtype. Therefore, applying heterologous expression systems to study phenobarbital's direct effects on specific ion channels—such as Na_V channel—would provide valuable mechanistic insights and help disentangle its multi-target actions.

3. Conclusions

Our discussion highlights the impact of small-molecule modulators on I_{Na}, revealing diverse underlying mechanisms that extend beyond their own original actions. Investigations conducted on excitable cells such as GH_3 or Neuro-2a cells have unveiled alterations induced by these small-molecule modulators on I_{Na}. This effect is pronounced during high-frequency stimulation (e.g., 20 or 40 Hz). The implications of these modulatory actions on I_{Na} variants are expected to offer promising prospects for pharmacological, therapeutic, and clinical applications.

Furthermore, previous studies emphasized a critical aspect: the impact of these small molecules (Table 1) on diverse activities of excitable cells, with a specific focus on their regulatory effects on I_{Na} during high-frequency stimulation. This encompasses multiple factors, including stimulus-secretion coupling, membrane excitability, and AP firing, as depicted in Figure 4. A comprehensive understanding of these effects will thus hold paramount importance in grasping the broader ramifications of these small-molecule modulators and their utility within medical and therapeutic contexts. Additionally, whether the cumulative inhibition of I_{Na} under high-frequency stimulation is isoform-specific for Na_V channels or region-specific within the brain requires further in-depth research.

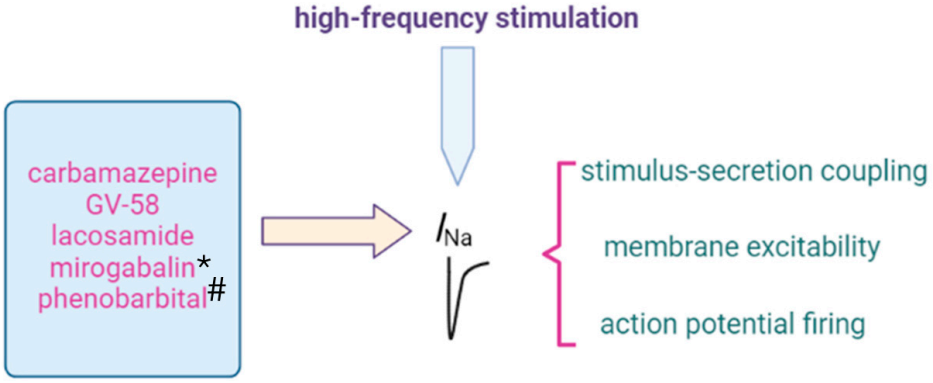


Figure 4. A simplified graph illustrating the effects of carbamazepine, GV-58, lacosamide, mirogabalin, and phenobarbital on I_{Na} under conditions of high-frequency stimulation. These effects lead to alterations in stimulus-secretion coupling, membrane excitability, and AP firing. * Mirogabalin alleviates neuropathic pain, whereas # phenobarbital exerts sedative, hypnotic, anesthetic, and anticonvulsant effects. The black-colored downward deflection in the center indicates the representative I_{Na} trace in response to a brief depolarizing step.

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Abbreviations

The following abbreviations are used in this manuscript:

AP	action potential
Ca_V channel	voltage-gated Ca^{2+} channel
GABA	γ-aminobutyric acid;
$GABA_A$ receptor	γ-aminobutyric acid type A receptor
$I_{K(DR)}$	delayed-rectifier K^+ current
$I_{K(erg)}$	*erg*-mediated K^+ current
$I_{K(M)}$	M-type K^+ current
I_{Na}	voltage-gated Na^+ channel current
$I_{Na(L)}$	late Na^+ current
$I_{Na(P)}$	persistent Na^+ current
$I_{Na(T)}$	Transient or peak Na^+ current
Na_V channel	voltage-gated Na^+ channel

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