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ION CURRENT TEMPERATURE DEPENDENCE OF Br-TYPE NEURON OF *HELIX POMATIA* L.

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The temperature dependence of neuron activity patterns and of neuron activity parameters is known both for vertebrate animals (BARKER and CARPENTER, 1970) and for invertebrate animals (KERKUT and TAYLOR, 1956). The change of ambient temperature will change the neuron resting potential (HODGKIN and KATZ, 1949), and a similar significant change will also be introduced in the repetition frequency of the cells (CARPENTER, 1967). Investigation of certain giant neurons showing burst activity proved the temperature dependence of the activity pattern: the characteristic activity pattern of the control disappears at low temperatures below 12°C and at high temperatures above 33°C (WACHTEL and WILSON, 1973; SALÁNKI et al., 1973).

Previous investigations carried out in our Institute (SALÁNKI et al., 1975) and also the work of other authors showed that probably several mechanisms were responsible for the slow periodical membrane potential change resulting in the burst activity pattern, and for the generation of the action potential.

Investigations of different ion content solutions also showed that the generation of slow waves and action potentials is dependent on the ions involved (JUNGE and STEPHENS, 1973; SALÁNKI et al., 1975). It is known that the ion current during voltage clamp measurements has a marked temperature dependence (HODGKIN et al., 1952). Recognizing the decisive role of temperature in the activity pattern, the purpose of our present investigations has been the determination of the temperature dependence in the Br-type RPal cell of *Helix pomatia* L.

Material and method

The isolated ganglion of *Helix pomatia L.* has been placed in a perfusion chamber having a volume of 3 cm^3. The temperature of the physiological solution and the temperature of the ganglion have been adjusted to the values of 7, 22 and 33 degrees Centigrade, resp. The temperature adjustment has been performed by PELTIER batteries driven by a special circuit (VÉRÓ, 1974a).

Glass microelectrodes filled with 2.5 M KCl having a resistance in the range of 4 to 7 Mohm have been used for recording the membrane and action potentials. Perfect compensation of the electrode potential was possible by the

use of a high input impedance negative capacitance amplifier. The voltage clamp measurement method (VÉRÓ, 1974b) was used for the determination of ion currents.

The components of the physiologicl solutions used in our experiments are given in the following *Table*

		Normal solution	Na^+ free solution	Ca^{2+} solution
NaCl	(mM)	51	—	51
KCl	(mM)	4.6	4.6	4.6
$MgCl_2 \cdot 6H_2O$	(mM)	12.0	12.0	12.0
$CaCl_2 \cdot 2H_2O$	(mM)	10.0	10.0	—
$NaHCO_3$	(mM)	2.3	—	2.3
Tris-HCl	(mM)	—	53.3	22.0

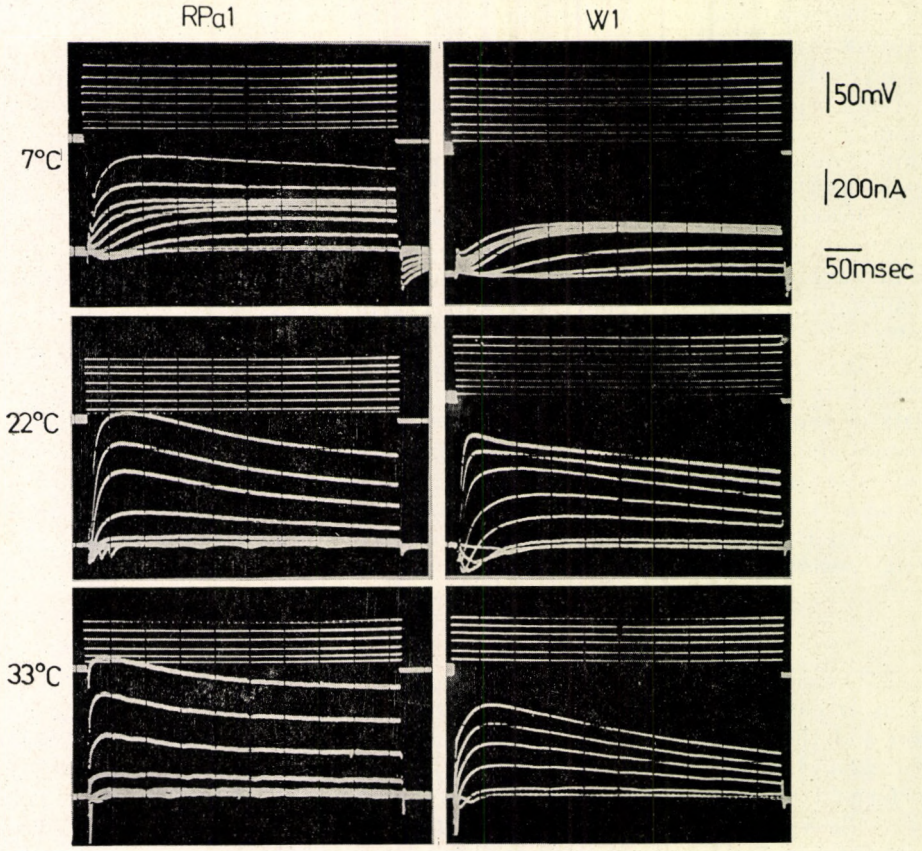

Fig. 1. Temperature effect on ion currents of PRa1 and W1 giant neurons

Results

1. Temperature effect on ion currents of RPal and Wl giant neurons

The ion current change of the RPa1 cell has been compared with the current change of the monomodal pacemaker neuron in the visceral ganglion, denoted by W1. *Fig. 1* shows the ion currents of cell RPa1 and W1 at different temperatures. It is seen that there is only a quantitative difference between the temperature dependences. The current changes due to temperature are adequately reflected by the votage-current characteristics plotted during the experiments. Results show that the highest inward current component of neuron RPa1 is 160 $\pm$ 10 nA at 22°C, 50 $\pm$ 11 nA at 7°C and 200 $\pm$ 20 nA at 33°C (see *Fig. 2*, left side). The outward current is first higher at the low temperature of 7°C than at 22°C and 33°C, but the derivative of the current-voltage characteristic shows a marked decrease for more positive voltages. For the W1 neuron, the highest value of the inward current is 190 $\pm$ 30 nA at 22°C, 33 $\pm$ 7 nA at 7°C and 220 $\pm$ 30 nA at 33°C. The outward current values are similar to those of the RPa1 neuron (*Fig. 2*, right side). *Fig. 3* shows the relative conductance change of the RPa1 neuron as a function of the membrane potential. It can be seen that the initial conductance for the outward current is high at 7°C, but the conductance is lower in the positive voltage range. The temperature dependence of the conductance for inward currents is not so marked.

The temperature change had not only effects on the value of the ion current but also on it's time dependence. *Fig. 1* shows clearly the inward current decay time increase at 7°C as compared with that at 22°C. At 33°C,

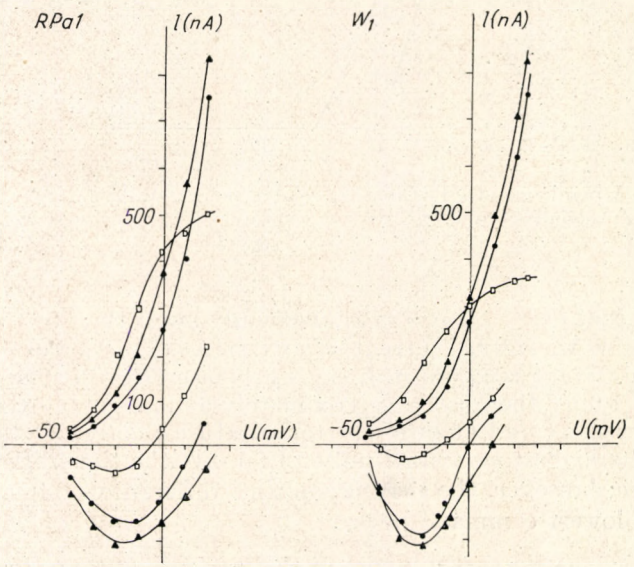

Fig. 2. Current-voltage characteristics of RPa1 and W1 neurons. □: 7°C, ○: 22°C, ▲: 33°C

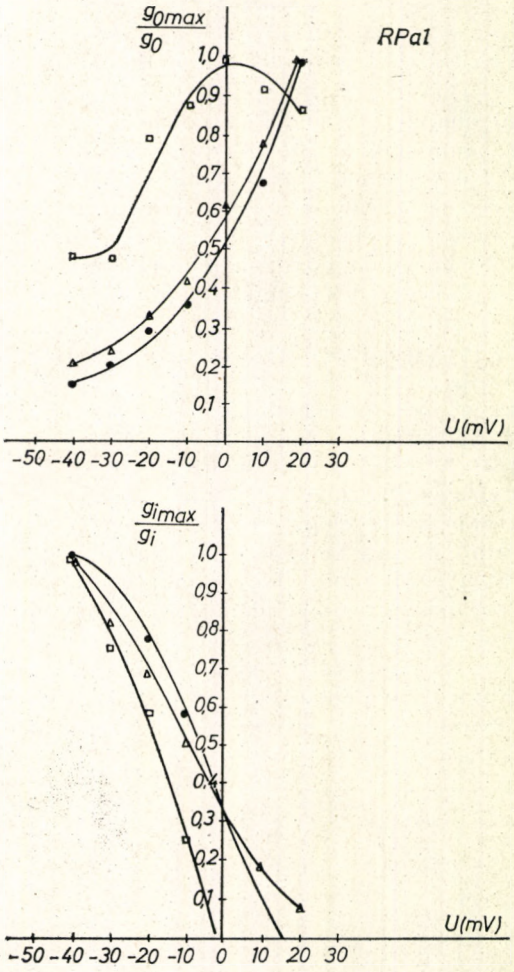

Fig. 3. Voltage dependence of RPa1 neuron relative conductances (g_i: Na^+ conductance, $g_{i\,max}$: highest Na^+ conductance, g_0: K^+ conductance, $g_{0\,max}$: highest K^+ conductance. □: 7°C, 0: 22°C, ▲: 33°C

the inward current decay time is substantionally lower than the decay time at 22°C. *Fig. 4* shows the inward current decay time as a function of the membrane potential for different temperatures. It can be seen that for the lowest command pulse of 10 mV there is an approximately 50 msec decay time difference between values measured at different temperatures, corresponding to a 50% change. For the highest command pulse of 90 mV, the time difference is only 2.5 . . . 3 msec; however, the percentage time difference is equal to that obtained for the lowest command pulse.

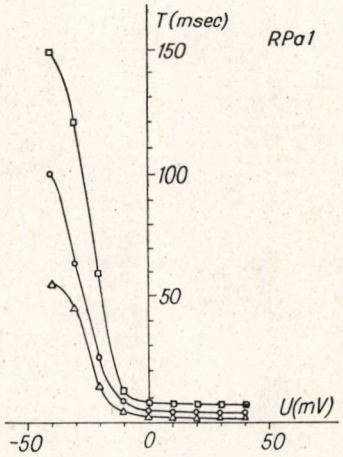

Fig. 4. RPa1 neuron inward current time durations as a function of membrane potential at different temperatures. □: 7°C, ○: 22°C, ▲: 33°C

2. Temperature effect on RPa1 neuron ion currents in Na^+-free and Ca^{2+}-free solutions

RPa1 neuron iron current data for physiological solutions having different ion contents have been reported in our previous investigations (SALÁNKI et al., 1975). Thus at 22°C, the inward current practically disappears in Na^+-free solutions, and shows a 30% decrease in Ca^{2+}-free solutions. The membrane current changes are identical to those obtained in normal physiological solutions; in this case, the control values have been those obtained with an ion-free solution at 22°C. Thus the inward current in the Na^+-free solution appears not even at 7°C. The inward current in the Ca^{2+}-free solution is decreased for lower temperatures and increased for higher temperatures by the same percentage as in normal physiological solution. In an ion-free solution, the temperature effect on the current time dependence will be the same as in normal solutions.

3. The effect of conditioning hyperpolarization on RPa1 neuron ion currents in normal physiological solutions

It is known that the outward current of some giant neurons has two components; a low delayed component which, though possessing some inactivation, is present during the whole period of the command pulse, and a fast component which precedes even the inward current and is characterized by complete inactivation; this fast component is produced by conditioning from a voltage level below the resting membrane potential (NEHER, 1971). This conditioning hyperpolarization has been investigated for the RPa1 neuron, and it was found that in the temperature range covered, the fast component is not present.

Further experiments have been carried out to investigate the slow outward current at different temperatures. A given constant voltage level

has been approached from different holding levels (conditioning hyperpolarizations). The initial conditioning value was equal to the membrane potential of −50 mV, the highest value was −150 mV, and the time duration was 250 msec. The outward current at 7°C showed an increase during the whole period of the command step, both with −50 mV and with −150 mV conditioning. At 22°C and with −50 mV hyperpolarization, the outward current attained it's steady state value in 50 msec. With higher hyperpolarization levels, a maximum appeared on the current-time plot. At 33°C, a maximum — though not so pronounced — was present even with −50 mV. This maximum tends to be more pronounced with higher conditioning, and the highest maximum value occurs with −150 mV hyperpolarization at t = 25 msec.

Fig. 5. shows the outward current maximum values and the approximately steady-state current values at the end of the command-step duration as a function of the holding level at different temperature. It is seen that at 7°C, the current value at the end of the command step duration represents simultaneously the highest value and the steady-state value. At 22°C, the highest deviation between the maximum and steady state current values (67%) is present with a holding level of −150 mV, but with −50 mV conditioning, the difference practically disappears. At 33°C, the highest deviation occurs at −150 mV conditioning (63%), while −50 mV will result in a current difference of less than 5%.

Discussion

It is known that the resting potential as determined from the equation of NERNST is temperature dependent. The potential difference effected by the Na^+ and K^+ pumps, which is also responsible for the generation of the resting potential, is temperature dependent too (GORMAN and MARMOR, 1970a; GORMAN and MARMOR, 1970b). The resting potential is further dependent on on the resting permeability of Na^+ and K^+ which are themselves temperature dependent parameters (MARCHIAFAVA, 1970; LIVENGOOD and KUSANO, 1972).

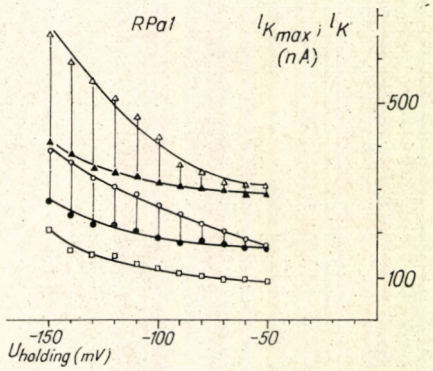

Fig. 5. PRA1 neuron outward current highest and steady state values at different temperatures as a function of the conditioning hyperpolarization.
□: 7°C, U_h = − 50 and − 150 mV; ●: 22°C, U_h = − 50 mV; ○: 22°C, U_h = −150 mV; ▲: 33°C, U_h = −50 mV; △: 33°C, U_h = −150 mV.

Thus the temperature effect on the membrane potential can also be verified indirectly. The membrane conductance in the case of different ions is proportional to the permeability (FRANKENHAUSER, 1963), so it can easily be concluded that the temperature dependence of ion currents is due to the change of conductance. Thus in the cases of RPa1 and W1 neurons too, the current decrease with decreasing temperature is caused by the decrease of Na^+ and K^+ conductance.

The temperature changes produced definite effects on the current function time parameters: at lower temperatures, the duration and the rise/fall times of the inward currents decreased. This implies that not only the Na^+ and K^+ conductance values are decreased but their time function is also changed. While the temperature effect on the conductance value is primarily determined by the highest time-independent conductance constant (HODGKIN and HUXLEY 1952), the temperature effect on the conductance time dependence is mainly effected by the membrane time constant change. The application of different conditioning hyperpolarizations showed that the activation and inactivation potential of the membrane is temperature dependent (MAGURA et al., personal communication). Our investigations showed that at 7°C, both the Na^+ and the K^+ activation is slow and nearly simultaneous. We believe that the current increase in time is primarily caused by the slow Na^+ activation. At higher temperatures, the Na^+ activation is substantially accelerated, and a significant increase of K^+ activity results in complete inactivation; thus the inactivation of the outward current is shown by the over-all current function. This proves that the temperature has different effects on the conductances of Na^+ and K^+; according to our investigations, Na^+ has a higher temperature sensitivity.

Our investigations also showed that the temperature dependence in case of an Na^+- and Ca^{2+}-free solution cannot be proved in this way. However, taking into account the role of these ions, especially that of Ca^{2+}, in the seasonal changes (BARKER and GAINER, 1973), the membrane properties are probably effected by the temperature dependent Ca^{2+} concentration in a manner which has not yet been cleared.

No specific feature of the RPa1 neuron which would distinguish this neuron showing a characteristic actvity pattern from other pacemaker neurons could be discovered. The RPa1 neuron parameters have a temperature dependence which is practically the same as that of other neurons with non-periodic activity patterns. The mechanism responsible for the Br-type activity pattern, though temperature dependent (SALÁNKI et al., 1973), cannot be determined by the methods outlined in this paper.

Summary

1. The magnitude of the inward current decreases, the duration of the inward current increases with temperature.

2. The outward current steady state value in the case of low command step values is highest at 7°C, and in the case of 50 mV and higher command steps, it is lowest at 7°C.

3. The K^+ conductance is decreased at low temperaturs in the case of command steps higher than 50 mV.

4. The conditioning hyperpolarization effect is temperature dependent; a temperature increase results in higher activation of both Na^+ and K^+, but they have a different decrease in their time durations. Thus at higher temperatures an increasing inactivation of the outward current can be ascertained.

5. The temperature dependence of currents measured with Na^+ and Ca^{2+} free solutions is not different from the temperature dependence of currents from the temperature dependence of currents measured with normal physiological solutions.

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HELIX POMATIA L. BR-TÍPUSÚ SEJTJE IONÁRAMAINAK HŐMÉRSÉKLETFÜGGÉSE

Vadász István és *Véró Mihály*

Összefoglalás

1. Hőmérséklet csökkenésekor a befelé irányuló áram nagysága csökken, időtartama nő.

2. A kifelé irányuló áram állandósult értéke kis feszültség ugrások esetén 7°C-on a legmagasabb, 50 mV-os és nagyobb kommand impulzusok esetén 7°C-on a legkisebb.

3. A K^+ vezetőképesség alacsony hőmérsékleten, 50 mV-nál nagyobb kommand impulzus esetén csökken.

4. Az előkondicionáló hiperpolarizáció hatása hőmérsékletfüggő; a hőmérséklet növelésekor mind a Na^+, mind a K^+ aktiváció mértéke nő, de itőtartamuk különböző mértékben csökken, így magasabb hőmérsékleten a kifelé irányuló áram növekvő inaktivációja figyelhető meg.

5. A Na^+ és Ca^{2+} ionok hiányában mért áramok hőmérsékletfüggése nem különbözik a normál fiziológiás oldatban tapasztalt hőmérsékletfüggéstől.