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Long-term nerve excitability changes by persistent Na^+ current blocker ranolazine

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HIGHLIGHTS

- Threshold tracking can non-invasively provide data on axonal membrane properties.
- Increased persistent Na^+ current (Na_p) is related to neurodegeneration.
- Threshold tracking monitored the excitability changes by ranolazine, a selective Na_p blocker.
- Ranolazine treatment demonstrated a long-term effect on suppression of Na_p.

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ABSTRACT

The persistent Na^+ current (Na_p) in peripheral axons plays an important functional role in controlling the axonal excitability. Abnormal Na_p is believed to contribute to neurodegeneration and neuropathic pain, and thus it is an attractive therapeutic target. To assess the chronic behavior of selective Na_p blockade, axonal excitability testing was performed in vivo in normal male mice exposed to ranolazine by recording the tail sensory nerve action potentials (SNAPs). Seven days after administering ranolazine i.p. (50 mg/kg) daily for 1 week, nerve excitability testing showed decreased strength-duration time constant in the ranolazine group in comparison to the control ($P < 0.03$). This change is explained by the long-term effects of ranolazine on Na_p. Importantly, ranolazine showed no effect on other ion channels that influence axonal excitability. Further study is needed to assess the chronic Na_p blockade as a useful therapy in peripheral nerve diseases associated with abnormal nerve excitability.

Abnormal peripheral nerve excitability accounts for a variety of symptoms, such as neuropathic pain, muscle cramps, muscle weakness, and fasciculations [33]. Axonal Na^+ channels are one of the most important factors to regulate the nerve excitability, the dysfunction of which has been reported in hereditary conditions such as erythromelalgia and in acquired conditions including diabetic polyneuropathy [12,19,24]. In addition to voltage-gated, fast activating and inactivating kinetics, Na^+ channels also demonstrate slow inactivation kinetics (persistent sodium current: Na_p) which modulate the availability of ion channels to open as a function of voltage [22]. Pathologically increased Na_p not only causes symptoms such as neuropathic pain, but it is also believed to lead to neurodegeneration and peripheral neuropathy [14,18,40,45]. Stabilizing Na^+ channels in inactivated states causes voltage- and frequency-dependent block that reduces neuronal firing [17]. Accumulation of intracellular Na^+ activates the reverse action of Na^+–Ca^{2+} exchanger that imports damaging levels of calcium into axons [9]. The function of the exchanger is augmented by Na_p [43]. Therefore, Na_p has been considered a useful therapeutic target in peripheral nerve diseases. The use of pan-Na^+ channel blockers such as mexiletine and lidocaine may be of limited use for regulating Na_p because of their adverse effects caused by blocking voltage-gated Na^+ channels. However, a new FDA-approved anti-anginal medication, ranolazine, selectively blocks cardiac and neuronal Na_p at therapeutic concentrations and has shown therapeutic effects in neuropathic pain [2,16]. Recently, we have shown ranolazine's acute in vivo effects on Na_p [34]. In that study employing nerve excitability testing, ranolazine was shown to block Na_p with a simultaneous suppression of slow K^+ current. The suppression of slow K^+ current was considered to be either compensatory or a result of nonspecific blockade by the ranolazine. Because the slow K^+ current consists of a steady outflow of K^+ ions, it reduces nerve excitability [6]. Given the potential useful clinical applications of

Abbreviations: *I*/*V*, current–threshold relationship; Na_p, persistent Na^+ current; RC, recovery cycle; SDTC, strength-duration time constant; SNAP, sensory nerve action potential; TE, threshold electrotonus.

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inhibiting Na_p, the knowledge of long-term sequel of ranolazine on nerve excitability is of importance. Therefore, the aim of the present study was to assess the ranolazine's long-term effects on axonal excitability, with a focus on Na_p and slow K^+ current.

The study was approved by the Beth Israel Deaconess Medical Center's Institutional Animal Care and Use Committee. Twenty male, 8-week-old, Swiss Webster mice (Charles River, Wilmington, MA) were equally divided into control and treatment groups. Electrophysiological studies were performed on the tail under 1.5% isoflurane anesthesia with the animal warmed on a heating pad to maintain a tail temperature at 32–34 °C throughout the studies. Of note, a recent animal study indicated that the use of isoflurane did not interfere with the function of neuronal Na^+ currents by a method similar to ours [4]. Sensory nerve action potentials (SNAPs) were recorded orthodromically by placing stainless steel, 30-gage, disposable needle electrodes as follows: the reference and active recording needles were placed 10 mm and 20 mm from the base of the tail respectively; the cathode and anode were placed 40 mm and 50 mm respectively distal to the active recording electrode, and the ground electrode placed midway between the stimulating and recording electrodes. The tail sensory nerve was specifically selected because recording from the foot caused significant stimulus artifact due to the short onset latency.

For neuronal excitability testing, stimulation was controlled by a PC running the QtracS program (Institute of Neurology, London), connected via a digital I/O device (National Instruments, Austin, TX) to a preamplifier (MEG-1200: Nihon Kohden, Tokyo, Japan) and a stimulator (DS-5: Digitimer, Letchworth, UK). Using 1-ms rectangular stimuli, the negative peak of the SNAP was recorded. For excitability tests, the TRONDNF multiple excitability recording protocol was used, which first records the stimulus–response curve and then uses the slope of the curve to track the stimulus needed to evoke a response of 40% of the maximum, defined as the standard "threshold" stimulus [5]. Stimulus–response curves, which were determined using a 1 ms duration test stimulus increased from zero until supramaximal potentials were attained. To record threshold electrotonus (TE), the unconditioned threshold on one channel was tracked, while the threshold at discrete points was determined on two other channels as follows: during and after 100 ms of hyperpolarizing and depolarizing currents, set to ±40% of the unconditioned threshold [44]. For the +40% depolarizing conditioning current, the difference of threshold changes between the greatest threshold reduction and at the end of the 100 ms conditioning pulse was defined as S2 accommodation. For the recovery cycle (RC), a supramaximal conditioning stimulus was given 19 times with delays ranging from 200 to 1.6 ms before the test stimulus provided on

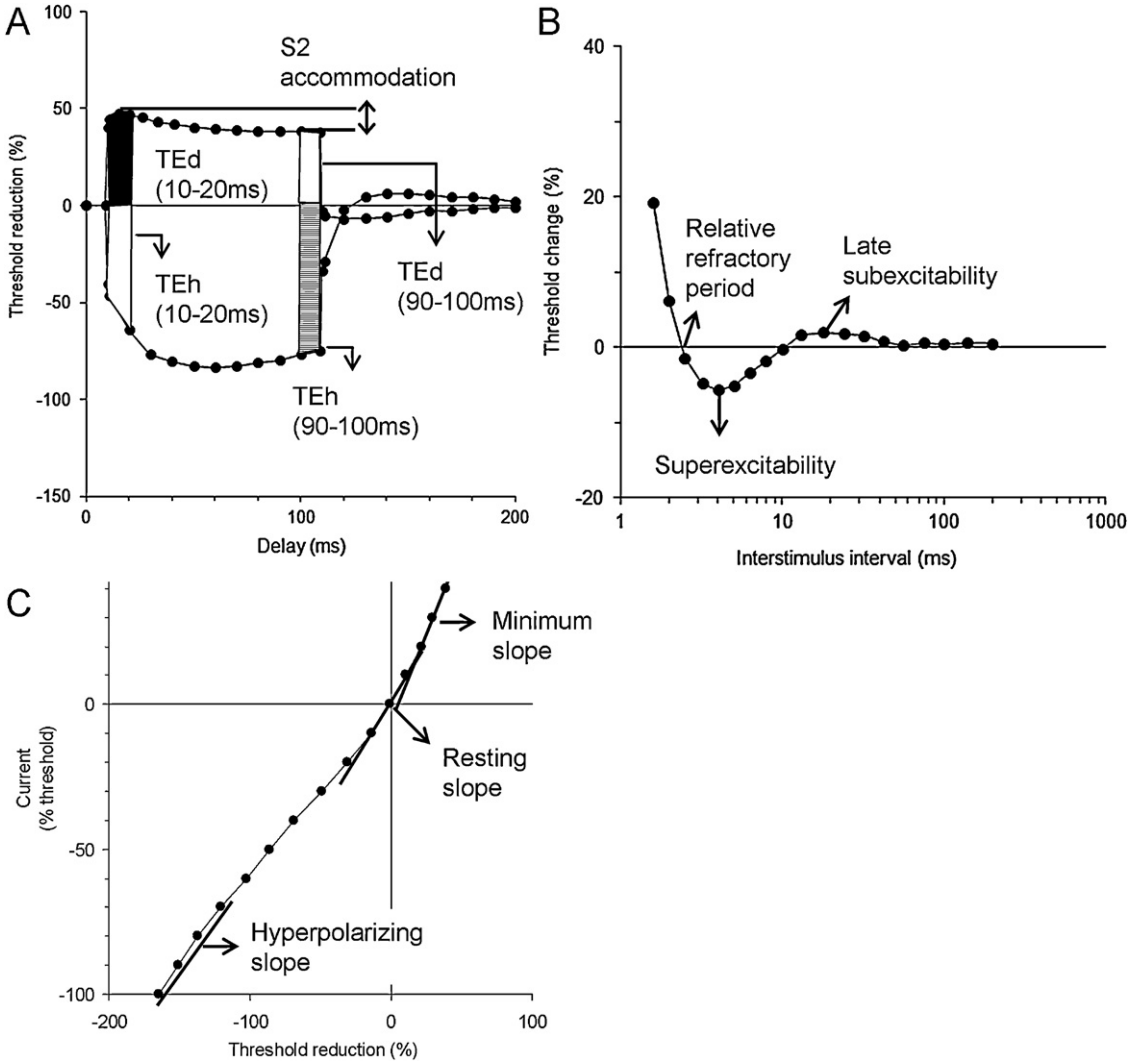

Fig. 1. Representative waveforms of the axonal excitability tests and the definitions of the parameters: threshold electrotonus (A), recovery cycle (B), and current–threshold relationship (*I*/*V*) (C).

Data were modified from Nodera and Rutkove [35].