Short communication

The Effect of Temperature on Ionic Currents in the Muscle Membrane of the Crayfish

J. POLEDNA, D. ZACHAROVÁ and M. HENČEK

Institute of Normal and Pathological Physiology, Centre of Physiological Sciences, Slovak Academy of Sciences, Sienkiewiczova 1, 813 71 Bratislava, Czechoslovakia

Since the classical paper of Hodgkin, Huxley and Katz (1952) it has several times been established that the ionic currents in the sodium type excitable membranes decrease and the action potentials increase in amplitude and duration with the decrease of temperature (for review see Hodgkin 1964; Huxley 1959; Cole 1972). The active electrical responses of the calcium type muscle membranes were also shown to increase with the decreasing temperature (Dudel and Rüdel 1968; Lipskaja and Zacharová 1977); the effect of temperature on calcium currents is, however, equivocal. Both a decrease (Rothberg 1953; Keynes et al. 1973) and increase (Goto et al. 1978) of inward currents were reported to occur in muscle membranes when the temperature was decreased.

We reexamined this problem in muscle fibres of the crayfish (*Astacus fluviatilis*) which are known to produce inward ionic currents by activation of calcium channels only (Henček and Zachar 1977). The calcium ionic currents, I_{Ca}, were recorded under voltage clamp conditions both in intact fibres and cut muscle fibre segments. The potassium currents in the former case were suppressed by TEA (150 mmol/l), or in the latter case by exposing the membrane to both K-free internal and external solutions. The methods as described elsewhere, were used i.e. Henček et al. (1969), Henček and Zachar (1977) for intact fibres and Hille and Campbell (1976) for cut muscle fibre segments. The preparation of muscle fibre segments from the crayfish muscle was the same as described by Zahradník and Zachar (1982). The temperature was maintained by means of Peltier thermoelectrical elements; the temperature was measured with a thermistor sited close to the fibre. When the temperature was changed by 5°C, it was stabilized within one minute. The measurements were performed 5 min after switching to a new temperature. The temperature range was 5—20°C.

Fig. 1a shows that the inward calcium current (Henček et al. 1978) was increased and prolonged and the outward potassium current was decreased when the temperature was decreased from 20°C—10°C, I_{Ca} decreased in some prepara-

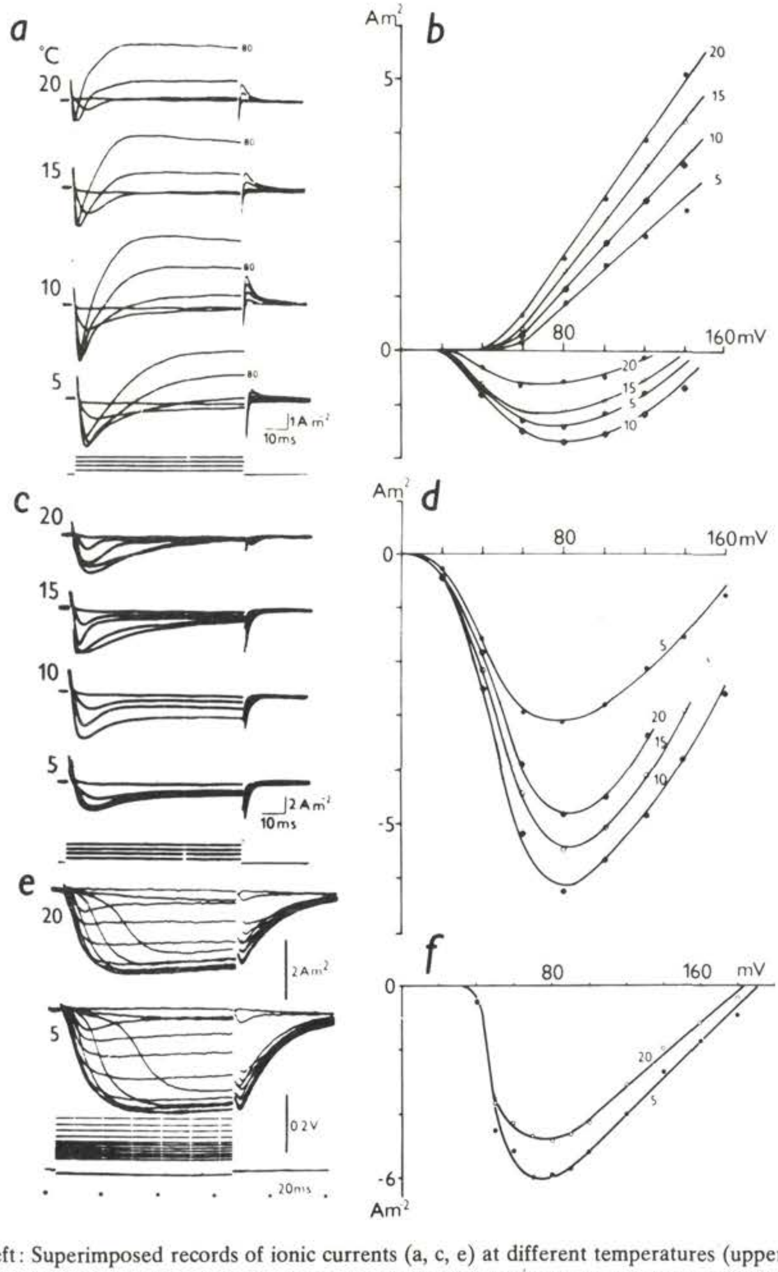

Fig. 1. Left: Superimposed records of ionic currents (a, c, e) at different temperatures (upper traces). Lower traces: rectangular voltage clamp pulses. Right: membrane currents-voltage relations of inward currents (b, d, f) and outward currents (b; positive currents). Numbers at the curves denote temperature (in °C). a, b: intact muscle fibre of the crayfish; c, d: intact muscle fibre in TEA^+ (150 mmol/l); e, f: cut muscle fibre segment. The internal solution contained (in mmol/l): 240 Cs glutamate, 1 $MgCl_2$, 0.01 $CaCl_2$, 1 EGTA and 4 ATP.

tions at temperatures below 5°C; this can be explained by phase transitions in the membrane lipid bilayer (Chapman 1975). The Arrhenius plot of the potassium conductance, G_K, on temperature is linear in the range from 5°C to 20°C. The calculated activation energy is 42.8 kJ.mol^{-1} ($p>0.005$).

Fig. 1c, d demonstrates that the increase in I_{Ca} is also present when the outward currents were suppressed in intact fibres with 150 mmol/l TEA^+. A new phenomenon became evident under these conditions at lower temperatures. The slow component of inactivation failed to relax completely; and the I_{Ca} was rather maintained at a definit steady level.

Also, in cut muscle fibre segments in K-free internal medium, the inward current increased with the decreasing temperature as demonstrated in Fig. 1e, f. The increase in I_{Ca} determined on five muscle fibre segments was 12,5 % at 15°C, 17,3 % at 10°C and 28,3 % at 5°C, in comparison with I_{Ca} determined at 20°C.

Two parameters of the inward current were evaluated: the time to peak (t_a) which characterizes activation, and the time constant of inactivation (τ_h) which was determined by the two-pulse method (Henček and Zachar 1977).

The Arrhenius plot of t_a (log t_a versus $1/T$, the reciprocal value of absolute temperature) represents a linear function from which the activation energy of 33.3 kJmol^{-1} ($p>0.005$) was calculated. The Arrhenius plots of τ_h at various depolarisation were also linear with the mean activation energy 16.8 kJ/mol ($p>0.05$).

The activation energies suggest that the rate of activation is considerably more prolonged than the rate of inactivation, even if the energy of inactivation was determined from the time to peak, which is partially influenced by inactivation. The difference between the activation energy of 33.3 kJ/mol and inactivation energy of 16.8 kJ/mol is, however, fairly distinct. It follows, that the maximum calcium conductance should decrease with the decrease in temperature. If instead the opposite effect is observed, namely I_{Ca} increase, the explanation should be looked for in other factors which determine the amplitude of the inward current. It has been reported by Kostyuk recently (1981) that Ca ions block the calcium channel from the internal site of the membrane at higher concentrations, when the temperature is decreased from 20°C to 5−7°C (10^{-7} mol/l or $10^{-3}-10^{-4}$ mol/l respectively). The I_{Ca} would then increase with the decreasing temperature due to the abolishion of the Ca^{2+} feed-back blocking mechanism. A role could be also ascribed to changes in Ca ions distribution at the membrane, which are suggested to occur when the temperature is changed (Apter and Koketsu 1960; Brading et al. 1961).

References

Apter J. T., Koketsu K. (1960): Temperature studies implicating calcium in regulation of muscle membrane potential. J. Cell. Comp. Physiol. **56**, 123—127

Brading A., Bülbring E., Tomita T. (1969): The effect of temperature on the membrane conductance of the smooth muscle of the guinea-pig taenia coli. J. Physiol. (London) **200**, 621—635

Chapman D. (1975): Phase transitions and fluidity characteristics of lipids and cell membranes. Quart. Rev. Biophys. **8**, 185—235

Cole K. S. (1972): Membranes, Ions and Impulses. Univ. California Press, Berkeley

Dudel J., Rüdel R. (1968): Temperature dependence of electromechanical coupling in crayfish muscle fibers. Pflügers Arch. **301**, 16—30

Goto M., Tsuda Y., Yatani A., Saito M. (1978): Effects of low temperature on the membrane currents and tension components of bullfrog atrial muscle. Jpn. J. Physiol. **28**, 211—224

Henček M., Nonner W., Stämpfli R. (1969): Voltage clamp of a small muscle membrane area by means of a circular sucrose gap arrangement, Pflügers Arch. **313**, 71—79

Henček M., Zachar J. (1977): Calcium currents and conductances in the muscle membrane of crayfish. J. Physiol. (London) **268**, 51—71

Henček M., Zachar J., Zacharová D. (1978): Membrane currents in a calcium type muscle membrane under voltage clamp. Physiol. Bohemoslov. **27**, 457—466

Hille B., Campbell T. C. (1976): An improved vaseline gap-voltage clamp for skeletal muscle fibres. J. Gen. Physiol. **67**, 265—293

Hodgkin A. L. (1964): The ionic basis for nerve conduction. Science **145**, 1148—1154

Hodgkin A. L., Huxley A. F., Katz B. (1952): Measurement of current-voltage relations in the membrane of the giant axon of Loligo. J. Physiol. (London) **116**, 424—448

Hodgkin A. L., Katz B. (1949): The effect of temperature on the electrical activity of the giant axon of the squid. J. Physiol. (London) **109**, 240—249

Huxley A. F. (1959): Ion movements during nerve activity. Annu. N. Y. Acad. Sci. **81**, 221—246

Keynes R. D., Rojas E., Taylor R. E., Vergara J., (1973): Calcium and potassium systems of a giant barnacle muscle fibre under membrane potential control. J. Physiol. (London) **229**, 409—455

Kostyuk P. G. (1981): Calcium channels in the neuronal membrane. Biochim. Biophys. Acta **650**, 128—150

Lipskaja E., Zacharová D. (1977): Conditions of the excitation-contraction link in crayfish muscle fibres at low temperature. Physiol. Bohemoslov. **26**, 457—458

Rothberg J. M. (1973): Temperature effects on the electrical characteristics of the barnacle muscle fiber. Amer. J. Physiol. **225**, 240—246

Zahradník I., Zachar J. (1982): Calcium currents in the muscle membrane of the crayfish in K^+-free internal environment. Gen. Physiol. Biophys. **1**, 457—461

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