ANNAL. BIOL. TIHANY	41	35–43	HUNGARIA 7974

THE Na-DEPENDENCE OF THE RESTING AND ACTION POTENTIAL IN THE GIANT NEURONES OF *LYMNAEA STAGNALIS* L.

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Received: 24th February, 1974

In the past years the role of Na^+ in giant neurones was investigated mostly from two aspects: *a)* its role in the generation of action potential, *b)* its role in the maintenance of resting potential.

The first question arose, when it was found in the central nervous system of some Gastropods that the action potential was maintained in Na^+-free solution for a long time. Some authors tried to explain this fact with a contribution of some other ions (Ca^{++}, Mg^{++}) (GERASIMOV et al., 1965; OOMURA et al., 1961; JERELOVA et al., 1972), others with an extracellular retention of Na^+ (KRASTS and VEPRINTZEV, 1972; MORETON, 1972).

The second problem emerges in the literature, whether the existence of an electrogenic Na-pump can be supported on a given object and if it can, in what degree does it contribute to the maintenance of the membrane potential (THOMAS, 1972), on the other hand, how large is the resting Na^+-permeability (HODGKIN and HUXLEY, 1952)

Regarding the giant neurones of *Lymnaea stagnalis*, a great mass of data has accumulated on the ionic mechanisms of the generation of potential. At least in one point these data agree, i. e. there are some neurones, which can maintain their action potential in Na^+-free solution for a long time (MAGURA et al., 1971; SATTELLE and LANE, 1972; KISS and SALÁNKI, 1973). However, up to now on the basis of their experiments only JERELOVA et al. (1972) have unequivocally expressed that on the examined neurones in the absence of Na^+ other ions, Ca^{++} and Mg^{++}, play a role in generating action potential. The other authors mentioned above believed in the presence of some kind of a Na^+-reservoire or restricted diffusion in the case of most of the neurones, nevertheless, they did not exclude the direct current-carrier role of Ca^{++} ions in the case of some of the neurones.

We wish to continue our examinations partly in this direction. It was supposed that if the Na^+-dependence of the potential generation of the identified cells really differs, then it should manifest itself in the blocking effect of TTX on the same neurones.

On the other hand, in one of our earlier works (KISS and SALÁNKI, 1973) we published such results, to the explanation of which the participation of an

electrogenic Na-pump in the maintenance of membrane potential was supposed. To confirm this hypothesis we performed some experiments with ouabain during the present work.

Method

Examinations were conducted on the identified giant neurones of the isolated central nervous system of *Lymnaea stagnalis* demonstrated on the scheme *(Fig. 1)* at room temperature. Membrane and action potentials were

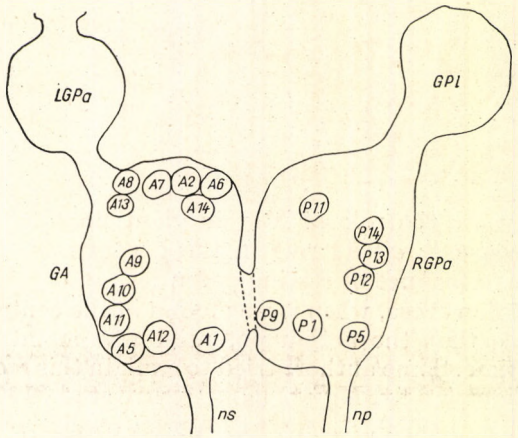

Fig. 1. Scheme of the abdominal and right parietal ganglion with the identified neurones. LGPa: left parietal ganglion; GA: abdominal ganglion; RGPa: right parietal ganglion; GPl: pleural ganglion; ns: nervus splanchnicus; np: nervus pallialis

recorded by glass microelectrodes filled with 2.5 M KCl. The membrane potential and its changes were measured after the compensation of tip potential of the electrode using a digital voltmeter. For the examinations we used an amplifier with FET input (Kiss et al., 1972). For injection of Na^+ to the intracellular space the "diffusion injection" technique was applied (Krnjevic et al., 1963). The good applicability of this method is supported by the data of Kerkut and Thomas (1964), as well as by Walker et al. (1971), according to which the K^+ and Cl^- outflow from a "leak" electrode with resistance lower than 5 MOhm displaced the equilibrium potential of the ACh-effect to a significant degree in one minute. The electrodes used by us for the injection were filled with 3 M NaCl and their resistance was 4—5 MOhm. At the preparation of Ringer and different ion-free solutions we proceeded according to an earlier description (Kiss and Salánki, 1973). Tetrodotoxin (TTX) and ouabain were dissolved in physiological solution and were applied in perfusion at a concentration 10^{-5}—10^{-4} M.

Results

I. Examination with TTX

In one of our earlier papers (KISS and SALÁNKI, 1973) three types of the identified neurones were distinguished on the basis of their sensitivity to the removal of extracellular Na^+. The activity of some cells ceased within 1—2 min (Al, A5 and P5), while that of the others ceased within 6—8 min. (All and P12), finally, there was a neurone whose activity was maintained for a long time (more than 30 min; Pl). For the purpose of the present experiments one representative of every type was chosen — Al, P12 and Pl neurone. On cells Al and P12 10^{-4} M TTX blocked the generation of spikes but did not affect that of cell Pl. If the perfusion was made with a concentration of 10^{-5} M, the effect may be characterized by a gradual row demonstrated in *Figs 2, 3* and *4*. On cell Al *(Fig. 2)* we may observe that after a quick (within 1 min)

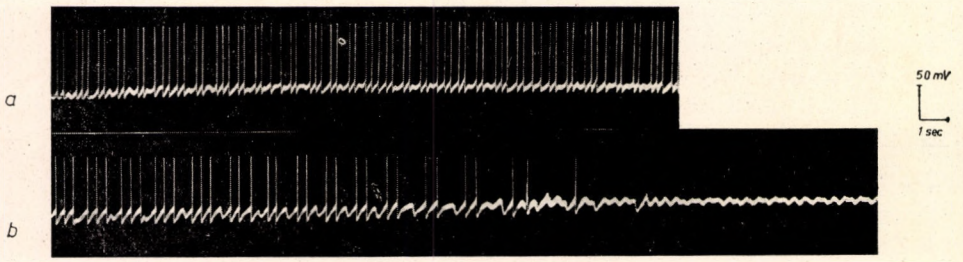

Fig. 2 a) Spontaneous activity of neurone Al *b)* 1/2 min. after the application of TTX. It may be seen that TTX inhibits within 1 min and after this IPSP-s are still maintained for a long time

blockade of the spontaneous activity IPSP-s are maintained for a long time. The blockade of activity is preceded by a diminution of the spike amplitude. On cell P12 the amplitude of the spike does not decrease so markedly, and the activity stops when the spike still has an overshoot. After this, the excitability of the cell membrane to an artificial depolarization is maintained for a long time, during which the amplitude of the evoked spike gradually diminishes, however, the overshoot is maintained (*Fig. 3*). Finally, on neurone Pl TTX is practically ineffective *(Fig. 4)*.

II. Examination with ouabain

The effect of 10^{-4} M ouabain causes no significant difference between the different identified neurones, so we give the mean of the effect measured on different cells. After the application of ouabain always gradually developing depolarization was observed, which reached its maximum value in the 3rd—4th min *(Fig. 5D)*.

In our earlier paper we drew attention to the fact that when recording was done by a microelectrode filled with NaCl, the K^+-free solution caused a greater hyperpolarization contrary to using a microelectrode filled with KCl (KISS and SALÁNKI, 1973). The greater hyperpolarization was explained by an electrogenic Na-pump-stimulating effect of a Na^+ outflow from the electrode. During the present work these measurements were completed by

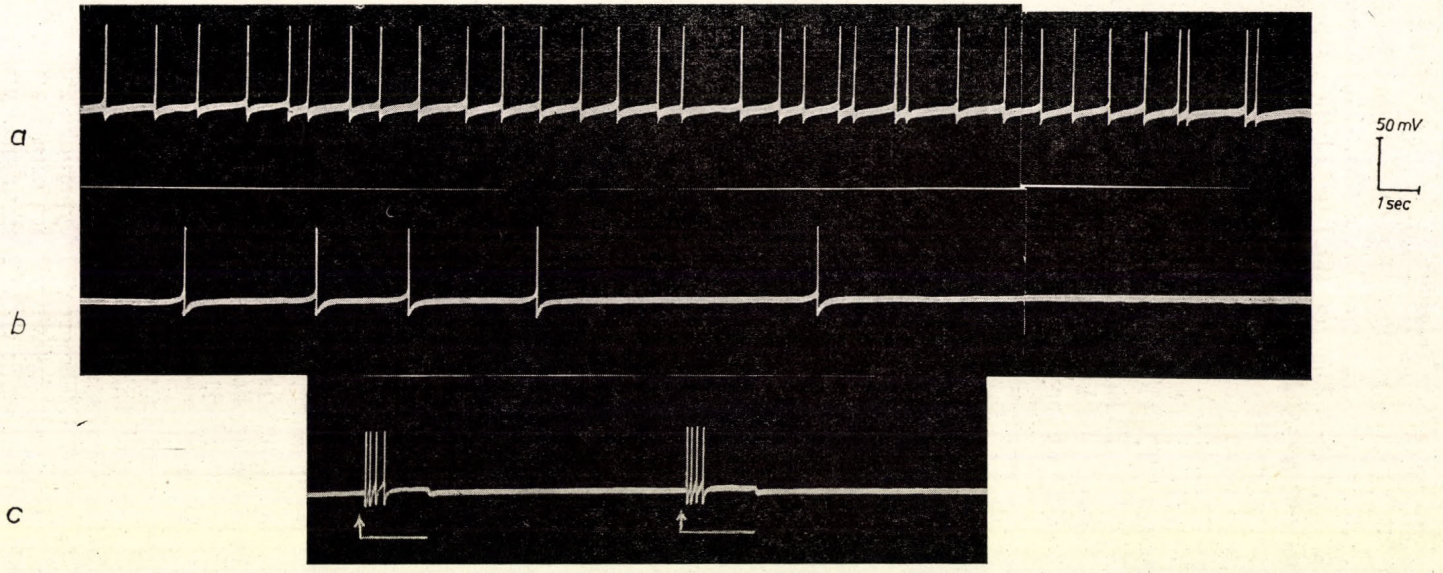

Fig. 3 a) Spontaneous activity of cell P12. *b)* Effect of TTX at the 4th min. *c)* Spike trains evoked by depolarization (↑) after 4 min. of TTX treatment

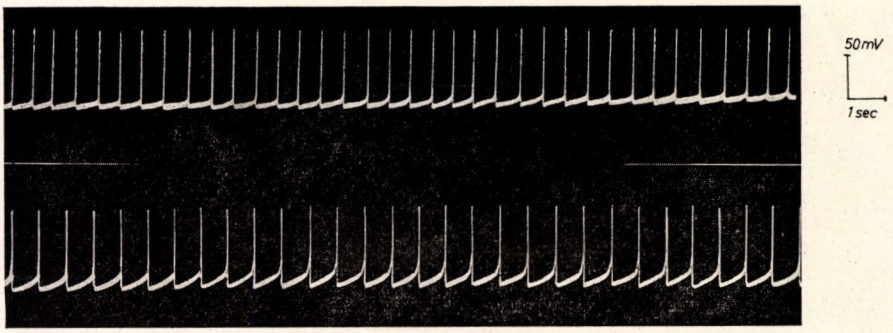

Fig. 4 a) Spontaneous activity of neurone P1 *b)* After TTX treatment lasting 20 min.

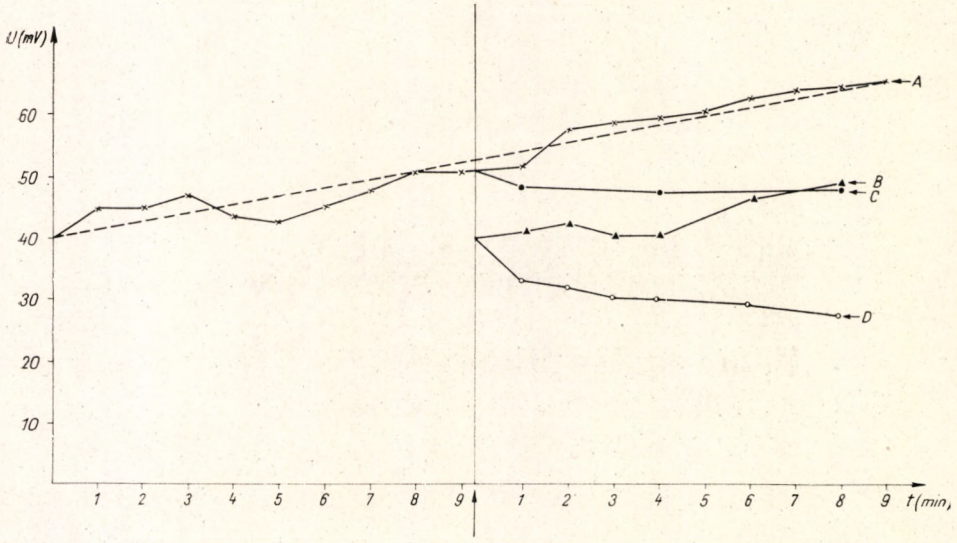

Fig. 5. Left: X — effect of electrode filled with NaCl on the membrane potential. *Right:* *A* — Simultaneous effect of the electrode filled with NaCl and the K^+-free Ringer; *B* — Recording in K^+-free Ringer with electrode filled with KCl; *C* — Simultaneous effect of the electrode filled with NaCl and the K^+-free Ringer containing ouabain. Dotted line is the approximating line of the curve, There is significant difference between the points of *A* and *C* curves and no significant difference between the points of the *B* and *C* curves; *D* — Effect of ouabain in normal Ringer using electrode filled with KCl

using ouabain. The electrode was kept in the cell for 10 min., while the change in the membrane potential was continuously registered *(Fig. 5)*. After 10 min the measurements were continued in two series. In the first case (A) the K^+-free Ringer, in the second case (C) K^+-free solution containing 10^{-4} M ouabain was tested.

As an effect of K^+-free Ringer, the hyperpolarization described earlier was obtained, however, the K^+-free solution containing 10^{-4} M ouabain contrarily caused a depolarization. There is no significant difference in K^+-free Ringer: either recording is done by an electrode filled with KCl, or by an electrode filled with NaCl in the presence of ouabain.

Regarding the Cl^--free Ringer, a hypothesis was described in our earlier work (KISS and SALÁNKI, 1973), according to which the burst activity developed here is a result of a depolarization caused by the absence of Cl^-, and consequently, an activation of an electrogenic Na-pump. This was controlled comparing the effect of Cl^--free Ringer and Cl^--free solution containing 10^{-4} M ouabain. For this investigation, neurones A10 and P1 were used, on which the Cl^--free solution caused the most marked changes (KISS and SALÁNKI, 1973). Also during the present investigations the regular rhythm became irregular in every case, in most of the cases a burst-like activity was obtained. After this

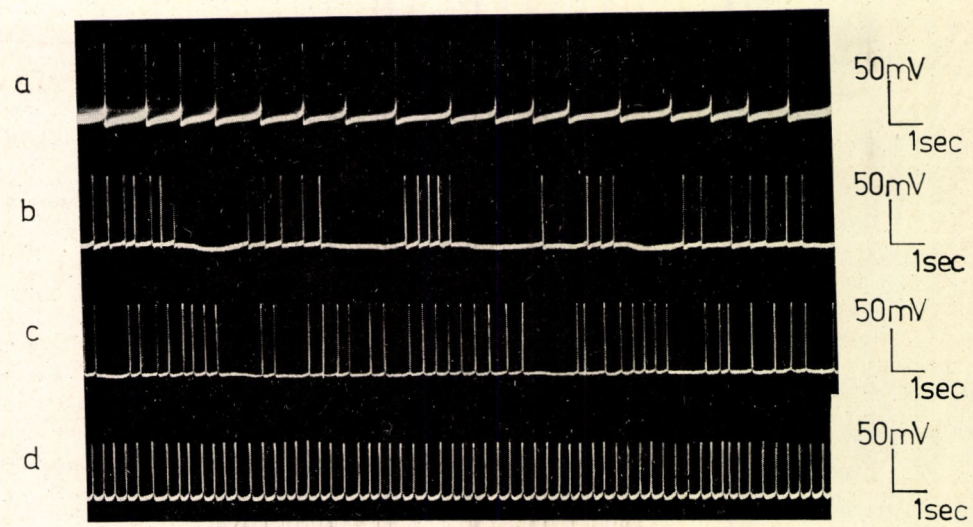

Fig. 6. Effect of ouabain in Cl^--free solution *a)* Control activity; *b)* Effect of Cl^--free solution; *c* and *d)* Effect of ouabain in Cl^--free solution at the first and the 4th min, respectively

perfusing with Cl^--free solution containing ouabain consequently the interburst hyperpolarization phases gradually became shorter, while in several minutes a continuous activity developed with a greater frequency than in the control *(Fig. 6.)*. Another type of reaction was obtained, when the normal Ringer was change at once to Cl^--free solution containing ouabain. In this case ouabain potentiates the depolarizing effect of the absence of Cl^-, and the activity after a temporary increase is inactivated at a depolarized level.

Discussion

Our results show that on the investigated neurones of *Lymnaea* the character of spontaneous activity is regulated in a complex manner by Na^+, K^+ and Cl^- concentration of the extra- and intracellular space. First of all, it may be established that regarding the generation of the pacemaker activity there are real differences among the identified neurones. This is shown by the coincidence of gradual rows which can be set up for the blocking effect of TTX and Na^+-free solution in the case of the three investigated neurones.

In the case of cell Al the action potential is obviously Na^+-dependent, since it is blocked in a very short time by Na^+-free solution and TTX. On the contrary, in the case of neurone Pl the very high resistance against TTX and Na^+-free solution suggests that the spike is much less Na^+-dependent. In this case the question of the current-carrier ion remains open henceforth, since in this cell we failed to confirm such a role of the Ca^{++}ion (KISS and SALÁNKI, 1973).

An essential new conclusion is obtained for cell P12: this neurone represents a third type between the above two extremes. Its action potential is Na^+-dependent, but besides some other ions (Ca^{++}, Mg^{++}) might play a role in the generation of the spike. The role of the latter two was found by JERELOVA et al. (1972) on other neurones of *Lymnaea*. The supposition of multiionic spike does not exclude that on the neurones of similar type in Na^+-free solution also passive or active Na^+ retention may occur, as MORETON (1972) and the present authors (KISS and SALÁNKI, 1973) considered probable, although SATTELLE and LANE (1972) contradict it on the basis of their histological observations.

The intracellular Na^+ concentration as well as the extracellular K^+ and Cl^- concentration affect the spontaneous activity mostly in an indirect manner through the control of the membrane potential. In our earlier paper we concluded that an electrogenic Na-pump may play an important role in the control of the membrane potential of the giant neurones of *Lymnaea*. However, this conclusion was drawn only in an indirect manner. During the present work the change in the membrane potential was registered continuously from the penetration with the electrode filled with NaCl, then the registration was continued in K^+-free solution. A surprising result was obtained that the K^+-free solution did not inhibit considerably the electrogenic mechanism: the rate of increase of the membrane potential remains the same, as was the case in the normal solution. In the literature the opinion has prevailed for a long time that the electrogenic Na-pump can be blocked by the removal of external K^+ (CARPENTER, 1970; THOMAS, 1972). However, contradictory data are also known, i. e. there is no inhibition, or its full development needs considerable time (AKIYAMA and GRUNDFEST, 1971). So the present result does not contradict every data of the literature. On the other hand, it is raised that the hyperpolarization obtained is the result of two effects, the electrogenic pump mechanism and the increase of K^+-concentration gradient, which effects are difficult to separate in the given case. However, the hyperpolarization caused directly by the absence of K^+ is not considerable, as it is known from SATTELLE and LANE's (1972) and our (KISS and SALÁNKI, 1973) work. The fact that if ouabain is added to the K^+-free Ringer the hyperpolarization does not develop, moreover, the membrane potential is slightly depolarized suggests the same. The amplitude of the last change does not depend on whether the recording is done by the electrodes filled with NaCl or KCl, this unequivocally suggests that the hyperpolarization obtained by the electrode filled with NaCl is a result of the electrogenic Na-pump stimulation.

Ouabain added to the normal Ringer caused much more depolarization (maximum 15 mV) while the same added to K^+-free Ringer the maximum depolarization was 6 mV. It is possible that here the increase in K^+ concentration gradient effects against the depolarization caused by the pump inhibition. Another explanation may be the anomalous K^+-dependence of the membrane potential (MARMOR and GORMAN, 1970; SATTELLE and LANE, 1972). According

to this at 0 extracellular K^+ concentration there is a much less difference between the measured membrane potential and that calculated by the GOLDMAN equation, than at a physiological K^+ concentration.

According to our earlier hypothesis concerning the effect of Cl^--free Ringer the depolarization, which occurs in the absence of Cl^-, activates the spike generation, but the activity of the electrogenic Na-pump increases during the time of the developed action potential train, which causes a hyperpolarization and accordingly a burst-like activity develops. The present results support this hypothesis, since ouabain fends off the development of the burst activity.

Summary

It has been established that there is a correlation between the different sensitivity of the neurones to the removal of Na^+ and to TTX.

Ouabain causes a depolarization in every cell, which may be resulted by a blockade of the electrogenic Na-pump. Further evidence of the existence of the electrogenic pump is the hyperpolarizing effect of Na^+ injected into the cell. Ouabain blocks this hyperpolarization. Applying K^+-free solution the absence of K^+ did not block considerably the electrogenic Na-pump within the interval investigated.

The burst activity developed in Cl^--free solution becomes a continuous activity under the influence of ouabain which supports the hypothesis that the electrogenic Na-pump may play a role in the development of burst activity.

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A NYUGALMI ÉS AKCIÓS POTENCIÁLOK Na-FÜGGÉSE *LYMNAEA STAGNALIS* L. ÓRIÁS NEURONJAIBAN

Kiss István és *Salánki János*

Összefoglalás

Megállapítást nyert, hogy a neuronok Na-elvonásra való eltérő érzékenysége és a TTX iránti érzékenység korrelációt mutat.

Ouabain minden sejten depolarizációt okoz, amely az elektrogén Na-pumpa blokkolásának eredménye lehet. További bizonyíték az elektrogén pumpa léte mellett a sejtbe injektált Na hiperpolarizáló hatása. Ouabain ezt a hiperpolarizációt blokkolja. K-mentes oldat alkalmazásakor a K-hiány a vizsgált intervallumon belül nem blokkolta számottevően az elektrogén Na-pumpát.

Cl-mentes oldatban kialakuló burst aktivitás ouabain hatására folyamatos aktivitássá alakul, ami alátámasztja azt a hipotézist, hogy a burst aktivitás kialakulásában az elektrogén Na-pumpának szerepe lehet.