



The Role of Ionic Currents in Peripheral Nerve Regeneration

Arash Abdolmaleki ^{1,*}, Saber Zahri ², Asadollah Asadi ², Tahereh Karimi Shayan ², Rahmatollah Parandin ³, Hussein A. Ghanimi ⁴, Muhammad Akram ⁵ and Muhammad Daniyal ⁶

¹Department of Bioinformatics, Faculty of Advanced Technologies, University of Mohaghegh Ardabili, Namin, Iran

²Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, Iran

³Department of Biology, Faculty of Sciences, Payame Noor University, Tehran, Iran

⁴College of Nursing, University of Al-Ameed, Karbala, 198, Iraq

⁵Department of Eastern Medicine, Government College University Faisalabad, Faisalabad, Pakistan

⁶Faculty of Eastern Medicine, Hamdard University Karachi-Pakistan, Karachi, Pakistan

*Corresponding author: Department of Bioinformatics, Faculty of Advanced Technologies, University of Mohaghegh Ardabili, Namin, Iran. Email: abdolmalekiarash1364@gmail.com

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Abstract

Various factors may result in peripheral nerve injury leading to permanent functional loss. Here, we review the role of calcium and potassium ions in peripheral nerve regeneration and repair. This narrative review of the literature collected its data by searching Google Scholar, PubMed, Elsevier, Springer, Wiley, EBSCO, Scopus, and Science Direct. Publications were searched with no particular time restriction from 1997 to 2021, including all types of study. About 100 relevant papers were found from 1997, 77 of which were selected for this study. Both beta subunits of sodium channels are expressed in peripheral neurons, and drugs that affect those channels may facilitate nerve repair. Riluzole is a sodium/glutamate antagonist which has recently entered clinical trials for spinal cord injury. Riluzole's neuroprotective effects are due to sodium channel blockade and, subsequently, the prevention of Ca²⁺ overflow. Besides, 4-aminopyridine (4-AP) is a neurotransmitter of potassium channel blockers that increases the rate of functional improvement following peripheral nerve damage by promoting remyelination. Verapamil is a calcium channel blocker that stimulates an endogenous anti-inflammatory response and reduces pro-inflammatory processes, thus causing pain modulation. Inhibition of ROCKs accelerate the regeneration and functional restoration after spinal-cord damage in mammals, and inhibition of the Rho/ROCK pathway has been additionally proven efficacious in animal models of stroke, inflammatory and demyelinating diseases, Alzheimer's disease, and neuropathic ache. Therefore, the neurite outgrowth of surviving neurons is necessary for nerve regeneration to reinnervate target tissue after nerve damage. One of the critical components of the damage response process is a local translation in axons, and it is critical for the regenerative outcome. On the other hand, it provides new axonal regrowth molecules and induces signals returning to the cell's soma to partake in regenerative pathways and survival.

Keywords: Peripheral Nerve, Sodium Channel, Potassium Channel, Calcium Channel, Ionic Current

1. Context

Peripheral nerve injury (PNI) is a typical trauma accounting for more than 3% of all traumatic injuries (1, 2). Thermal, chemical, mechanical, or pathological damage may result in severe PNI, leading to permanent motor loss. The nerve may be pinched, stretched, partially sliced, or even entirely severed in this form of injury. The Peripheral nervous system (PNS) tends to regenerate after mild nerve damage spontaneously (3, 4). When the nerve is completely severed without surgical mediation, regeneration is typically ineffective and functional recovery is incomplete (5, 6). Surgical intervention is required when there is a severe defect in the peripheral nerve, which involves transplanting a graft between the proximal and distal ends

of the nerve (7, 8). Unfortunately, the functional recovery of a peripheral nerve after grafting is always sub-optimal.

Axons must have the intrinsic capacity to regrow to regenerate the peripheral nerve, and the distal environment must be tolerant of the axons' regrowth; besides, target tissues must embrace the restored axons (9, 10). Several factors play a role in the progress of nerve regeneration after surgical repair. Neuronal ionic imbalance is one of the most significant inhibitory factors prolonging neuronal recovery (3, 11). However, while many other variables have been considered and analyzed, little evidence is available on the impact of ionic currents on regeneration. Here, we explore the role of ionic currents in peripheral nerve regeneration.

2. Mechanism of Peripheral Nerve Regeneration

After peripheral nerve damage, proliferating and reactive Schwann cells produce growth elements, cytokines, and growth-associated proteins, which play vital roles in axon regeneration and nerve repair (12). It has been located that exogenously administered glial cell line-derived neurotrophic factor (GDNF) will increase the myelination in axons. Tacrolimus (FK506) has been shown to enhance nerve regeneration in the setting of axotomy, autograft restoration, and allograft repair (13).

Rho kinases (ROCKs) are serine/threonine kinases that are essential in fundamental tactics of migration, cell proliferation, and cell survival. Inhibition of ROCKs accelerate the regeneration and functional restoration after spinal-cord damage in mammals, and inhibition of the Rho/ROCK pathway has been additionally proven efficacious in animal models of stroke, inflammatory and demyelinating diseases, Alzheimer's disease, and neuropathic ache. Besides, ROCK inhibitors consequently can prevent neurodegeneration and stimulate neuroregeneration in various neurological disorders (14). Experiments on rat sciatic nerve have also shown that calcium alginate has a protective role in nerve regeneration due to its beneficial interaction with inflammatory cells and Schwann cells (15). In another study to determine changes in the expression of sodium channels (VGSCs) during sciatic nerve injury, the results showed a positive adjustment of Nav1.3 and a lower adjustment of Nav1.7, Nav1.8, and Nav1.9 (16).

3. Factors Restricting the Effectiveness of Peripheral Nerves Regeneration

Regenerating transected nerves is a complex process influenced by: (1) the form and timing of the repair; (2) surgical procedure used for the repair; (3) graft size and the type of nerve graft used; (4) lesion position; and (5) patient's age (17-19).

4. Protection of Nerve Cells Against Apoptosis

Functional recovery can be reduced by losing the number of neurons after injury, therefore, prevention of apoptosis is maximized (20-23). Neurons undergoing apoptosis can be observed one week after injury in animal models (24, 25), and more than 40% of dorsal root ganglion neurons die two months after injury (26). Motor neuron death increased in a rat model with the proximity of the injury site to the spinal cord (27, 28). Some strategies to

preserve healthy neurons capable of entirely reinnervating target tissues are critical for the best functional recovery after nerve injury. At present, surgical nerve repair after nerve damage helps limit the death of neurons. However, pharmacological agents may also help preserve neurons (22, 29). Most cells are destroyed due to the destructive microenvironment of damaged tissues, which includes oxidative stress, ionic imbalance, lack of growth factor, and inflammatory response, all promoting apoptosis. Multiple strategies are warranted to avoid apoptosis best and get the best functional recovery (9, 30, 31).

5. The Function of Ion Currents in Nerve Regeneration

5.1. Sodium Ionic Current's Function in Nerve Regeneration

The molecular targets for medications that reduce pain and treat heart arrhythmias, epilepsy, and bipolar disorder are all sodium channels. Type 1 transmembrane glycoproteins with an extracellular N-terminus and a cytoplasmic C-terminus are beta subunits of the sodium channel (31). The beta subunits of the sodium channel also modulate channel expression and control channel gating. They also form links via ankyrin and spectrin to the intracellular cytoskeleton. In both the central and peripheral nervous systems, the beta subunits of sodium channels are expressed (32-34). In this regard, riluzole is the anticonvulsant benzothiazole for managing amyotrophic lateral sclerosis (ALS) and is one of the neuroprotective sodium/glutamate antagonists (35-37). Its neuroprotective effects are due to sodium channel blockade and, subsequently, the prevention of the overflow of Ca^{2+} (38). Riluzole has been successfully used with animal models to minimize symptoms by modulating presynaptic glutamate release via blocking the voltage-gated sodium channels (35, 39). The use of Riluzole in vitro in adult dorsal root ganglion neurons is known to have a neuroprotective effect by promoting the neurites outgrowth in terms of number, length, and branch (28, 40, 41). Neurite outgrowth is necessary for nerve survival and regeneration after nerve damage to reinnervate target tissue.

Moreover, riluzole inhibits neuro-excitotoxicity in animal models of neurological damage and can mitigate nerve root pain shortly after administration. Also, it can limit the development of synaptic dysfunction in nerve roots to the spinal cord (42). Riluzole has recently been officially licensed to treat motor neuron disease, and its influence on damaged peripheral nerves in the regeneration processes is being investigated (3, 43, 44).

5.2. Calcium Ionic Current's Function in Nerve Regeneration

Calcium ions are the regulators of the second messenger of several metabolic pathways. In addition, calcium ions influx into the presynaptic terminal is necessary for neurotransmission. Indeed, axon transaction initiates a broad depolarizing voltage discharge that travels back to the soma and induces vigorous spiking activity and sustained depolarization (45).

Verapamil is a calcium channel blocking agent. In addition to its effects on the cardiovascular system, several laboratory studies have demonstrated its role in peripheral nervous system regeneration by decreasing scar formation (46, 47). However, it is not clear if the topical administration of verapamil would be effective after *in vivo* surgical nerve repair to reduce scar formation in the nerve tissue (48). Verapamil's anti-scar effects are due to stimulating an endogenous anti-inflammatory response and decreasing pro-inflammatory activity, thus causing nerve regeneration (49).

Verapamil may prevent scar development by inhibiting the transduction of signals inside and outside of fibroblasts and preventing collagen and extracellular matrix production, thus reducing biological activity in cells (48). The key to an effective damage response is a local translation in axons, which is critical for the regenerative outcome. Also, verapamil provides new axonal regrowth molecules. On the other hand, it induces signals that they are returning to the soma cell, which are helpful in the path of restoration and survival.

Rapamycin is another drug of interest in the recovery of injured nerves. The mammalian target of rapamycin (mTOR) expression is increased at the injury site and regulates the axons mechanism in a peripheral lesion (50, 51). Furthermore, mTOR pathway destruction affects nerve regrowth in the PNS. A sudden and widespread injection of calcium into the axoplasm occurs at peripheral nerve injury, resulting in a surge of depolarization along the axon to the soma and disrupting gene expression. In addition to this reverse signaling, calcium aggregates at the injury site and causes the cytoskeleton's calpain-dependent structures to be rearranged, promoting membrane re-sealing, which relies on calcium-regulated proteins. Moreover, the rise in local calcium causes mitogen-activated protein kinase (MAPK) signaling reversed by dual leucine zipper-bearing kinase (DLK) phosphorylation. This, in turn, provokes gene expression (52, 53). A dinin-mediated "fast" transmission, which depends on motor-based axons, follows this "slow" signaling in a matter of hours. This increases calcium load, incorporating the extracellular signal-regulated kinases (ERK1/2) pathway in the pancreatic stem cells (PSC), which appears to be one of the most

significant factors in nerve recovery. During a rise in the local calcium concentration, the mitochondria suddenly release excess calcium ions in the injured peripheral nerve. This causes significant mitochondrial dysfunction and reactive oxygen species (ROS) production in mitochondria enriched with axons. As a result of calcium overload, H_2O_2 is released by the mitochondria of injured neurons and causes neurotransmission by signaling ERK1/2 (54).

Degenerative structural and molecular modifications at the site of damage are caused by the peripheral nerve injury (PNI) cascade. The calcium influx into Schwann cells occurs soon after the mechanical injury due to amputation and oxygenation (55). *In vitro*, the early proliferation of Schwann cells is induced by calcium. It also reaches the axoplasm of weakened axons where calpain, a critical protease for axonal degradation, is activated. The entry of calcium into the axon is also essential for new growth cones to evolve.

A controlled concentration of Ca^{2+} may be required for nerve regeneration, indicating that Ca^{2+} channel blockers can improve recovery (56). Increased calcium exchange activates intracellular cascades and proteins controlling genes, such as signal-regulated kinase (ERK) and N-terminal protein kinases extracellular signal-regulated protein kinases family of MAPK. Besides, ERK 1/2 is activated 20 minutes after damage. The MAPK triggering occurs six hours later to increase calcium, neuroglia, and fibroblast growth factor (FGF-2) levels. Activation of p38 MAPK, as one of the several significant molecules for Wallerian degeneration, happens later and destroys myelin. In this cascade, the low current activates the C-jun signal transcription factor, which regulates Schwann cell reaction to damage. The inactivation of C-jun in Schwann cells is also affected by axon regeneration, leading to the loss of the necessarily increased expression of specific neurotrophic factors linked to C-jun. Rapid activation of ERK 1/2 is a prerequisite for Schwann cell proliferation. ERK 1/2 and other transcription factors, such as activating transcription factor 3 (ATF3), are vital for forming axons.

Calcium channels are divided into high voltage-activated (HVA) channels and low voltage-activated (LVA) channels, depending on their activation voltage and conduction. As known, HVA and LVA channels have different gate and drug characteristics. 1, 4 dihydropyridine antagonists (DHPs) bind preferentially to HVA channels, and DHP agonists help increase the open time and the single-channel conductance (57). Interestingly, specific HVA calcium channels exhibit distinct tissue preferences and various sensitivities to DHP and other toxin antagonists. This has contributed to the discovery of several HVA channels.

DHP-sensitive channels are present in many cells and have a long activation period, hence named DHP calcium channels or L-type (LTCC) (9, 58). The CTX-sensitive calcium channels are pronounced because of their location in the nervous system, also known as N-type channels. In cerebellar Purkinje cells, omega-AGA-sensitive was first observed, referred to as P-type channels. In addition to these three types of HVA, specific calcium channels were not susceptible to any of those antagonists and were classified as R-type-resistant channels. Among the LVA channels, only one kind of calcium channel is registered, named the transient calcium channel (also referred to as T-type channel). T-type channels are similar in different phrases and antagonist-resistant properties to type L channels. However, they are distinguished from L-type channels by single-channel conductivity and activation at low membrane potential. The various sensitivities to various antagonists of HVA and LVA channels demonstrate the ability of these antagonists to change the calcium conduction preference in different cells (59, 60).

5.3. Synaptic Calcium

Previous studies have shown evidence for centralized improvements in calcium and extracellular regulation during neurodegenerative events, such as Alzheimer's, Parkinson's, Huntington's diseases, and ALS (61-63).

5.4. Mitochondrial Calcium

TOM70 is an outer membrane mitochondrial translocase (TOM) subunit that imports mitochondrial proteins. Decreased TOM70 interaction with IP3 receptors decreases the role of ER-bound IP3 in mitochondrial calcium transport. Significantly reduced production of mitochondrial calcium decreases mitochondrial respiration, affects bioenergy of cells, causes autophagy, and prevents reduced TOM70 cells from proliferating (64, 65).

5.5. Calcium Channels and Neuropathic Pain

Expression in cell membranes of T-type CaV3.2 calcium channels and their activation in the dorsal root ganglion (DRG) damaged neurons in mice with neuropathic pain increase after spared nerve injury. Antisense inhibition may decrease mechanical allodynia 14 days after spared nerve injury, suggesting that CaV3.2 T-type calcium channels play a role in damaged DRG-mediated neurons (66). The CaV3.2 membranes of medium-sized DRG neurons play a considerable function in neuropathic pain (67).

5.6. CaV3.2 Channel Molecular Pathways Impaired After Spared Nerve Injury

There are two possibilities about how it occurs. The first possibility is an inflammatory mediator, such as interleukin-6, which is transmitted from activated macrophages and glial cells to its receptors to regulate CaV3.2 in damaged DRG neurons. The second possibility is via neurotrophic cytokines, such as ciliary neurotrophic factor and leukemia inhibitor. Such factors may regulate the traffick in weakened DRG neurons of T-type CaV3.2 channels through the Janus kinase (JAK) and ERK signaling pathways (68).

5.7. Potassium Ionic Current's Function in Nerve Regeneration

In experiments, it is vital to block the expression of potassium channels to control the concentration of peripheral potassium ions (69). In this regard, 4-aminopyridine (4-AP) is a blocker of potassium channels with the chemical formula C₅H₄N-NH₂ (70). Medical efficacy for 4-AP has been shown in treating neurological disorders such as multiple sclerosis (71). Besides, 4-AP mechanisms of action are revealed by its effect on enabling impulse conduction in demyelinated axons where it blocks K⁺ channels that would allow K⁺ to leak out of the axons. That, in turn, allows axons to restore the degree of depolarization needed to propagate action potential (72).

Recently, it has been shown that 4-AP is a potent small molecule with neurodegenerative effects that improve both the speed and degree of clinical regeneration following acute peripheral nerve damage by promoting remyelination (73).

5.8. Potassium Channels

In nearly all types of eukaryotic cells, potassium channels are expressed and active in many physiological processes in electrically stimulated and non-excitable cells (74, 75). Depending on the components of the vital structure, potassium channels can be broken into three groups: (1) inwardly rectifying potassium (Kir); (2) two-pore domain potassium (K2P); (3) and voltage-gated potassium channel (Kv). Members of the Kir family are primarily split into three major evolutionary classes of primary channels invertebrates and found in almost all cells. G protein-activated channels as K⁺ subfamily channels are expressed in neurons, and Kir channels represent ATP modulation in epithelial and glial tissues. These channels are strongly expressed in the CNS and have a role in depression, epilepsy, and other neurological disorders (76, 77).

5.9. Potassium Currents and Neuropathic Pain

In painful neuropathies, extreme irritability of primary afferent neurons is a typical finding associated with reduced K current in various animal models. In all nerve cells, K currents are the dominant external currents, contributing to cell over-polarization. As a result, cell excitability is decreased. Based on their kinetics and drug susceptibility, K currents are approximately classified into three categories: (1) slow-inactivating sustained K-current (IK), (2) fast-inactivating transient current (IA); and (3) slow-inactivating transient current (ID). The most critical factor for activity, possible threshold, waveform, and firing frequency is the IA current, indicated by voltage-gated potassium channels (Kv). Neuropathic pain's origin is not observed with changes in excitability and Kv4.1 expression in the primary afferents of injured neurons (78).

5.10. Potassium and Peripheral Nerves

The family of KCNQ genes encodes potassium voltage-gated channels as Kv7 channels (Kv7.1 - Kv7.5). Kv7.2 and Kv7.5 channels are located in nervous tissue, while Kv7.1 is located in the heart and smooth muscle. In dorsal root and trigeminal ganglion nerves, Kv7.2 and Kv7.3 are expressed. Biological agents, such as intracellular signaling of muscarinic acetylcholine receptors, can influence Kv7 channel function. Phospholipase-mediated Kv7 channel repression has been documented to trigger peripheral inflammatory pain (79, 80). A powerful mechanism of neuropathic pain is believed to be the reduced expression of Kv7.2 channels in primary afferent nerves. Because of the high excitability of the nerve membrane, Kv7 channels have been researched to treat neurological diseases. Compounds that amplify the neuronal Kv7 channels may have an inhibitory effect on the onset of seizures and neuropathy. This suggests that synthetic compounds bind to Kv7 channels and deform channel opening, which may effectively treat neuropathy (81).

6. Conclusion

One of the main impediments to a successful outcome after nerve repairs is the changes in the distal part of the nerve. Surgical nerve repair after nerve damage is used to avoid the death of neurons; however, pharmacological influences may also be helpful for neuronal safety. In this regard, Riluzole's neuroprotective effects are due to sodium channel blockade and, subsequently, the prevention of Ca²⁺ overflow. Also, 4-aminopyridine is a small solid molecule with neuro-regenerative properties that improve both the speed and degree of functional regeneration by facilitating remyelination after acute peripheral

nerve injury. Furthermore, verapamil, a calcium channel blocker, activates the endogenous anti-inflammatory response. The neurite outgrowth of surviving neurons is necessary for nerve regeneration after nerve damage to reinnervate target tissue. One of the critical components of the damage response process is a local translation in axons, which is critical for the regenerative outcome. On the other hand, it provides new axonal regrowth molecules and induces signals that they are returning to the cell's soma to partake in regenerative pathways and survival. In Schwann cells, dorsal ganglion neurons (DRG) are two to three times more common following axonal damage without C-jun activation. The inactivation of C-jun in Schwann cells is also affected by axon regeneration due to the loss of the necessarily increased expression of specific neurotrophic factors leading to the existence of C-jun.

7. Future Perspectives

Intensive research is needed to evaluate drugs effective in regenerating peripheral nerves and evaluate ionic currents associated with it, especially in humans.

Footnotes

Authors' Contribution: All the authors have the same contribution.

Conflict of Interests: The authors declare that they have no conflict of interest.

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