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INTRACELLULAR POTENTIALS DURING PROTRACTED RECORDING FROM THE NEURONES OF *HIRUDO MEDICINALIS*

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Studying the changes of resting potential during protracted leading is regarded to be of general importance in biology. SOLOGUB (1961) reported that the resting potential changes during protracted leading in such a way that it is comparable to the generation of the action potential. The initial steady level of resting potential is followed by a slow and then a fast depolarization. The only difference is that resting potential does not return to the initial level. Little is known about the changes in resting potential althought such information could contribute to the more exact understanding of cell functions. The changes of resting potential were investigated on the spinal ganglia and muscle fibers of the frog during protected leading (WOODBURY, 1958; KURELLA, 1959; SOLOGUB, 1961; 1965; CAREY and CONWAY, 1954; BOYLE and CONWAY, 1941).

The present study was undertaken to clear up the changes of the resting potential in *Hirudo medicinalis* giant neurones as well as the effect of the physiological solutions with different Na^+-concentrations during protracted leading.

Material and method

For the experiments the giant neurones of *Hirudo medicinalis* were used. These cells are situated on the dorsal surface of the large glia-bundle in the ganglia (COGGESHALL and FAWCETT, 1964). The registration of the resting potential was carried out from the cells of the 2nd—4th ganglia following the cerebral ganglion. After cutting the isolated ganglion was put to a small chamber (1.5 cm^3) supplied with perfusion. The physiological solution reported by NICHOLLS and KUFFLER (1965) was used. In Na^+-free solution the Na^+ ions were substituted by saccharose in an equimolar quantity. In the experiments glass microelectrodes filled with 2.5 M KCL were used their resistance varied between 10—40 MΩ. The experiments were performed at room temperature (20—24 °C).

Results

The resting potential was registered from 82 neurones, the mean value is indicated in *Table I*. As one can see from *Table I* the level of the resting

TABLE I

Mean value of initial resting potential in the neurones of Hirudo in different months (±SE)

Month	Number of neurons	Resting potential mV
March	16	41.5±2.3
April	36	30.5±0.9
May	30	37.5±1.0
Total	82	35.5±0.7

potentials measured by us was somehow lower than the values known from the literature (ECKERT, 1963; GERASIMOV and AKOEV, 1967;). The lowest levels were found in the month of April, while in May with some mV higher values resting potential were observed referring to seasonal fluctuations. According to the statistical analysis the difference was significant between the levels of resting potential measured in the months of April and May ($P = 0.01$).

The giant neurones of *Hirudo* are characterized with the high frequency of the action potentials, however, the interval between the single spikes is not constant. The magnitude of the action potential never exceeded the resting potential and its value was 22 ± 5.7 mV. The amplitude of action potential showed great variability even on the same ganglion. The duration of the action potentials was 10—20 msec.

After omission 50 per cent of the Na^+ ions from the physiological solution the amplitude of the action potentials was reduced in average by half of its initial value, so that it corresponded to 10 ± 2.9 mV. The complete deprivation of the Na^+ ions led to the elimination of the spike generation *(Fig. 1)*. The process was reversible as the original level of the resting and action potential reversed after returning to the normal saline.

In normal physiological solution the giant neurones of *Hirudo* maintained their activity for 40—210 minutes. The time required for decreasing of the

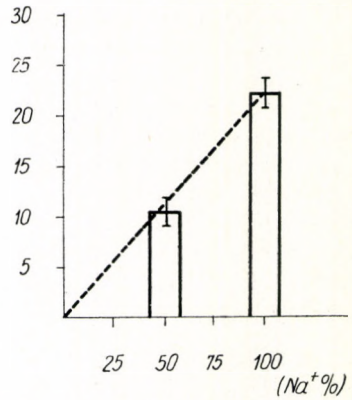

Fig. 1. Comparison of the amplitudes of action potentials in a solution containing different concentrations of Na ions

resting potential to zero depended on many factors. The changes of resting potential measured at the first 20 minutes are presented in *Table II*. The results are divided into three groups according to the resistance of the microelectrodes (17 ± 3, 24 ± 3.8 and 28 ± 5 MΩ, respectively) used for recording. Three types of the initial reaction were described: 1. the resting potential decreased, 2. the resting potential remained unaltered, 3. the resting potential increased.

TABLE II

Changes of the resting potential in the first 20 min of leading for three groups of neurones grouped according to the resistance of the microelectrodes (R_e) ($\pm$SE)

Groups of neurones	Number of neurones	R_e MΩ	Time of leading (min)						
			0	3	5	7	10	15	20
I	11	40—30	36.6 ±1.3	38.0 ±1.1	39.2 ±0.9	41.2 ±0.7	38.6 ±1.4	37.3 ±1.0	35.9 ±0.7
II	17	30—23	34.0 ±1.4	—	34.0 ±1.4	—	35.6 ±1.6	—	—
III	17	15—10	32.0 ±1.0	—	23.4 ±0.8	—	22.0 ±0.8		21.5 ±1.0

The initial hyperpolarization of the resting potential was observed in the cases when microelectrodes of high resistance (30—40 MΩ) were used. The characteristic phasic changes took place first of all at high resting potential leve *(Fig. 2)*.

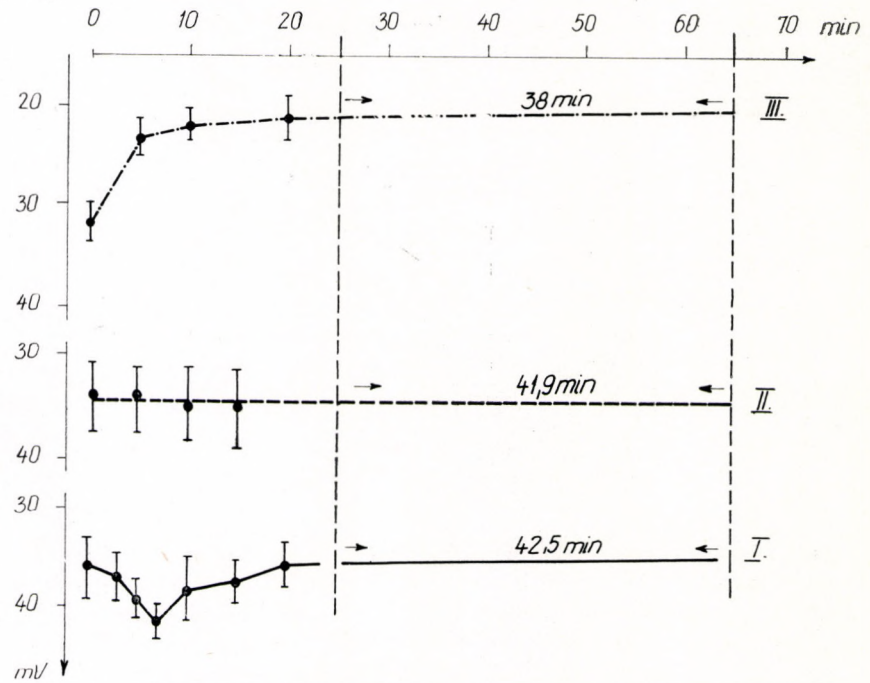

Fig. 2. Three variants of the initial alteration of resting potential and the stable phase

The values of resting potentials grouped according to the intial alteration can be seen in *(Figs 3, 4* and *5)*. In 82 per cent of all the cases the fast depolarization and in 18 per cent reversal of the resting potential were observed. The magnitude of the reversed potential was +2—7 mV. The low number

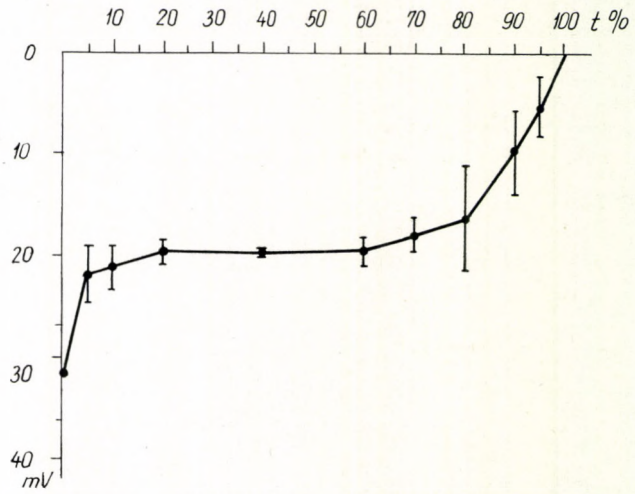

Fig. 3. Changes of resting potential level depending on the time of leading off. The whole period of leading was taken for 100 per cent (first group)

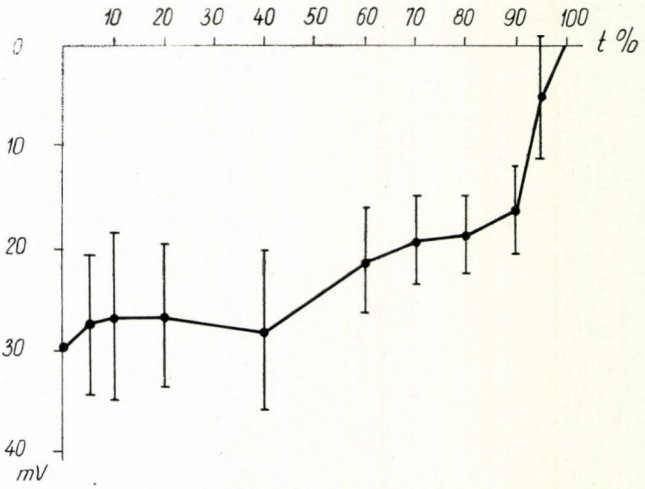

Fig. 4. Same as in *Fig. 3* for the second group of neurones

of the potential reversal probably bear relation to the fact that overshoot was rarely registered even in normal state, and even when the overshoot was present it reached only a few mV. Three characteristic curves of resting potential can be seen in *Fig. 6* originating from prolonged registration. On curve A the reversal of the action potential on the curve B the fast depolarization, while on curve C the slow depolarization phase can be observed.

The alterations of the resting potential were studied further in 50 per cent Na^+-deprivation *(Fig. 7)* as well as in Na^+-free solutions *(Fig. 8)*. In *Fig. 8* the hyperpolarization can be seen arising after the substitution of

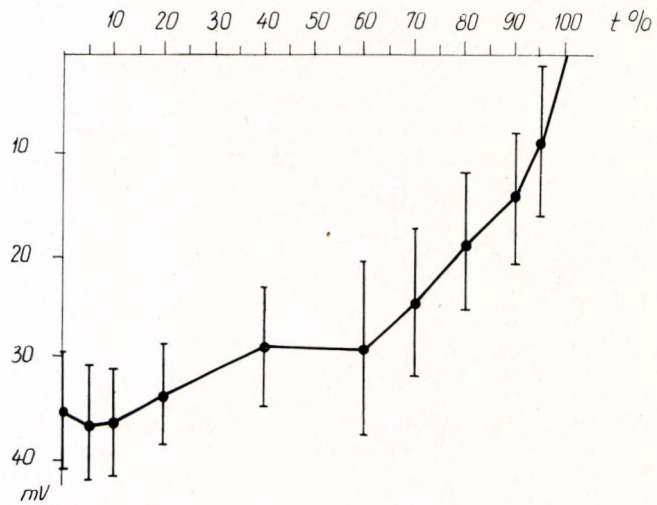

Fig. 5. Same as in *Fig. 3* for the third group of neurones

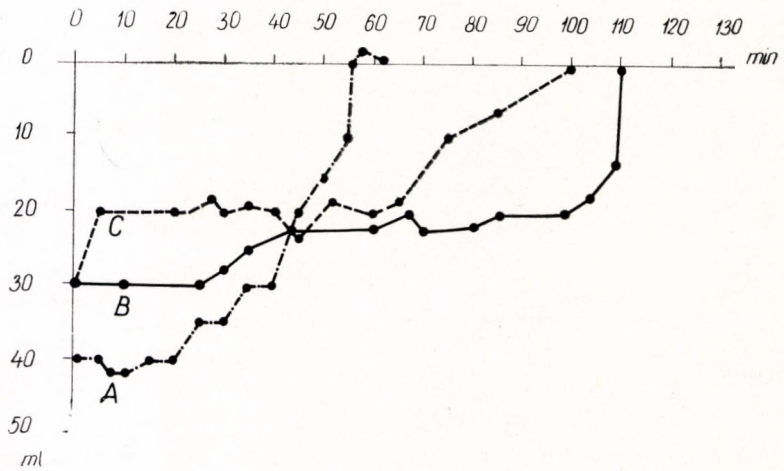

Fig. 6. Three characteristic curves of alteration of the resting potential

the solution (arrow), being by 12 per cent higher than the initial level. In the Na^+-free solution the fast depolarization phase of the resting potential was not observed. The resting potential dropped to zero through slow depolarization.

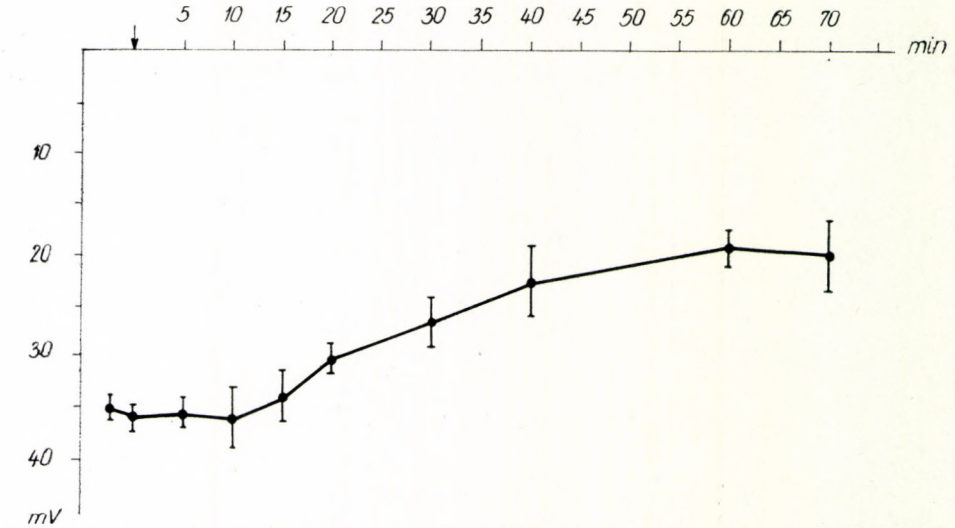

Fig. 7. Changes of the resting potential in the solution containing 50 per cent of the initial Na ions

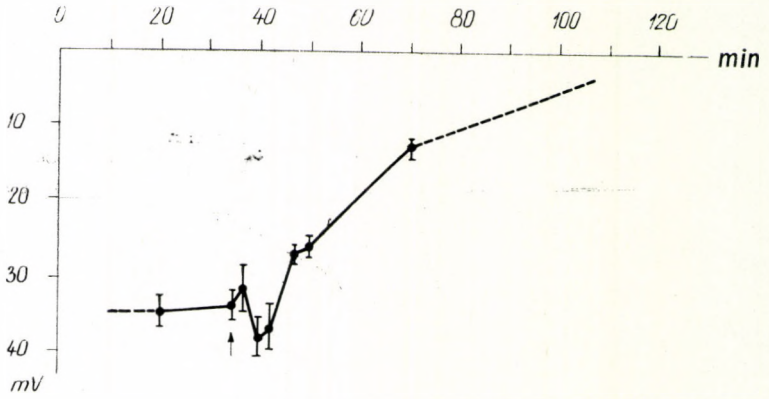

Fig. 8. Changes of the resting potential in Na^+-free solution

Discussion

On the basis of our and literary data it is supposed that the alteration of the resting potential during protracted leading and the generation of action potentials are based on the same ion mechanisms. According to earlier data (Sologub, 1965a) in the alteration of resting potential the following phases can be differentiated *(Fig. 9)*:

I. initial hyperpolarization of the resting potential,
II. stable level of the resting potential,
III. slow depolarization phase exceeding up to the critical level,
IV. fast phase of depolarization,
V. reversal phase of the resting potential.

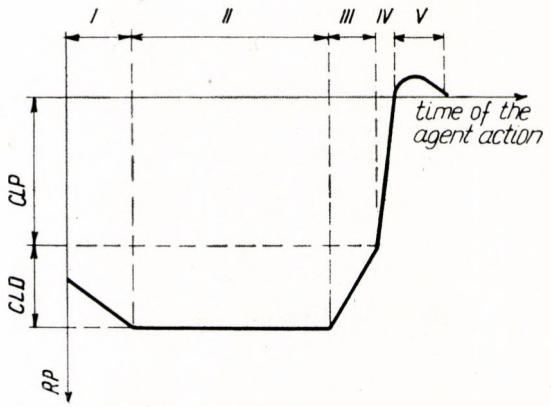

Fig. 9. Phases of the changes of resting potential (SOLOGUB, 1965a)

The first phase demonstrated in *Fig. 2* can have three different forms depending on the resistance of the electrodes, i.e. on the damage of the surface membrane. The hyperpolarization arising in this phase is ascribed to be activation of Na/K pump as well as to the increase of metabolism (WOODBURY, 1958). According to other authors (WEDENSKY, 1901) the phenomenon is connected with the positive trophic influence of the weak stimulation and the increase of the metabolism. At the second phase the ion gradient was not altered significantly. As usual this phase was the longest. The third phase is the phase of slow depolarization, which is a consequence of the Na influx into the cell. It is suggested that the Na influx is connected with the deterioration of the function of the Na pump and it refers to the exhaustion of the energy-reverses. Having reached the critical level of depolarization (15—20 mV) the resting potential alters suddenly (fast depolarization) and this process can be compared with the generation of the action potentials (fourth phase).

Our experiments proved the significant role of Na ions in the maintenance of the resting potential. Fast depolarization was observed only in the solution containing Na ions. Similar changes were not found in the Na^+-free medium. On the basis of the above data it is suggested that for the alteration of resting potential during protracted leading off the Na ions are responsible.

Both our results and literary data (WOODBURY, 1958; DRAPER et al. 1963) prove that the resting potential changes by the same way in different cells during protracted leading and also the alterations under the influences of outward environment are the same.

Summary

1. During protracted leading the resting and action potential of the giant neurones of *Hirudo medicinalis* were studied in normal physiological solution, in solution containing 50% of initial concentration of Na^+ ions and in Na^+-free solution.

2. The amplitude of the action potentials was lower than that of the resting potentials and proved to be in direct ratio to the extracellular Na

concentration. In Na-free solution the generation of the action potentials ceased. The action potentials of *Hirudo* neurones proved to be Na^+-dependent. Na ions play an active role also in setting the level of the resting potential.

3. Depending on the resistance of the microelectrodes three variants were established in the intial alteration of the resting potentials: initial increase, stable level and initial decrease. The initial hyperpolarization observed after penetrating the microelectrodes with high resistance into the cell was connected with the increases of the activity of Na/K pump and the intracellular metabbolism.

4. Fast depolarization of the resting potential and the reversal were observed only in the presence of Na ions. Thus the depolarization and the reversal are caused by the influx of Na ions at the end of the protracted leading.

5. It is suggested that the slow alterations of the resting potential and the fast changes leading to the generation of action potential are based on same ion mechanisms.

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A PIÓCA INTRACELLULÁRIS POTENCIÁLJÁNAK TANULMÁNYOZÁSA HOSSZÚ IDEJŰ ELVEZETÉS ALATT

Sologub M. I. és *Kiss Tibor*

Összefoglalás

1. Vizsgálták a pióca *(Hirudo medicinalis)* óriás neuronjainak nyugalmi és akciós potenciáljait hosszú idejű elvezetés alatt normál Ringer oldatban, 50% NaCl-t tartalmazó oldatban, valamint NaCl nélküli oldatban.

2. Az akciós potenciál amplitúdója kisebb a nyugalmi potenciálnál és egyenes arányban függ az extracelluláris Na koncentrációtól. Na-ion nélküli oldatban az AP generálása teljesen megszűnik. A pióca óriás neuron AP-i Na-ion függők, azonkívül a Na-ionok részt vesznek a RP szintjének meghatározásában is.

3. A mikroelektródák ellenállásától függően a RP kezdeti változásainak három variánsát állapították meg: kezdeti növekedés, stabil szint és kezdeti csökkenés. A nagyellenállású elektródák sejtbe való behatolását követő kezdeti hiperpolarizációt a metabolizmus és a Na—K pumpa aktivitásának növekedésével hozzák összefüggésbe.

4. A RP gyors depolarizációját és reverzióját csak Na-ionok jelenlétében mutatták ki. Tehát a gyors dolpearizációt és a reverziót a Na-ionok kiáramlása okozza a hosszú idejű elvezetés végén.

5. Feltételezik, hogy a RP lassú változásait, valamint az AP alatt végbemenő gyors változásokat azonos ion mechanizmus hozza létre.