ELSEVIER

Biochimica et Biophysica Acta 1511 (2001) 391–396

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

www.bba-direct.com

Nonlinear temperature modulation of sodium channel kinetics in GH_3 cells

Arthur D. Rosen *

Department of Biological Sciences, Purdue University, West Lafayette, IN 47907, USA

Received 3 November 2000; received in revised form 13 February 2001; accepted 16 February 2001

Abstract

The effect of temperature on sodium channel function was examined in GH_3 cells, using the whole cell patch-clamp methodology. Specific parameters examined were current-voltage relationships, activation time, and inactivation time. For the temperature range studied, 23–37°C, there was no change in the current-voltage relationship. A linear response to temperature was seen in the inactivation time constant, τ_h. The activation time constant, τ_m, was clearly nonlinear, with a sharp discontinuity at 28°C. This nonlinearity was especially evident at lower membrane voltages. These findings are consistent with the hypothesis that membrane structural changes, which occur during the thermotropic phase transition, are capable of influencing the function of the intramembranous portion of the channel. Caution should, therefore, be exercised in extrapolating data on channel function obtained at room temperature to physiological temperatures.

Keywords: Temperature effect; Membrane channel; Phase transition

1. Introduction

The kinetics of ion channel function, as with all biological processes, is temperature dependent. Chemical and enzymatic processes involved in channel function are expected to be sensitive to temperature changes, and since ion channels are intimately associated with membrane lipids [1], their function might also be altered by any temperature dependent physical change of the membrane's lipid matrix. The extent of processes such as thermal expansion and diffusion would be minimal over the temperature range allowable in biological systems, but thermotropic reconfiguration of lipid structures [2] might significantly alter the function of imbedded ion channels. The effects of temperature on ion channels have been studied in several nonmammalian systems [3–6], but less attention has been paid to the influence of temperature on channel function in the mammalian cell. Sodium channel studies that have been done concentrated on the effect of temperature on fast and slow inactivation rates. Most studies of channel function have been carried out at room temperature, i.e., 22–23°C, with the operational assumption that changes in kinetics may be described as a simple function of temperature [7]. Recently, the activation kinetics of mammalian calcium channels has been shown to exhibit a distinct discontinuity between 27 and 32°C [8]. The present study was carried out to

* Fax: 765-494-0876;
E-mail: arosen@bilbo.bio.purdue.edu

0005-2736/01/$ – see front matter
PII: S0005-2736(01)00301-7

accurately quantify the effects of temperature, from 23 to 37°C, on voltage-dependent sodium channel function in GH_3 cells. These cells are of rat pituitary origin and exhibit large TTX sensitive sodium currents, typical of a neuronal phenotype [9], but do not elaborate neurites in culture. This obviates the potential problems involving spatial clamp of rapidly activated inward sodium current. In addition, since GH_3 cells are maintained in culture, they are particularly suitable to the whole cell patch-clamp methodology used in this study.

2. Methods

2.1. Cell cultures

Proliferating GH_3 cells were grown in 100 mm polystyrene culture dishes at 37°C in a 5% CO_2 environment. The growth medium contained DMEM supplemented with 10% FCS and penicillin/streptomycin. As the cells approached confluence, they were split and replated at 10% of their initial density. At the same time, a number of 35 mm polystyrene culture dishes were established to provide sufficient dishes of cells for recording on any given day. All recordings were carried out between 2 and 5 days of plating to minimize any variability due to cell health.

2.2. Temperature control

Bath temperature was controlled with a 34.2 mm diameter circular thermofoil heater (Minco Products), with an 11.4 mm diameter central opening. This device was cemented to a larger epoxy glass composite base and fit neatly under a 35 mm culture dish, while allowing visual access to those cells situated over the central aperture. Since the heater itself was only 0.05 mm thick, it did not make physical contact with the floor of the dish, thereby eliminating the possibility of movement associated with thermal expansion of the heater's resistive element. Current for the heater was supplied by a proportional temperature controller (Omron Electronics) with an analog output. This arrangement prevented the introduction of switching transients into the recording system. Temperature sensing was by means of a small, insulated thermocouple situated 1–2 mm from that cell from which recordings were being carried out. The overall stability of this system was ± 0.1°C from the set point.

2.3. Recording conditions

Immediately prior to recording, the growth medium in a 35 mm culture dish was slowly exchanged with a 37°C solution of 140 mM NaCl, 5 mM $MgCl_2$, 10 mM HEPES, titrated with NaOH to a pH of 7.2. The dish was then placed on the thermoregulated portion of the microscope stage and allowed to settle to the initial setting for that day's recording.

Patch pipettes were pulled from borosilicate glass to an outer diameter of approx. 2 μm and lightly fire polished. The electrode was filled with a solution consisting of 120 mM CsCl, 10 mM K-EGTA, and 10 mM HEPES, titrated with KOH to a pH of 7.2. These electrodes had resistances of 2–4 MΩ and, combined with the use of series resistance compensation, voltage-clamp errors due to high access resistance were minimized. Membrane current was recorded with an Axopatch 1-D patch-clamp amplifier. Command voltages for this amplifier were generated with HEKA Pulse software, running on a Macintosh 840AV computer. Current responses were digitized at 40 kHz on an Instrutech ITC-16 analog-to-digital converter and processed for display by the Pulse software.

Under slight positive pressure, the electrode tip was lowered to the cell surface while its resistance was continuously monitored by measuring the current in response to application of a 10 mV, 10 ms pulse. Once the electrode touched the cell, a small negative pressure was applied to form a seal of at least 1 GΩ. Following seal formation, the tissue within the pipette was broken with an additional negative pressure or with a voltage zap (1 V for 5 ms). The membrane was initially clamped to a holding potential of −80 mV, and inward sodium current was assessed by the application of a series of 100 ms pulses, in 5 mV increments, from the holding potential to +40 mV. Correction for any linear leakage currents was accomplished with a P/4 subtraction protocol. The leak-subtracted data were displayed, along with on-line analysis of the current-voltage relationships for that cell. Data acquisition was de-

layed until the current-voltage relationship stabilized. This typically occurred 5 min after the cell was clamped, the time needed for the electrode solution to dialyze the intracellular solution. Once stable, data were stored for off-line analysis. After all recordings were obtained from a given cell, 1 μM of TTX was added to the preparation and test pulses again applied.

2.4. Temperature management

Recordings were made at 1°C increments, from 23 to 37°C. To ensure that recordings were carried out under stable conditions, the bath temperature was increased at a very slow rate (0.5°C/min) and maintained at the higher temperature for 2 min before recording. Because of the amount of time involved in establishing temperature stability, it was not possible to record from the same cell at all temperatures. Nevertheless, this protocol guaranteed that there were no thermal gradients during recording and that cell temperatures were virtually identical from one experiment to another.

2.5. Data analysis

Current-voltage relationships were computed online. Activation and inactivation time constants were computed on the stored data by fitting the sodium current at each voltage step with a general Hodgkin-Huxley formalism with a defined number of activation gates (three) and inactivation gates (one). This m3h model provided an excellent fit to the data. The time constants τ_m and τ_h thus generated define the activation and inactivation kinetics, respectively.

3. Results

Technically satisfactory data were obtained from 16 cells. In all cases recordings were carried out at

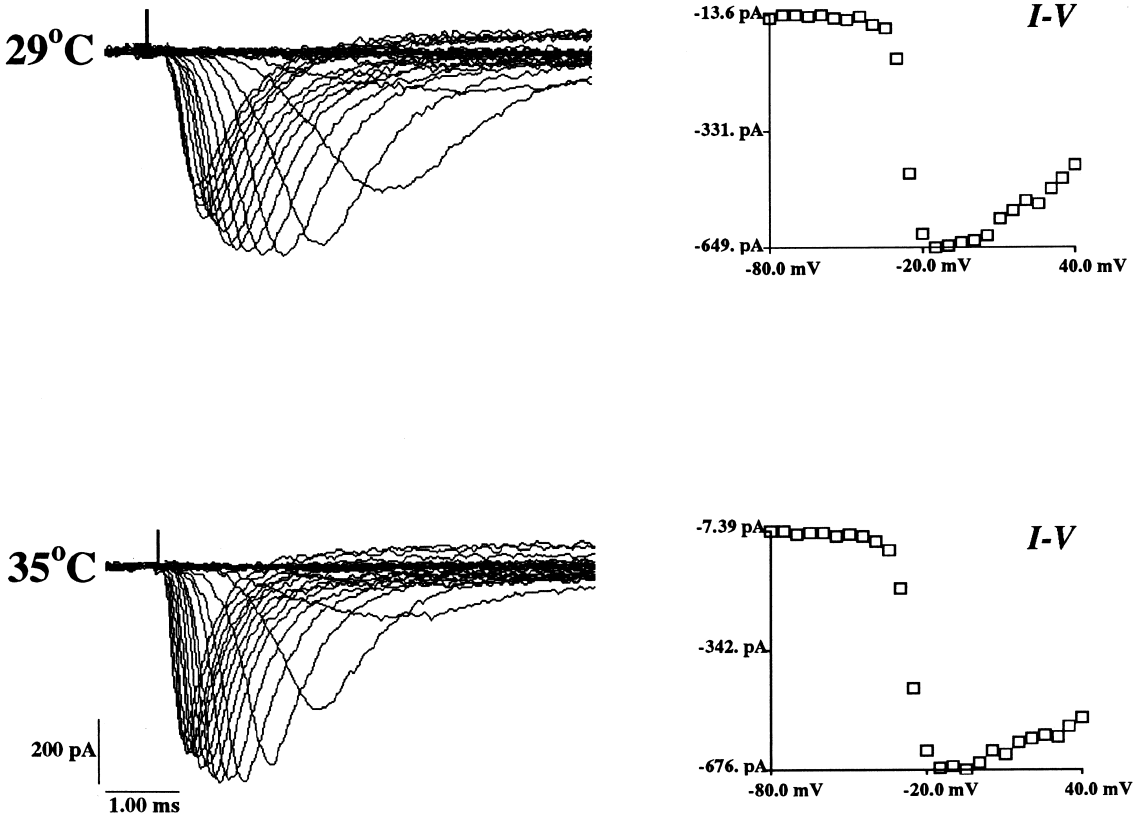

Fig. 1. Sodium currents recorded from the same cell at 29°C and 35°C, with corresponding current-voltage plots. Increase in activation and inactivation rates is evident. No change in current-voltage relationships.

two or more temperatures and, in four cells, at more than five temperatures. In every cell studied, there was a complete block of inward current following the introduction of TTX. The threshold for sodium current was −35 mV.

3.1. Stabilization time

Current-voltage plots revealed peak current initially at −5 mV but, during the first 5 min after clamping, there was a 10 mV shift toward a more negative value, which then remained constant. This shift in peak is probably secondary to dialysis of the intracellular fluid [10]. Since the threshold voltage remained at −35 mV, steep voltage dependence for activation was evident in the delayed current-voltage plots. The parameters recorded in this study were consistent with those described in the literature for GH_3 cells [11].

3.2. Effect of temperature

The time courses of sodium currents were obviously sensitive to temperature. This is evident in Fig. 1, which illustrates sodium currents for the same cell at 29°C and 35°C. Changes in temperature, however, had no effect on the current-voltage relationships.

The changes in activation and inactivation rates illustrated in Fig. 1 were quantified by fitting a Hodgkin-Huxley m3h function to all of the data. This was carried out for each membrane voltage step, over the range of −25 mV to +40 mV. For membrane voltages of −20 mV to +40 mV the values of both τ_m and τ_h were sufficiently uniform at each temperature to justify use of mean values. Fig. 2A illustrates the mean τ_m, at −20 mV as a function of temperature. A distinct discontinuity is evident at 28°C. In order to further examine this discontinuity, τ_m was plotted against both temperature and membrane voltage. This is shown in Fig. 3. It is clear from that graph that although there is a decrease in the value of τ_m with increasing temperature at all membrane voltages, the 28°C discontinuity was evident only at lower membrane voltages. The Q_{10} for τ_m was calculated for each membrane voltage step using the mean values of τ_m derived from 63 trials in 16 cells. This was done for two temperature

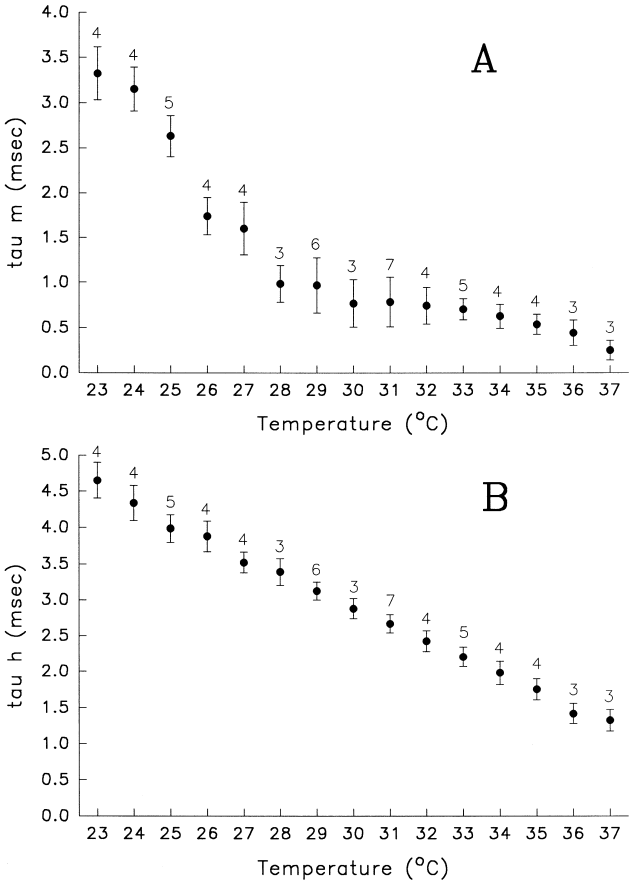

Fig. 2. (A) Mean values of τ_m, at membrane voltage of −20 mV, as a function of temperature. Numbers above error bars indicate number of trials at that temperature. Discontinuity seen at 28°C. (B) Mean values of τ_h, at membrane voltage of −20 mV, as a function of temperature. Numbers above error bars indicate number of trials at that temperature. No discontinuities seen.

ranges, 23–28°C (24 trials) and 29–37°C (39 trials). The calculated values are shown in Fig. 4. At the lower membrane voltages there are two distinct curves that merge at 5 mV. At and above that membrane voltage the mean Q_{10} is 1.98 with a standard error of 0.10.

The mean τ_h at a membrane voltage of −20 mV was a linear function of temperature. This is shown in Fig. 2B. Although there is a decrease in the value of τ_h at higher temperatures for all membrane voltages, no discontinuity was evident. The Q_{10} for τ_h was also calculated for each membrane voltage step using the mean values of τ_h derived from 63 trials in 16 cells. The calculated values are shown in Fig. 5. There was little variability at each membrane voltage

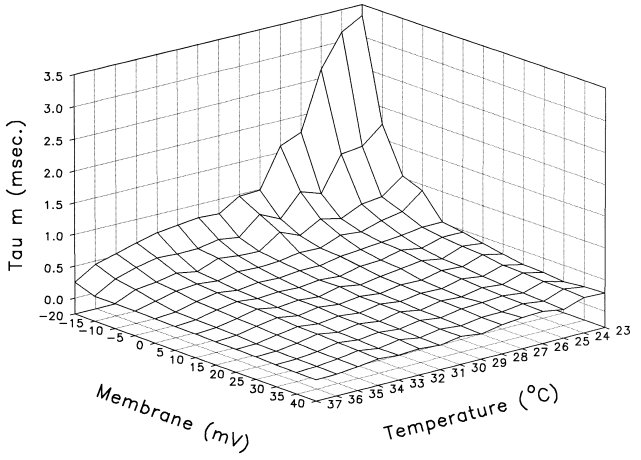

Fig. 3. Cumulative data, mean values of τ_m plotted against temperature and membrane voltage. Discontinuity in activation time constants evident at 28°C, especially at lower membrane voltages. Number of trials at each temperature as in Fig. 2.

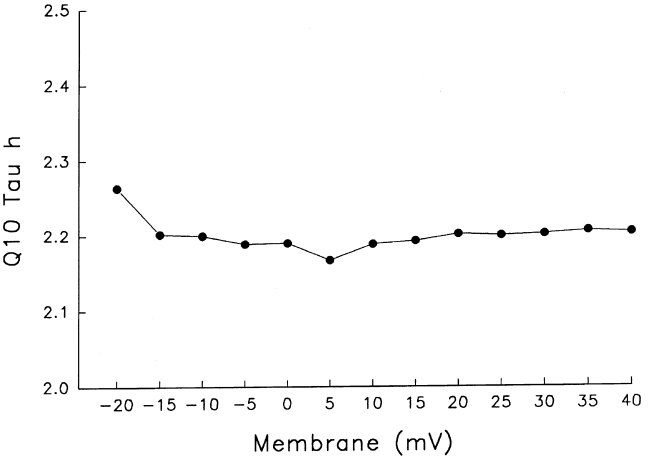

Fig. 5. Q_{10} values for τ_h, calculated after averaging the values of τ_h from 63 trials, as a function of membrane voltage for the temperature range of 23–28°C. The mean Q_{10} was 2.2 with a standard error of 0.02.

step. Over the temperature range of 23–37°C, the mean Q_{10} was 2.2 with a standard error of 0.02.

4. Discussion

Higher temperatures will enhance all biological processes by providing additional energy for chemical reactions. The relationship between temperature and biological effect is traditionally described by the multiplicative constant, Q_{10}, which describes the consequence of a 10°C increment in temperature. Over a narrow range, the value of Q_{10} does provide a satisfactory approximation, but the use of this constant assumes a linear relationship over the temperature range being examined. In the present study, the inactivation rate of voltage gated sodium channels was temperature dependent and the rate of change of τ_h, as a function of temperature, was constant over the full range of temperatures studied. Therefore the use of the descriptive constant Q_{10}, for τ_h, is appropriate.

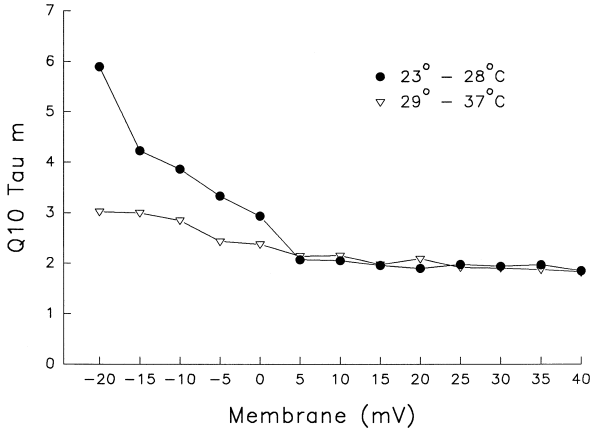

Fig. 4. Q_{10} values for τ_m, calculated after averaging the values of τ_m from 63 trials, as a function of membrane voltage for two temperature ranges, 23–28°C and 29–37°C. Q_{10} values are the same at and above 5 mV, where the mean is 1.98 with a standard error of 0.10.

The activation rate of voltage gated sodium channels was clearly temperature dependent, but the rate of change as a function of temperature was not constant. This was especially evident at lower activation voltages, presumably because the gating kinetics at higher voltages are sufficient to overshadow the effects of temperature.

The discontinuity in the temperature dependence of activation time at 28°C is an especially interesting observation. Channel activation involves several coupled steps, each with its own kinetics. When the kinetics for each process is linear, the overall activation kinetics will be linear. A change in the rate of one process, even when the new kinetics for that process remains linear, will introduce nonlinearity into the overall activation kinetics. Discontinuities in the temperature dependence of channel conductance and gating kinetics have been reported, and attributed to the influence on the rate of channel opening either by changes in the membrane's fluidity [3,12–14] or to a thermotropic phase transition of the

membrane's lipids [5,8]. The transition between gel and liquid-crystal phases of biological membranes is well known [2]. This reversible alteration in membrane structure, which occurs over a narrow temperature range, is due to the abrupt rotameric disordering of the lipid acyl chains at the phase transition temperature [15]. This is associated with a decrease in intermolecular interaction, thereby increasing membrane fluidity, as well as inducing structural changes within the lipid bilayer and alterations in membrane lipid-protein interactions. These changes could influence the intramembranous portion of embedded ion channels by introducing conformational changes to that portion of the protein involved in one or more of the coupled steps involving activation. Sodium channel activation is due to a sequence of voltage induced changes in the S4 segment of the α-subunit [16]. This subunit is a 260 kDa protein located entirely within the membrane where it would be vulnerable to thermotropic transitions.

No evidence of discontinuity in the temperature dependence of the inactivation rate was seen in this study. Fast inactivation of the sodium channel has been shown to involve an intracellular protein [17] and it has been proposed that this protein functions by occluding the channel pore in the membrane's cytoplasmic domain [18]. Such an intracellular mechanism would be unaffected by physical changes within the membrane.

The findings of the present study are compatible with the hypothesis that membrane structural changes, which occur during the thermotropic phase transition, are capable of influencing the function of the intramembranous portion of voltage sensitive sodium channels, while having no effect on those portions of the channel that do not lie within the membrane per se. These physical processes are nonlinear and coexist with the linear effects of temperature on the activation energy of chemical and enzymatic processes. Because of the complexity of temperature dependent changes in sodium channel function, care must be exercised in extrapolating data on channel function from one temperature to another.

References

[1] D. Chapman, Phase transitions and fluidity characteristics of lipids and cell membranes, Q. Rev. Biophys. 8 (1975) 185–235.
[2] D.L. Melchior, J.M. Steim, Thermotropic transitions in biomembranes, Annu. Rev. Biophys. Bioeng. 5 (1976) 205–238.
[3] J.E. Kimura, H. Meves, The effect of temperature on the asymmetrical charge movement in squid giant axons, J. Physiol. 289 (1979) 479–500.
[4] C.L. Schauf, Temperature dependence of ionic current kinetics of Myxicola giant axons, J. Physiol. 235 (1973) 197–205.
[5] W. Schwartz, Temperature experiments on nerve and muscle membranes of frogs. Indications for a phase transition, Pflug. Arch. 382 (1979) 27–34.
[6] E. Van Lunteren, K.S. Elmslie, S.W. Jones, Effects of temperature on calcium current on bullfrog sympathetic neurons, J. Physiol. 46 (1993) 81–93.
[7] A. Cavalie, T.F. McDonald, D. Pelzer, W. Trautwein, Temperature induced transitory and steady-state changes in calcium current of guinea pig ventricular myocytes, Pflug. Arch. 405 (1985) 294–296.
[8] A.D. Rosen, Temperature modulation of calcium channel function in GH3 cells, Am. J. Physiol. 271 (1996) C863–C868.
[9] D.R. Matteson, C.M. Armstrong, Na and Ca channels in a transformed line of anterior pituitary cells, J. Gen. Physiol. 83 (1984) 371–394.
[10] F.N. Quandt, Recording sodium and potassium currents from neuroblastoma cells, in: T. Narahashi (Ed.), Ion Channels of Excitable Cells, Academic Press, New York, 1994, pp. 3–20.
[11] J.M. Fernandez, A.P. Fox, S. Krasne, Membrane patches and whole-cell membranes: a comparison of electrical properties in rat clonal pituitary (GH_3) cells, J. Physiol. 356 (1984) 565–585.
[12] G.D. Fischbach, Y. Lass, A transition temperature for acetylcholine channel conductance in chick myoballs, J. Physiol. 280 (1978) 527–536.
[13] S. Higiwara, M. Yoshii, Effect of temperature on the anomalous rectification in the membrane of the egg of the starfish, *Mediaster aequalis*, J. Physiol. 307 (1980) 517–527.
[14] T. Narahashi, A. Tsunoo, M. Yoshii, Characterization of two types of calcium channels in mouse neuroblastoma cells, J. Physiol. 383 (1987) 231–249.
[15] J.F. Nagle, Theory of the main lipid bilayer phase transition, Annu. Rev. Phys. Chem. 31 (1980) 157–195.
[16] C.M. Armstrong, Ionic pores, gates, and gating currents, Q. Rev. Biophys. 2 (1975) 179–210.
[17] C.M. Armstrong, F. Bezanilla, E. Rojas, Destruction of sodium conductance inactivation in squid axons perfused with pronase, J. Gen. Physiol. 62 (1973) 375–391.
[18] C.M. Armstrong, F. Bezanilla, Inactivation of the sodium channel. II. Gating current experiments, J. Gen. Physiol. 70 (1977) 567–590.