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# Effects of Laser-Induced Hyperthermia Treatment on Ionic Permeability of Myelinated Nerve

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**Summary.** The effect of laser-induced hyperthermia on the ionic permeability of nerve membranes was studied using the nodes of Ranvier in amphibian myelinated nerve as a model. To effect a photothermal modification of nerve membrane functions, controlled laser irradiation consisting of a 5-sec thermal pulse was applied to the nodal membrane, increasing the temperature to a maximum of 48–58°C at the node. Major electrophysiological changes observed in the nodal membrane following laser-induced hyperthermia were a differential reduction of the sodium and potassium permeability, an increase in the leakage current, and a negative shift on the potential axis of the steady-state Na inactivation. There was no significant change in the kinetics of ion channel activation and inactivation for treatments below 56°C. The results suggest that a primary photothermal damage mechanism at temperatures below 56°C could be a reduction in the number of active Na channels in the node, rather than a change in individual channel kinetics, or in the properties of the lipid bilayer of intervening nerve membrane. A differential heat sensitivity between the noninactivated and the inactivated Na channels is also suggested. For the treatments of 56°C and above, a significant increase of membrane leakage current suggests an irreversible thermal damage to the lipid bilayer.

**Key Words** node of Ranvier · laser-induced hyperthermia · ion channel · myelinated nerve · Na inactivation · thermal damage

## Introduction

The conduction of neural signal in myelinated nerve is sensitive to external interventions, such as heating, cooling, UV- radiation, among others. Due to the thermodynamic dependence of ionic permeability, system temperature is one of the most important parameters in determining nerve membrane function. Consequently, there has been an intense interest in temperature effects on nerve membrane current, in which it has been shown that the temperature effect was largely reversible when the original temperature was restored (Frankenhaeuser & Moore, 1963; Schwarz, 1979; Schwarz & Eikhof, 1987). In these studies, the $Q_{10}$ factors of various kinetic parameters in different temperature ranges have been calculated and compared.

In contrast, when the nerve is exposed to temperatures beyond the physiological range, adverse effects on nerve function are present, causing irreversible damage to nerve conduction, manifested as conduction heat block. Evidence accumulated from both experimental and theoretical studies has pointed to a nerve conduction blocking temperature of 45°C or above (Klumpp & Zimmermann, 1980; Ritchie & Stagg, 1982) however, the heat block mechanism is not fully understood. In particular, it is not known whether the heat block is a threshold process or whether there is a graded response with respect to temperature.

To date, the adverse effects of high temperatures on nerve conduction have been studied mostly on whole nerve, using the compound action potential to characterize the effects. Although it is advantageous to study the adverse heating effects at the cellular or sub-cellular level, relatively few studies have been done on the irreversible thermal modification of ionic permeability in excitable nerve membrane. This is largely due to the experimental difficulty in achieving an efficient and stable temperature control at the cellular level. In this respect, fast laser heating offers an advantage for the study of functional damage to nerve membrane at temperatures above the physiological range, because the laser enables us to increase the temperature to the desired level very swiftly, and to keep the temperature of the nodal membrane constant during the irradiation.

Previous in vivo studies in our laboratory of pulsed Nd-YAG laser effects on whole nerve conduction in the sciatic nerve of the rat showed a differential effect on slow and fast myelinated nerve fibers (Lin, Wesselmann & Rymer, 1990; Wesselmann, Lin & Rymer, 1991), which could reflect different

heat sensitivity in different-sized fibers. Our data suggested that the differential suppression in fast and slow nerve fiber conduction after the laser treatment was due to preferential laser action on the function of the nodes of Ranvier. One possible damage mechanism is that some membrane proteins operating as ion channels are denatured by the heat action at such a temperature. Alternatively, the lipid bilayer membrane could also be subject to irreversible phase transition after high temperature exposure. Based on these earlier results, the primary objective of this paper is to elucidate the mechanisms of the change in nerve membrane function after brief exposures to laser-induced hyperthermia.

To achieve this experimental objective, a novel irradiation control scheme was designed to generate a constant temperature pulse. The unique geometry and small size of the nodal membrane of myelinated nerve also proved to be advantageous for the approximation of a uniform temperature distribution in the nodal membrane.

## Materials and Methods

### Nerve Preparation

Single myelinated nerve fibers were isolated from the sciatic nerves of large frogs (*Rana pipiens*, 3–3.5″) and mounted in a nerve chamber using a procedure similar to that described by Stämpfli and Hille (1976). The voltage-clamp instrument replicated the design of Nonner (1969). The Ringer's solution consisted of 115 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, and 5.0 mM MOPS titrated with NaOH to pH = 7.4 at 15°C. After the fiber was mounted in the chamber, the solution in the side pools was changed to 120 mM KCl to depolarize the adjacent nodal membranes. In most of the experiments, the internodal segments were not cut in order to maintain the integrity of the nerve fiber. No effort was made to discriminate between sensory and motor nerve fibers.

### Voltage Clamp

At the beginning of the experiment, the holding potential ($E_h$) was adjusted to give a steady-state Na inactivation of about 0.7. The holding potential was assumed to be -70 mV in the analysis. All membrane potentials are given as deviation from this holding potential, i.e., $V = E - E_h$. The voltage-clamp circuit was balanced to remove bias current at holding potential prior to hyperthermic treatments. During the entire experiment, the node of Ranvier was perfused with pre-cooled solution at 15°C at a rate of 10 ml/hr.

A calibration factor of 20 MΩ was used for the resistance between the center pool and the recording pool (commonly referred to as $R_{ED}$) to obtain the absolute value of nodal membrane current. The membrane current recorded from the nodes was filtered by a 10 kHz, 8-pole Bessel low-pass filter and sampled at 25-μsec intervals. The data acquisition and processing were performed by an Apple Macintosh IIcx microcomputer with data acquisition board (MIO-16, National Instrument, TX) installed in the system. The experimental protocols and data analysis were programmed in LabVIEW (National Instrument, TX).

Leakage current was estimated by taking the average of 32 current responses to a 40 mV hyperpolarization voltage step. The averaged result was scaled for the subsequent leakage subtraction. The steady-state Na inactivation, $h_\infty(V)$, curve was estimated using the conventional two-pulse method (Hodgkin & Huxley, 1952).

### Laser Irradiation

Temperature in the nodal membrane was controlled by a feedback mechanism as shown in Fig. 1*A*. The output of a continuous-wave Nd-YAG laser (model ML880, Lasersonics, CA) with a wavelength of 1.32 μm was delivered to the nodal membrane through a fast shutter (nm Laser Products, CA) with short induction delay. The power of the laser beam measured at the shutter output was 1.2–1.4 watts. The laser beam was then tightly focused to a spot of about 100 μm in diameter centered around the node of Ranvier under study. The shutter was controlled by a pulse generator with a variable duration adjustable from 4.5 to 8.0 msec. When activated, the circuit compared the measured temperature with the command temperature and generated an oscillating square wave with frequency proportional to the difference voltage that triggers the pulse generator. A small thermocouple of 35 μm in diameter was placed in the center pool of the nerve chamber about 75 μm beneath the normal position of the node for temperature measurement. Since the center pool of the nerve chamber was also the active pool for recording membrane current, an isolation amplifier was used to separate temperature measurement from the voltage-clamp circuitry in order to avoid unnecessary ground loop and the induction of excessive capacitance by thermocouple wires. The temperature gradient in the solution adjacent to the node of Ranvier was 0.25°C/100 μm. Thus, the temperature distribution in the nodal membrane (which has a typical diameter of 15 μm and gap width of 1 μm) can be considered as uniform.

A typical temperature pattern in the node is shown in Fig. 1*B* in which a 5-sec temperature step was applied. The rise time from the base temperature of 15°C to the target temperature of 50°C was less than 150 msec. At the plateau portion of the temperature record, variation around the target value was less than 0.2°C. The rate of temperature drop after the temperature step was determined by heat dissipation and thus was a much slower process. Nevertheless, upon termination of thermal pulse the temperature decreased to below 30°C within 200 msec. Assuming that there is no irreversible modification of membrane current below 30°C, the pattern can be considered as a thermal pulse. Temperature elevation in the side pools of the nerve chamber during laser treatment was less than 1°C, indicating that possible temperature effect on the recording electrodes in the side pools was negligible (Moore, Holt & Lindley, 1972).

A 5-mW HeNe laser was used in the laser apparatus for aiming the near-IR beam. The aiming of laser to the nodal membrane typically took less than a minute, and the HeNe beam was then blocked to avoid possible spurious HeNe effects.

In a typical experiment, a sequence of 5-sec thermal pulses with temperature ranging from 48 to 58°C in step increments of 2°C was applied sequentially to the nodal membrane at an interval of about 5 min between thermal treatments. Membrane currents were recorded beginning at 30 sec after each thermal pulse when the temperature in the nodal membrane had returned to 15°C. Leakage current and $h_\infty$ curve were determined using the potential

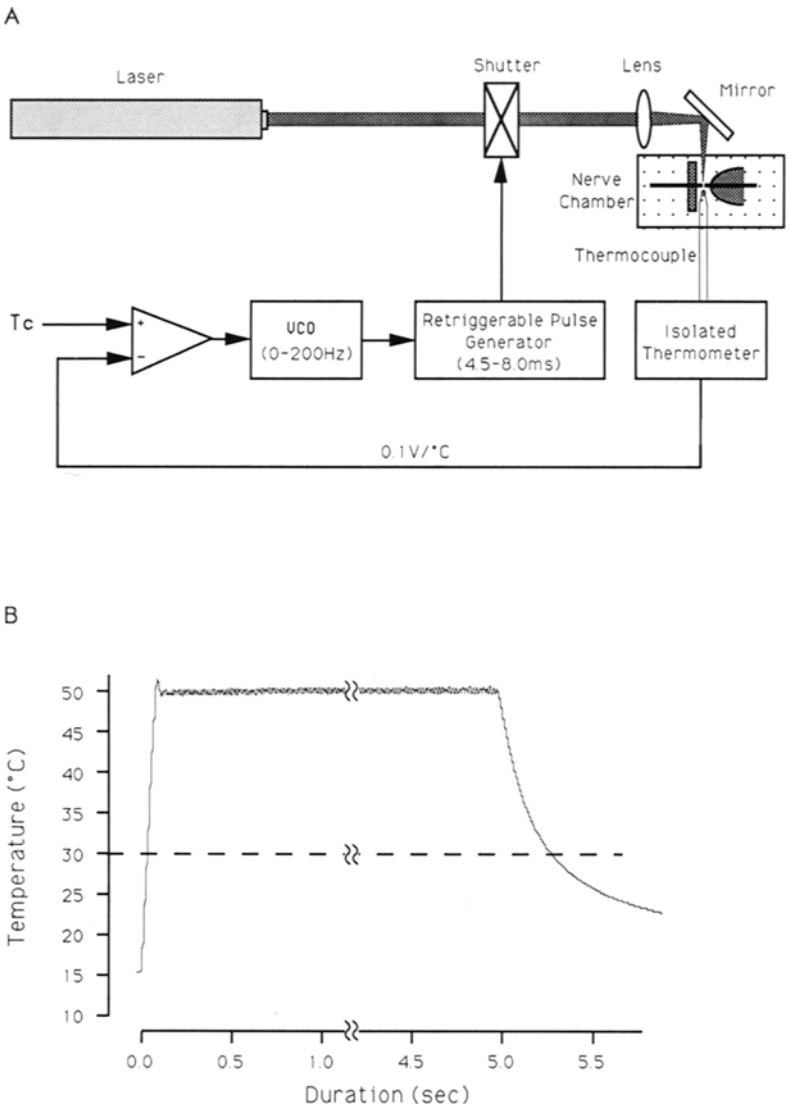


**Fig. 1.** (*A*) Block diagram of laser irradiation control apparatus. A 10-watt 1.32 $\mu$m Nd-YAG laser beam is passed through a fast shutter with a maximum operational speed of 200 Hz. Due to the small size of the shutter aperture, the laser power after passing through the shutter was reduced to 1.2–1.4 watts. The output from the isolated thermometer is compared with a temperature command signal ($T_c$). A voltage-controlled oscillator (VCO) sends a periodic square wave with its frequency proportional to the difference between $T_c$ and $T_m$. A retriggerable pulse generator accepts the input from the VCO and controls the opening of the shutter. (*B*) Temperature response measured by the thermocouple installed in the center pool of the nerve chamber where the node of Ranvier is situated when a 5-sec 50°C step command was given to the irradiation control apparatus. The response had a rise time of 150 msec, an overshoot of 2°C, and a control range of ±0.2°C. After the cessation of laser irradiation, the temperature decreased to below 30°C within 250 msec.

pulse protocol described above. In addition, two series of membrane current recordings induced by potential step to different depolarization potentials were taken with and without a 50 msec, 50 mV hyperpolarization potential prepulse.

## Data Analysis

The kinetic analysis of the membrane current was performed by scaling and curve-fitting. The scaling process adjusted the current traces to be compared to have the same peak amplitude. A change in current kinetics can be identified by overlapping the scaled traces.

In the curve-fitting procedure, the sodium current inactivation was assumed to fit two exponential processes, as proposed by several authors (Chiu, 1977; Nonner, 1980; Ochs, Bromm & Schwarz, 1981; Benoit, Corbier & Dubois, 1985). Therefore, the decaying portion of the membrane currents after leakage current adjustment was fitted by the equation

$$I_{ion} = I'_{Na}[g \exp(-t/\tau_{h1}) + (1 - g)\exp(-t/\tau_{h2})] + I'_K[1 - \exp(-t/\tau_n)]^3$$

where $I'_{Na}$, $\tau_{h1}$, $\tau_{h2}$, $g$, $I'_K$, and $\tau_n$ are the fitted parameters. The time to peak inward current ($t_p$) was also measured.

The steady-state inactivation curves of sodium current were fitted to the Boltzmann relation

$$h_\infty(V) = \frac{1}{1 + \exp[(V - V_h)/k]}$$

where $V_h$ is the potential at which $h_\infty = 0.5$ and $k$ is the slope factor.

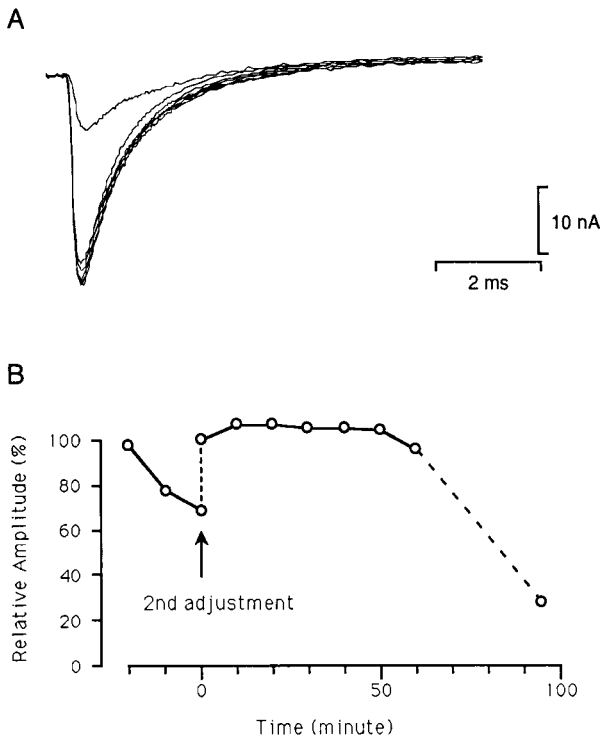


**Fig. 2.** Long-term stability of the voltage-clamp recording. (*A*) Overlapped membrane current traces elicited by 60 mV depolarization potential steps. The nearly identical traces were recorded every 10 min in 60 min after the second adjustment of holding potential and balance of voltage clamp, whereas a significantly lower amplitude trace was recorded 95 min after the adjustment. (*B*) Relative changes of the amplitude of the peak inward current.

## Results

### General Description: Stability of the Preparation

Previously, in studying the ultraviolet (UV)-radiation effects on single myelinated nerve nodal currents (Fox, 1974), a spontaneous rundown of membrane currents was identified and adjusted in the final result for the quantitative derivation of Na current sensitivity to UV treatment. Such phenomena were not observed in the present study. Rather, after an initial settlement period of about 15 min, during which the balance of the voltage-clamp circuit was readjusted, the membrane current remained stable for 60–90 min, before a fast deterioration occurred (Fig. 2). The reason for the discrepancy between the present observations and the earlier UV experiments is unclear. However, due to the stability of the membrane current recording, the rundown phenomenon was not taken into consideration for data analysis.

For temperatures below 48°C, the membrane current usually remained unaffected after 5-sec hyperthermic treatments. For instance, in three trials, initiated from an initial temperature 15°C, a 30-sec exposure to 46°C did not have a persistent effect on the membrane current. On the other hand, after the nerve membrane was exposed to 48°C or higher temperatures for 5 sec, there was a consistent irreversible suppression of both the peak inward current and the steady-state outward current. The action of hyperthermia on membrane currents appeared to be acute due to the lack of an obvious delayed effect after the heat treatment.

Since the early inward current is carried by sodium ions, while the delayed current is carried mostly by potassium ions (e.g., *see* Stämpfli & Hille, 1976), the changes in the amplitude of the peak inward current and late steady-state outward current were assumed to represent the change in sodium and potassium permeability, respectively.

The experimental protocol was selected to start from 48°C with 2°C increments for each subsequent treatment until the nodal membrane was demonstrably damaged. Total disruption of the electrical properties of nodal membrane was characterized by a more than 10-fold increase in resting membrane conductance. This occurred at 56°C treatment in three nodes and at 58°C in nine other nodes.

### Na and K Permeability

With 5-sec treatments between 48 and 54°C, there was a differential response in the Na current and K current as illustrated in Fig. 3. The Na current began to show a more pronounced decrease than the K current starting at the 50°C treatment. Significant decrease of the K current started after 54°C treatment. Following 56°C treatment, both Na and K currents were totally abolished.

For treatments below 54°C, the change in the leakage current and the amplitude of the capacitive transient was less than 10%, indicating that the electrophysiological properties of membrane were changed very litte. However, there was an increase in the leakage current immediately before the detrimental treatment, which can be observed as an increase in negative holding current (bottom panel, Fig. 3). This phenomenon usually served as an early indication of membrane disruption in our experiments.

The differential nature of the Na and K current response to laser hyperthermia is best demonstrated by comparing their relative change in the current amplitude after each treatment. Since the depression of ionic currents is voltage independent, the current

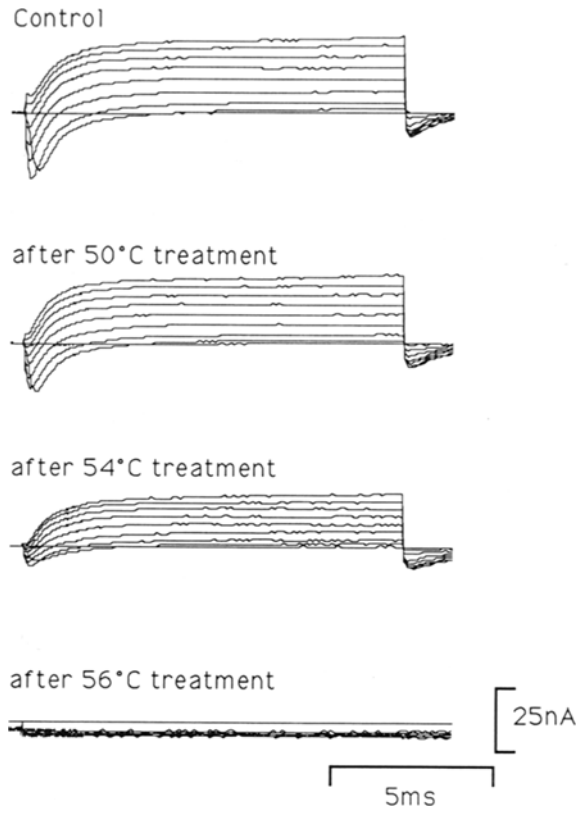


**Fig. 3.** Effects of hyperthermia treatments on ionic currents. Families of voltage-clamp current in response to a single depolarizing potential steps of 20 to 180 mV in 20-mV increments following treatments of 5-sec hyperthermia at the indicated temperatures. The holding potential was set to let $h_x = 0.7$ at the beginning of the experiment. Temperature 15°C, node #532. With thermal pulse treatment from 50 to 56°C, there was a gradual decrease in the amplitude of the membrane currents. There was a differential response to laser hyperthermia in the Na current and K current. After 50°C treatment, Na current began to show a decrease while the K current remained unchanged. Significant decrease of the K current started after 54°C treatment. The active ionic currents were totally abolished after the 56°C treatment. A possible damage to the membrane lipid bilayer was indicated by the induction of a negative holding current by the hyperthermic treatment.

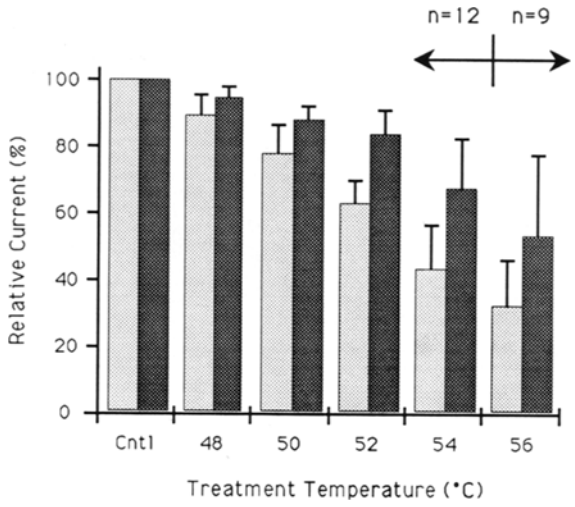


**Fig. 4.** Averaged results of the hyperthermic suppression of the early transient inward Na current and late stable outward K current. The Na current was evaluated using the current traces elicited by $V = 60$ mV, whereas the evaluation of the potassium current made use of the membrane current elicited by $V = 120$ mV. (▩)K, (□)Na.

recordings elicited by $V = 60$ mV and 120 mV steps were used to evaluate the relative change of Na and K currents, respectively. The averaged results of the relative amplitudes of ionic currents following successive laser hyperthermia treatments are illustrated in Fig. 4. The rate of decrease of the potassium current was routinely less than that of the Na current. The decrease of sodium current began at lower temperatures, starting from the initial 48°C exposure up to the detrimental exposure of 54 or 56°C. On the other hand, significant hyperthermic effects on the potassium current were confined to a small range of temperatures, between 54 and 56°C.

The variability of the thermal depression of ionic currents between experiments was higher after 54°C and 56°C treatments. Since these temperatures were very close to the detrimental temperature range, the treatments at these temperatures would result in a more variable degree of change in current amplitude because of the higher probability of damage.

A steep reduction of potassium current was accompanied by a significant increase in resting holding current, suggesting a deterioration of nodal membrane rather than a direct thermal damage to K channels themselves. Since the damage temperature of potassium current is close to the membrane total disruption temperature, the steep reduction of potassium current could not always be detected before total disruption. It is even possible that the K channel has a higher thermal damage threshold than the lipid bilayer, and the steep decrease of K current is a secondary effect due to a leaky membrane that is not able to sustain the electrochemical gradient necessary for ionic currents.

The current-voltage plots of the Na current (Fig. 5*A*) and the K current (Fig. 5*B*) showed a gradual decrease in the slope following the treatments with progressively higher temperature pulses. The reversal potential for sodium current ($E_{Na}$) was not altered by laser hyperthermia except after a detrimental temperature exposure to 56°C. The positive-slope portion of the *I-V* plots remained linear after the treatments of up to 54°C, indicating that the depression of ionic currents was not voltage dependent,

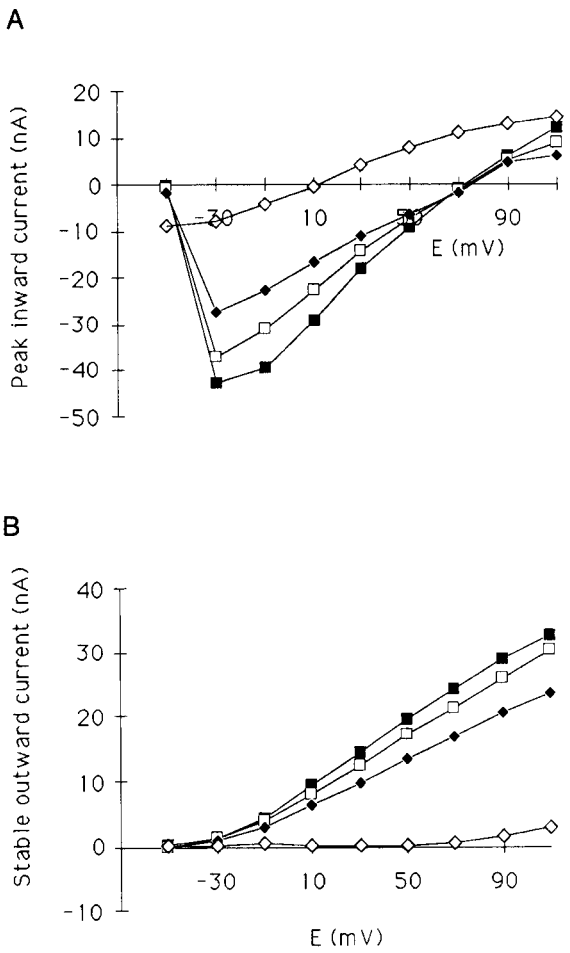


**Fig. 5.** Current-voltage plots for the sodium current (*A*) and potassium current (*B*). The slope of these *I-V* plots gradually decreases after subsequent treatment with progressively higher temperature. The reversal potential of the Na current appeared to be stable before the final detrimental treatment; node# 532. (■) Control, (□) 50°C, (◆) 54°C, (◇) 56°C.

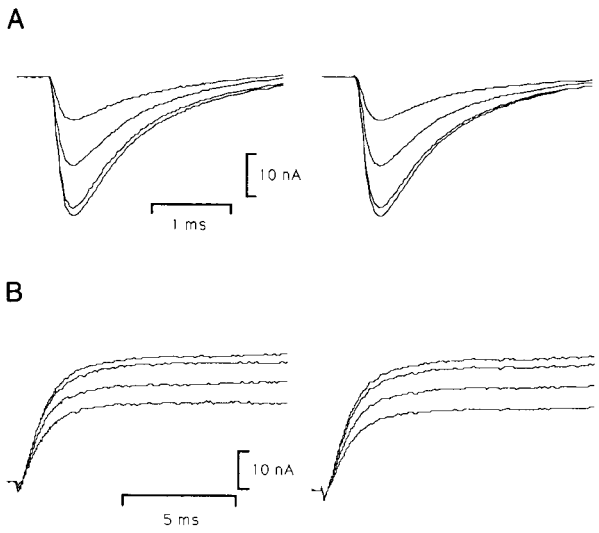


**Fig. 6.** Progressive decrease of early inward current (*A*) and late outward current (*B*) following hyperthermia treatments of 48, 50 and 52°C. Membrane currents were elicited by a depolarizing voltage step with amplitude of V = 60 mV and 100 mV. The right panels show the scaled current traces of the control. The scaled traces have the same peak amplitude as the actual recordings; any difference in the current kinetics can be identified by comparing the scaled and the actual traces. It is obvious from the figure that there were virtually no changes in the kinetics of Na and K currents after successive hyperthermic treatments. Temperature 15°C, node #537.

i.e., the relative change of ionic current amplitude was the same for any membrane potential. In conclusion, the decrease in current amplitude reveals a voltage-independent depression of ionic permeability.

## Ionic Current Kinetics

The change in ionic current kinetics was evaluated using the scaling of current traces and numerical curve-fitting from the membrane currents elicited by voltage steps of $V = 60$ mV and $V = 100$ mV. Figure 6 shows one of the results by scaling the control recordings with numbers corresponding to the proportional change in peak amplitude. The left panels are the actual recordings, whereas the right panels are the current traces obtained by scaling the control according to the actual relative peak amplitudes. It is obvious from these results that there was virtually no change in both the kinetics of the early and the late portion of membrane currents. The numerical curve-fitting also showed virtually no difference in ionic current kinetics after successive hyperthermic treatments. In conclusion, the hyperthermia treatments did not have a profound effect on the activation and inactivation kinetics of both K and Na currents until after the 56°C exposure.

On the other hand, the steady-state sodium inactivation curve ($h_\infty$) was shifted progressively to the hyperpolarization direction after successive hyperthermia treatments (Fig. 7*A*). Although the $h_\infty$ curve shifted consistently to the negative potentials, the shift was not significant until after the treatment of 54°C or greater, where the hyperthermic suppression of Na current was also significant. As shown in Fig. 7*B*, the average shift of 12 experiments was less than 4 mV for the treatments up to 52°C. A more significant shift could be observed after the 54°C treatment. The slope factor ($k$) of the $h_\infty$ curve was little altered by the thermal treatment.

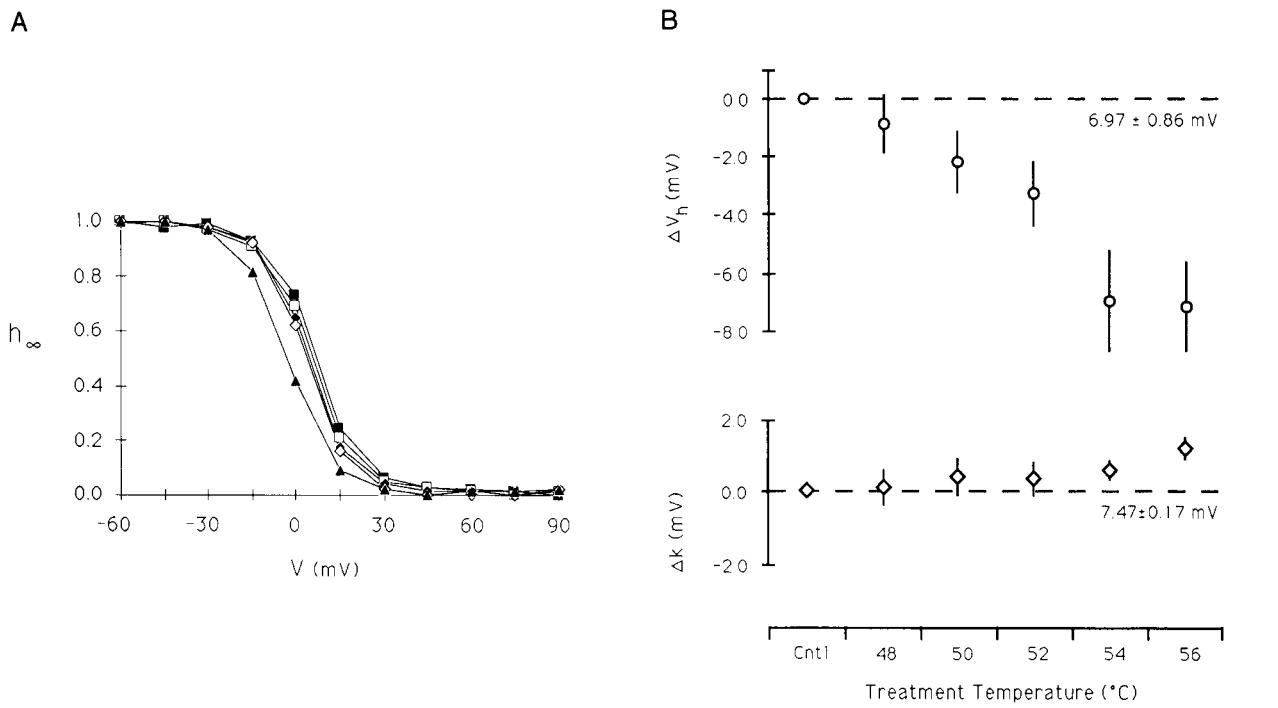


**Fig. 7.** (*A*) Steady-state inactivation ($h_\infty$) curves following subsequent laser hyperthermia treatments. The curve showed a consistent shift toward the negative potential axis after successive treatment. (*B*) the averaged change in $V_h$ and $k$. The curve shifted in voltage axis in the hyperpolarization direction after each laser treatment with a significant shift occurring after 54°C treatment. $V_h$ and $k$ were the fitted parameters of the $h_\infty$ curve to the equation $h_\infty(V) = 1/\{1 + \exp[(V - V_h)/k]\}$; node# 532. (■) Control, (□) 48°C, (◆) 50°C, (◇) 52°C, (▲) 54°C.

### Leakage Current

The increase in the leakage current could result from leaky membrane or open ion channels induced by thermal treatments. In order to characterize the nature of the induced leakage current, five experiments were performed following the same protocols with the ionic currents blocked by 500 nM tetrodotoxin (TTX) and 12 mM tetraethylammonium (TEA). As shown in Fig. 8, after thermal treatment with temperatures higher than 52°C, the leakage conductance increased. The *I-V* plots for leakage current after successive hyperthermia treatments cross one another at approximately 0 mV membrane potential, suggesting that the leakage was a voltage-independent ohmic component, and was not mediated by the known voltage-dependent ion channels. Since the ion channels were pharmacologically blocked before laser treatment, the increased leakage current is more likely to arise from leaky nodal membrane induced by thermal treatments.

## Discussion

A brief description of the observed phenomena is summarized in the Table. The major changes in the nodal membrane currents induced by 5-sec exposures to laser hyperthermia at 48–54°C were a differential reduction of the sodium and potassium permeability and a shift of the steady-state Na inactivation. After the treatment with temperatures above 54°C, the membrane became leaky, and the chemical equilibrium was lost, so that little active responses could be detected in this region. With even higher temperature treatment, the membrane was totally destroyed and its electrical behavior became purely resistive.

Of the most interest is the range of temperature from 48 to 54°C, where a differential suppression of Na and K currents was prominent while the electrical properties of the membrane were not altered. Because of the lack of change in current kinetics and reversal potential after hyperthermia treatments over this temperature range, the irreversible nature of the ionic current suppression could best be related to structural change of ion channels, rather than to imbalance of the electrochemical gradient or ionic environment. Furthermore, the retainment of kinetic behavior and reversal potential after the hyperthermic treatments appears to rule out alterations of either channel opening probability, or the magnitude of single-channel current; it is likely that the hyperthermia treatments induced an irreversible partial damage to ion channel population, and the thermally damaged ion channels are in a nonconducting closed state.

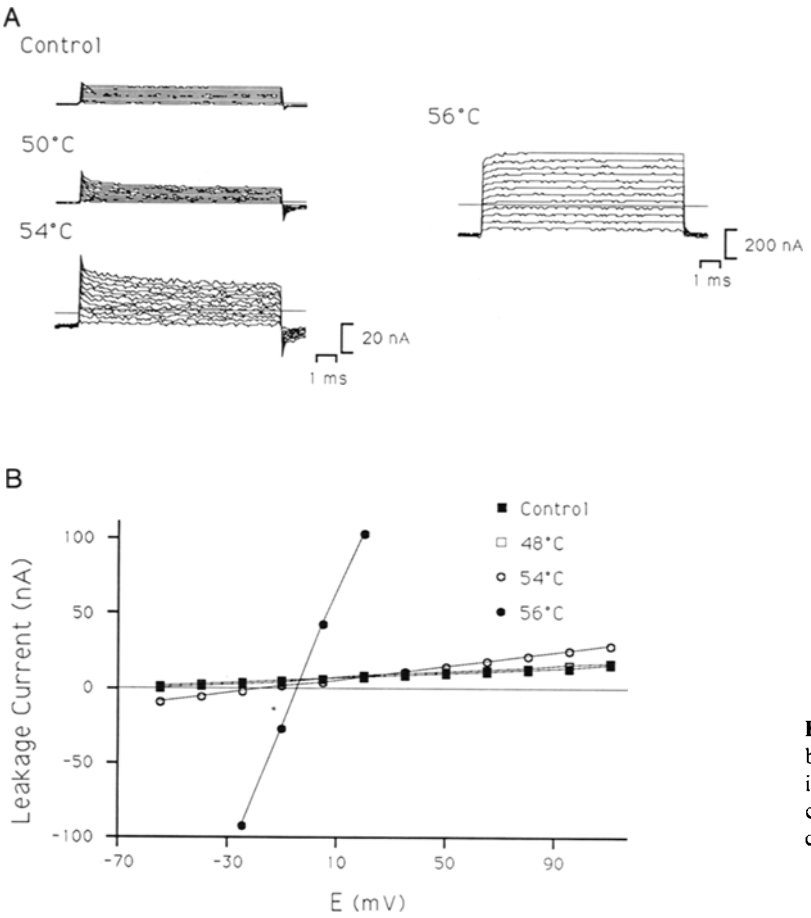


**Fig. 8.** (*A*) Families of leakage currents induced by voltage step of $V$ = 15 to 180 mV in 15-mV increment. Notice the difference in the calibration scales. (*B*) *I-V* plots of the leakage currents shown in *A*.

**Table.** A summary of the major observations after the 5-sec laser-induced hyperthermia treatments in different temperature ranges

| Temperature | Observations |
|---|---|
| <48°C | no effect |
| 48–54°C | differential suppression of Na and K currents |
| | no change in leakage current |
| | no change in current kinetics |
| | slightly reduced $h_\infty$ |
| 54–56°C | leaky membrane |
| | significantly reduced $h_\infty$, change of $E_{Na}$ |
| | significant reduction of K current |
| >56°C | complete damage |
| | very leaky membrane |
| | no detectable active ionic currents |

The differential heat sensitivity of Na and K currents may be related to the number of ion channels or to their thermodynamic energy. The number of ion channels in frog nodes of Ranvier has been determined by many different methods (e.g., Nonner, Rojas & Stämpfli, 1975; Sigworth, 1980). Despite the variable data reported by different authors, a reasonable estimate for the number of ion channels, found in a review by Stämpfli (1981), showed 30,000 sodium channels at resting potential per node, and 55,000 potassium channels per node. In view of the roughly comparable number of Na and K channels, it is unlikely that the difference in the number of channels would result in a differential heat sensitivity. Instead, it is more likely that the differential thermal effect is determined mainly by differences in ion channel conformation and by its interaction with the membrane environment. Therefore, the present data provide an indirect evidence that the K channels are thermodynamically more stable than the Na channels in the frog nodes of Ranvier.

The simultaneous decrease in sodium permeability and steady-state inactivation which was prominent in the present study has also been observed with a diverse variety of agents acting on the nodal membrane such as cooling (Chiu, Mrose & Ritchie, 1979; Schwarz, 1986; Murray et al., 1990), UV radiation (Schwarz & Fox, 1977) and x-ray radiation (Schwarz & Fox, 1979). Although it has been suggested that the simultaneous decrease could be

separated into two independent processes in the cooling and UV radiation studies (Chiu et al., 1979; Schwarz & Fox, 1977), more recent evidence showing that Na channels with h-gate in different configurations may react differently to external agents (Hof & Fox, 1983; Salgado, Yeh & Narahashi, 1985) could lead to the hypothesis that the hyperthermia-induced reduction in both sodium permeability and $h_\infty$ is mediated by the same mechanism.

Suppose that the inactivated Na channels are not damaged by the thermal treatment, the reduction of the noninactivated channels will reduce the $h_\infty$ at constant holding potential, since the h-parameter in Hodgkin-Huxley formulation simply denotes the proportion of the noninactivated Na channels in the whole functional Na channel population. Further support came from a theoretical thermodynamic derivation by Conti (1986) which suggested that the Na channels in the inactivated state have a lower mean entropy than in the closed and open states and, therefore, a higher degree of structural order. In this respect, the inactivated Na channels should be less sensitive to heat treatment based on absolute reaction rate theory.

The phenomenon of phase transition of nerve membranes has been the subject of extensive studies. After high temperature exposures, the lipid bilayer changes from crystalline gel phase to liquid-crystalline phase which is thinner, more permeable, and has higher capacitance (Kind et al., 1982).

The phase transition of nodal membrane should induce a change in ionic current kinetics as a result of the change in the molecular interaction between the lipid bilayer and the ion channels. Although many studies have suggested the influence of lipid environment on ion channel permeability (Chiu et al., 1979; Schwarz, 1979; Kirsch & Sykes, 1987), no quantitative results have been reported so far. A voltage dependence of ionic permeability would be another indication of changes in the physical properties of the lipid bilayer and its interaction with ion channels (Woodhull, 1973). Since our results did not show significant change in the ionic current kinetics or the leakage current up to 54°C treatment, and the induced depression of ionic permeability was not voltage dependent, it is unlikely that the irreversible phase transition was induced with 5-sec thermal treatments of 48–54°C. After the treatments at 54°C and above, the increase in the resistive leakage current is a strong indication of phase transition of the nodal membrane.

In the present laser irradiation setup, since the size of the laser spot (~100 $\mu$m) is much larger than the nodal gap (typically 1 $\mu$m), the internode and paranode were also covered in the laser treatment area. However, these regions contribute very little to ionic current as the ion channels are densely aggregated in the nodal membrane. In addition, the refractive coefficient of myelin is higher than unmyelinated nerve membrane (Svaasand & Ellingsen, 1983), causing less energy absorption. The phase transition of myelin at high temperatures has been studied but it was observed only in the dehydrated form (Ladbrooke et al., 1968; Andjus et al., 1988). Consequently, the inclusion of the internodal and paranodal regions in the irradiation should not result in significant changes in the ionic permeability in the nodal area.

The interpretation of the hyperthermia effects is complicated by the possible existence of multiple Na and K channel types, since different channel types may have different heat sensitivity and will cause the overall ionic permeability thermal response to show a broad distribution in the temperature domain. A more complicated situation would be that some channel types have overlapping heat sensitivity, making it hard to discriminate their response to hyperthermia treatments.

In the amphibian nodes of Ranvier, most authors agreed that there is only one type of sodium channel with several transition states (Chiu, 1977; Ochs et al., 1981; Schmidtmayer, 1985; Jonas et al., 1989). In contrast to the Na channel, multiple types of K channels have been identified in amphibian nodes of Ranvier, based primarily on the difference in their inactivation characteristics when bathed in high K concentration solutions (Dubois, 1981; Jonas et al., 1989). However, the activation properties of these different K channel types are still unclear, and the number of K channel types that can be detected in normal K concentration Ringer's solution is not known. Since our experiments were done with the node bathed in normal Ringer's solution and activated with relatively short voltage steps, we chose to use the conventional Hodgkin-Huxley type of K current in our analysis. A detailed analysis of the tail current would be required to differentiate the hyperthermia response of different potassium channel types.

Since at present there is a lack of data for temperature effects on nodal membrane currents at higher temperature range, and since lasers can be an effective heating source for studying membrane currents at high temperature, the results of the present study could provide information for establishing safety standards in medical laser applications to neural tissue, for exploring new therapeutic modalities, and for further elucidating the heat block mechanism in nerve conduction.

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