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CONTRIBUTIONS TO THE MECHANISM OF ACTIVATING LIGHT CATION EFFECT ON GLOCHIDIA

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Adductor response of mussel larva (*Anodonta*-glochidium) was observed in the presence of alkali and alkaline earth metal ions. Previously, we (LÁBOS and SALÁNKI 1963) reported about the K^+ ion evoking an extremely long lasting rhythmic activity and have shown some features of Na^+, Li^+, Cs^+, Rb^+, Ca^{2+} and Mg^{2+}-e effects, too. Furthermore, the difference between K^+- and tryptamine-responses in the susceptibility to cholinotropic pharmacons and SH-inhibitors also was pointed out (LÁBOS and his co-workers 1964a, 1964b).

In the present paper we attempt to answer some of the problems of K^+-effect from a pharmacological aspect. Mainly the tonic response of larvae was examined. We focussed our attention on the following questions:

1. activity-sequence of ions and its changes,
2. ion-antagonisms and synergisms,
3. metabolic inhibitors and ion-effect,
4. heavy water isotope-effect.

Method and materials

In groups of mussel-larvae rhythmic contractions were counted and the number of closed glochidia was noted by a method described previously (LÁBOS and SALÁNKI 1963). In general, observations were compared originating from experiments with at least 100—100 glochidia. As different measures of effects the number of contractions accomplished by 100 larvae in 1 or 10 minutes (a/min, Σa), the ratio of closed glochidia after the application of the agents with t minutes (c_t) were used.

The applied substances were: KCl, NaCl, $CaCl_2$, $MgCl_2$, RbCl (BDH), CsCl (Fluka), LiCl (BDH), $SrCl_2$ (Merck), ouabain (Ph. Hg. IV.), 2,4-dinitrophenol, (2,4-DNP; Reanal), 2,4-DNP-hydrazine (Reanal; 2,4-DNPH), NaF, KCN, NaN_3 (BDH), digitonin (BDH), cetylpyridine-bromide (EGYT) papaverin-HCl (Chinoin), ethanol, parachloromercuri-benzoic acid (PCMB; Light), N-ethyl-maleimide (BDH; NEM), D_2O, NaSCN, veratrine-sulphate (Merck), histamine-diHCl (Light), guanidine-HCO_3 (Fluka), tryptamine-HCl (Fluka), serotonin-creatinine-sulphate (Fluka; 5-HT), dopamine-HCl (Fluka), d-tubocurarine-HCl (d-TC; Schuchardt), tetramethylammoniumiodide (BDH;

TMA), tetraethylammoniumchloride (BDH; TEAC), tetra-n-butylammoniumiodide (BDH; TnBA). Experiments were carried out on about 30,000 glochidia. The concentrations refer to the salts.

1. The order of activity of alkali metal ions

The sequence of the effect concerning the dose-effect curves of rhythmic activity *(Fig. 1)* is the following:

$$Rb^+ > K^+ > Cs^+ > Li^+ > Na^+$$

but the order concerning the peak-values and average of the mentioned curves is different:

$$K^+, Cs^+ > Rb^+ > Li^+, \quad Na^+$$

The rhythmic activity was characterized by the amount of reversible contractions performed by a given duration (10 min). There is a maximum site on the dose-effect curves owing to the tonic closure occurring at high concentrations. The K^+ and Cs^+ curves are striking, but the curves of Rb^+, Na^+ and Li^+ run through lower values.

The tonic closure was characterized by the ratio of closed animals in a given time after the application of a given concentration (1—100 mM). The activity-sequence mainly depends on these two factors. For low concentrations and short durations the

$$Rb^+ > K^+ > Cs^+, \quad Na^+, \quad Li$$

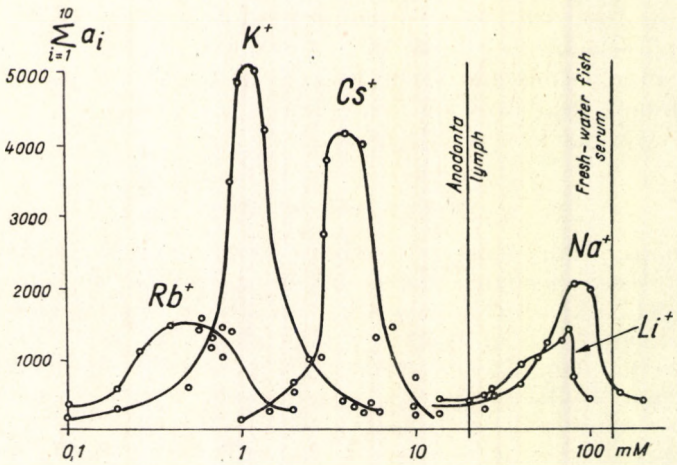

Fig. 1. Dose-effect curves of alkali metal ion chlorides. *Abscissa:* concentration in mM/lit. *Ordinate:* number of contractions of 100 animals in 10 min (each point represents result originating from observations on 100 animals). Vertical lines mark the osmotic pressure of *Anodonta* lympha and host-fish-blood. Populations from November

1. ábra. Dózishatás görbék alkáli fémkloridok jelenlétében. *Abszcissza:* koncentráció mM/lit-ben. *Ordináta:* a 10 perc alatt teljesített ritmikus kontrakciók száma 100 állatból álló csoportban (minden pont 100 állaton végzett megfigyelés eredménye). A függőleges vonalak az anyai limfa, ill. a gazdahalak vérének ozmotikus nyomását jelentik. Novemberi populációk

sequence is typical. At such conditions Na^+ and Li^+ do not cause contracture and for this reason the order cannot be demonstrated.

In solutions of higher concentrations of all the alkali metal ions and after a longer time a secondary relaxation is produced. The secondary attribute is used to distinguish from the relaxation following the phasic contractions. It is chiefly expressed in the case of Li^+, then Na^+, K^+, Rb^+, and Cs^+ in the following. The order observable in 50 mM solutions and after 120 min is partly the consequence of this fact:

$$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$$

The relation of Li^+ and Na^+ in the 70—100 mM range of concentrations firstly is $Li^+ > Na^+$, then it becomes reversed *(Fig. 2)*. For this reason the curves of different ions cross each other whether the ratio of closed larvae is plotted against time or concentration. Thus, there are the following crosses: $K^+ - Cs^+$, $Rb^+ - Cs^+$, $Na^+ - Li^+$. The crosses can be observed at higher values of time and concentration carrying out measurements at increasing values of concentration and time, respectively. For example in an experiment in November the times of $K^+ - Cs^+$ inversion in 1—1.5—2 mM solutions are 46, 20 and 15 minutes. All inversions are not concerned to the secondary relaxation. In general after the critical time and concentration values the effect may

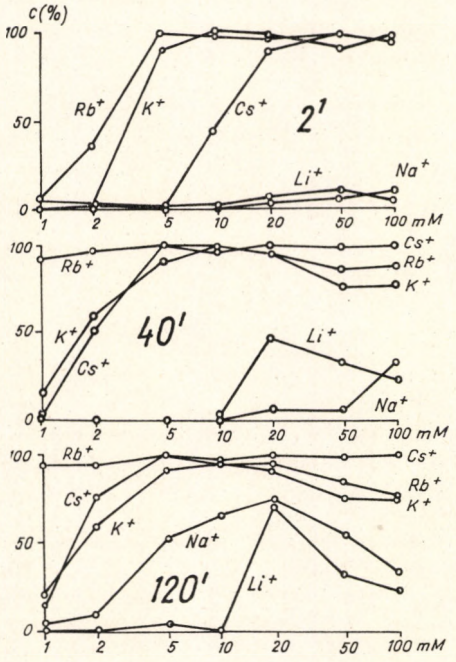

Fig. 2. Tonic effect of alkali chlorides. *Abscissa:* concentration in mM/lit. *Ordinate:* ratio of closed larvae after 2, 40 and 120 min of the salt application. Populations from April

2. ábra. Alkáli fémkloridok tónus-kiváltó hatása. *Abszcissza:* koncentráció mM/literben. *Ordináta:* tónusarány az anyag adását követő adott időpontokban (2, 40, 120 min). Áprilisi populációk

increase in the case of K^+ and Cs^+. However, the $Rb^+ - Cs^+$ and $Na^+ - Li^+$ inversions are related to the secondary relaxation. The course of curves of tonus-ratio and the site of inversions is varying in different populations, which are discussed as concerned to the susceptibility changes.

Summarizing the observed sequences:

$$Rb^+ > K^+ > Cs^+ > Na^+,\ Li^+$$

$$Rb^+ > Cs^+ > K^+ > Na^+,\ Li^+$$

$$Cs^+ > Rb^+ > K^+ > Li^+ > Na^+$$

$$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$$

One can observe that the Li^+ and Cs^+ anomaly appear under different conditions.

2. The sequence of the effect of alkaline earth metal ions

The order of ability to evoke the rhythmic action among the alkaline earth metal ions is: $Ba^{2+} > Sr^{2+} > Mg^{2+} > Ca^{2+}$. The capacity to cause tonus has a different sequence: $Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$. In 60 min 50 per cent of the total tonus can be achieved by 70 μM $BaCl_2$ *(Fig. 4)*, 50 mM $SrCl_2$, 50 mM $CaCl_2$; for $MgCl_2$ this concentration is significantly higher than 100 mM. Tonus evoking capacity of Ca^{2+} and Sr^{2+} is very close to each other. Usually the two curves are intersected by each other *(Fig. 3)*.

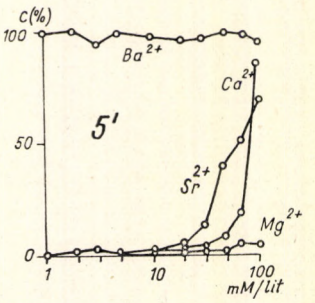

Fig. 3. Tonic effect of alkaline earth metal ions plotted against concentration; (c_5) values
3. ábra. Alkáli földfémionok tónuskiváltó hatásának (c_5) koncentrációfüggése

The initial activation described for $MgCl_2$ (LÁBOS and SALÁNKI 1963) was not observed in the case of $CaCl_2$, $SrCl_2$, $BaCl_2$. According to an experiment carried out on 100 animals 100 mM $MgCl_2$ caused an initial closure lasting for 46 $\pm$25 sec.

3. Antagonistic and synergetic effect of cations

The glochidia can adapt themselves to a wide range of osmotic pressure (fresh water 0.05 atm; maternal lymph 0.5 atm; serum of fishes 3.5 atm) and ionic composition of the medium. They are in closed state in the fish-blood,

but in opened state in the mussel lymph continuously. These facts give reason to examine the effect of ion-mixtures. The activator ion obtained from the K^+, Rb^+, Cs^+ Ba^{2+} group, the inhibitor is in the Na^+, Li^+, Mg^{2+}, Ba^{2+} group.

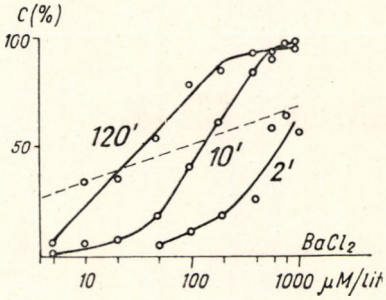

Fig. 4. Tonus-ratio in 5—1000 µM/lit $BaCl_2$ after 2, 10 and 120 minutes. *Abscissa:* concentration in µM/lit. *Ordinate:* tonus-ratio in per cent. The effect of each concentration was observed on 100 animals

4. ábra. $BaCl_2$ 5—1000 µM/lit. koncentrációinak tónuskiváltó hatása az adást követő 2., 10. és 120. percben. *Abszcissza:* koncentráció µM/lit.-ben. *Ordináta:* tónusarány %-ban. Minden koncentráció hatását 100 állaton vizsgáltuk

3—1. K^+/Na^+ and related antagonisms

The contracture inhibiting effect of Na^+ and Li^+ may demonstrate in an experiment of 120 min if a not too concentrated KCl solution is used causing tonus alone, too. The effect depends on the ratio of counter-ion-concentrations and also on the absolute quantities. Thus at 53 mM NaCl concentration the Na^+/K^+ ratio was changed between 0.1—1.8. The maximal relaxation was reached at $Na^+/K^+ = 0.76$. In such a case the effective range of Na^+ concentration is very closed.

In less concentrated KCl to achieve an approximately equal inhibition 10—25 mM NaCl was needed. In such a case the Na^+/K^+ ratio must be above 10. Thus at conditions 3 mM KCl and $Na^+/K^+ = 2.2$ is not significant inhibition; in 2 mM KCl and at $Na^+/K^+ = 11.2$ the K^+, Rb^+ and Cs^+-tonus can be inhibited markedly *(Fig. 5)*. The inhibitory effect of NaCl has a transitional phase of 2—10 min, then the inhibition becomes constant.

At the same concentrations and ratios there is an order of counter-ion-pairs according to which the inhibition increases. Thus in the case of *Fig. 5.*

ion-pair	Rb^+/Na^+,	Rb^+/Li^+ >	Cs^+/Na^+,	Cs^+/Li^+	K^+/Li^+ >	K^+/Na^+
inhibition	6%	13%	42%	35%	45%	84%

3—2. K^+/Ca^{2+} and K^+/Mg^{2+} antagonism

Similarly to the previous cases the antagonism can be observed only if the inhibitor ion concentration is not too high and it will not take part in a synergism. The tonus evoked by KCl of 2 mM is half-inhibited in 1 min by 3—3.5 mM, in 120 mM min by 1—1.5 mM $CaCl_2$. The marked quickness of $CaCl_2$-effect is remarkable. It is stronger than that of NaCl. Using 100—100

glochidia and 2 mM KCl then concentrations of $CaCl_2$ were applied. The inhibition is increasing along an S-shaped curve. The linear stretch of it can be approached. The percentage inhibition at 1 and 120 min can be expressed by the following regression-equations: $i_1 = 84-64$ lg $[Ca^{2+}]$ and $i_{120} = 55-80$ lg $[Ca^{2+}]$. The $CaCl_2$ concentration is given in mM/lit.

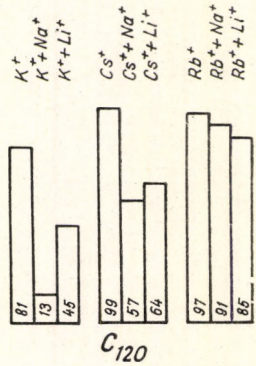

Fig. 5. Tonus-ratio values after 120 min. Each column represents observations on 100 animals. Concentrations: KCl, RbCl, CsCl 1,5 mM; NaCl 22.5 mM

5. ábra. Tónusarány a 120. percben. Minden oszlop 100 állat megfigyelése alapján készült. KCl, RbCl, CsCl 1,5 mM; NaCl 22,5 mM

At the same conditions 3—5 times higher concentrations are necessary from $MgCl^2$ than from $CaCl_2$ in order to obtain equal effect. The observation at the 120th minute shows that 5 mM $MgCl_2$ is equipotent with 1—1.5 mM $CaCl_2$.

Summarizing the ion-ratios inhibiting half the 2 mM KCl-effect we get: $Na^+/K^+ = 6.8$; $Ca^{2+}/K^+ = 0.5-0.8$; $Mg^{2+}/K^+ = 2-3$.

3—3. Synergisms among the cations

Using some of the cation combinations in which ions with similar effect (K^+, Rb^+, Cs^+, Ba^{2+}) are mixed appear real potentiations. However the similarity is only a necessary but is not a sufficient condition of the synergism.

In order to demonstrate the phenomena we applied concentrations of ineffective or of partial effect. The combinations of 20 μM $BaCl_2$, 200 μM RbCl, 500 μM CsCl and 500 μM KCl were used. In the given populations these concentrations show nearly equal effect. The total equipotentia can be hardly realized, thus the conclusions are semiquantitative. The sum of the effect of different ions compared to the effect of combinations generally proved to be higher than 1:

Rb^+/Cs^+	K^+/Rb^+	K^+/Cs^+	K^+/Ba^{2+}	Ba^{2+}/Cs^+	Ba^{2+}/Rb^+
3.92	3.02	2.16	1.18	0.76	0.55

The effect is the average tonus-ratio at the first 120 min.

4. The action of metabolic inhibitors on the K^+-tonus

20—25 μg/ml ouabain, 2,4-DNP, 2,4-DNPH are not effective. NaF in 100 μg/ml concentration has a small effect.

NaN_3 and KCN in suitable concentrations produce significant effect. The pure effect of KCN is disturbed in high concentrations mainly by the alkalic hydrolysis and not by the presence of K^+. The application of buffer solutions also has a disturbing effect. Thus the KCN-action was evaluated only in low concentrations. 57—67 per cent contracture was produced in 1.5 mM KCl after 1—120 min. The development of the contracture was observed without and with 15—300 μM KCN *(Fig. 6)*. It seemed to be typical for the KCN-effect

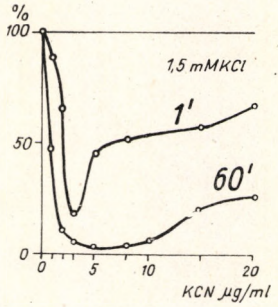

Fig. 6. KCN-effect. *Abscissa:* KCN-concentration. *Ordinate:* tonus-ratio expressed by per cent of cyanide-free control. Each concentration was tested on 100 animals

6. ábra. KCN-hatás. *Abszcissza:* KCN-koncentráció. *Ordináta:* tónusarány a $CN^=$mentes kontroll %-ában kifejezve. 100—100 állat koncentrációnként

that at 5 μg/ml (75 μM/lit) concentration there is an almost total inhibition after 60 min. The inhibitory effect in lower and higher concentrations is the smallest; half inhibition is at 20 and 225 μM/lit KCN. At low concentrations (15 μM KCN) sometimes an increase of tonus was observed. The KCN-effect is significant already in the 10th min, moreover, after 1—1.5 min develops a steady state. Thus 80—85 per cent steady state inhibition is produced by 45—120 μM/lit KCN. At 2 mM KCl concentration only 40 per cent inhibition was observed. To clarify the significance of the KCl concentration a dose-effect curve of KCl was produced without and with 75 μM/KCN between 0.5 and 2.5 mM KCl *(Fig. 7)*. The curve is shifted by the KCN parallel to the direction of higher concentrations. The shift increases between 1 and 30 minutes. Simultaneously the parallellism of the curves grew worse.

Depending on the KCl and NaN_3 concentrations potentiation and inhibition of the KCl-effect could be observed *(Fig. 8)*.

Applying 20—70 mM KCl concentrations which cause secondary relaxation 50—100 μg/ml NaN_3 decreases this relaxation thus the level of the tonus was higher (110—270%) than in the control. This potentiated tonus was inhibited by 0.1 mM $CdCl_2$ even below that of the control.

The necessary condition of tonus-inhibition with NaN_3 is the sufficiently small KCl concentration. Thus the tonus evoked by 1—2 mM KCl may be inhibited by 50—100 μg/ml NaN_3. Consequently, there must be a KCl concen-

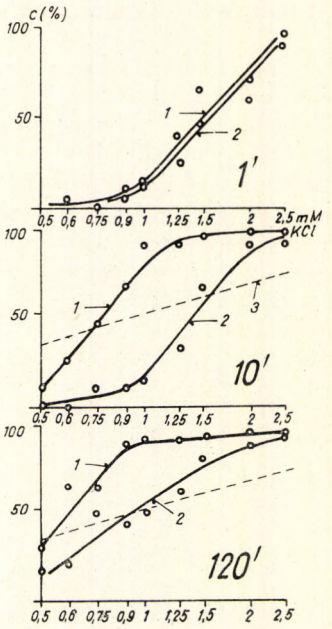

Fig. 7. KCN-effect. *Abscissa:* KCl-concentration. *Ordinate:* c_t (%) value 1—0 μM, 2—75 μM KCN. Each KCl concentration was tested on 100—100 glochidia

7. ábra. KCN hatás. *Abszcissza:* KCl koncentráció, *ordináta:* c_t(%) értéke 1—0 μM, 2—75 μM KCN. 100 glochidium minden KCl koncentrációnál

tration at which the tonus cannot be inhibited by a given concentration of NaN_3.

Tonus caused with 1.5 mM CsCl and 0.6 mM RbCl is also blockable by NaN_3 at similar conditions to those mentioned above.

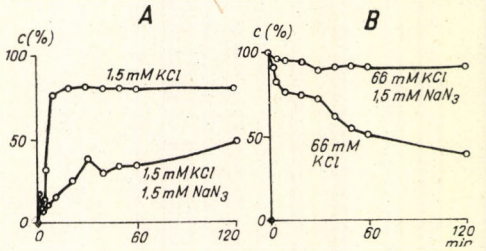

Fig. 8. Inhibitory (A) and potentiating (B) effect of NaN_3 on K^+-tonus. *Abscissa:* time. *Ordinate:* tonus-ratio. 100—100 animals for each solution

8. ábra. A NaN_3 K-tónus-gátló (A) és potencírozó (B) hatása. *Abszcissza:* idő, *ordináta:* tónusarány. 100—100 állat oldatonként

5. The action of surface active agents on the potassium tonus

Digitonin alone perceptibly increases the change of contracture already in 1 μg/ml (0.8 μM/lit) concentration. After 120 min, with the application of this agent, the ratio of closed larvae is about 10%. In spite of this it inhibits the tonus evoked by 1 mM KCl already in very low concentration. It seems to be evident that similarly to some other tonus evoking agents the inhibition of tonus can be realized only at low concentrations. We have observed half-inhibition in the 60th min with 0.4—0.8 μM/lit digitonin. There is a moment (between 5 and 10 min) at which the inhibition of 1 mM KCl-effect is at its maximum (95%).

Cetyl-pyridine-bromide (CPB) alone already in 0.5—1 μg/ml concentration increases perceptibly the ratio of closed animals after 30 min. Similarly to digitonin CPB also can block the K^+-tonus in this low concentration. But the inhibition reaches 50% only in the first 10 minutes then the steady state value is only 10—25%.

In low concentration ethanol also blocks the KCl-tonus. The measure of this inhibition at nearly steady state conditions, i.e. after 60—120 min is 35—60 %. This effective range of ethanol concentration is very narrow because the known tonus evoking action appears.

0.2—0.4 mM papaverin practically entirely overthrows the 100 per cent tonus evoked by 2 mM KCl

6. The effect of SH-inhibitors

Owing to the bad solubility of PCMB in water the combination of KCl and PCMB without Na^+ can hardly be realized because NaOH must be used. In such a case Na^+ disturbs the KCl-effect.

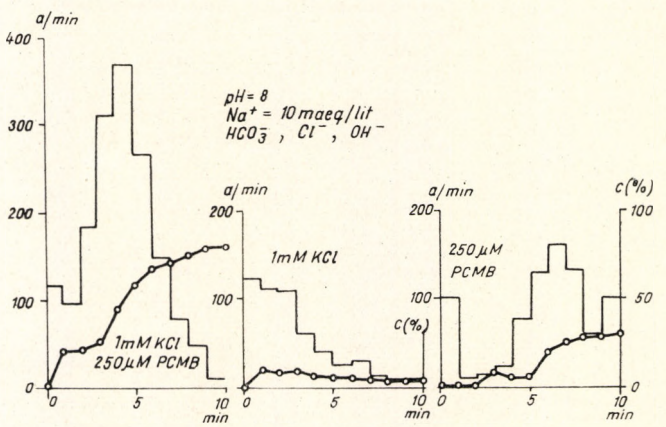

Fig. 9. PCMB-effect. *Abscissa:* time in minutes. *Ordinates:* tonus-ratio, c (%) and frequency of rhythmic activity, a/min resp. 100—100 animals

9. ábra. Paraklórmerkuribenzoát (PCMB) hatása. *Abszcissza:* idő, *ordináták:* tónus-arány c(%), illetve a ritmikus aktivitás frekvenciája (a/min). 100—100 állat

PCMB alone possesses activity increasing and tonus evoking effects. Applying 250 μM PCMB together with 1 mM KCl in the presence of Na^+ ions we observed activity of high frequency and tonic closure. One part of this originates from the own effect of PCMB but there is a real potentiation, too *(Fig. 9)*. A possible alternative is still the protection against the antagonistic effect of Na^+.

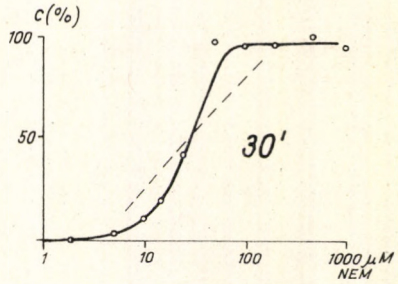

Fig. 10. NEM-effect. *Abscissa:* concentration. *Ordinate:* tonus-ratio. 100—100 animals for each concentration

10. ábra. N-etilmaleimid hatása. *Abszcissza:* koncentráció, *ordináták:* tónusarány. 100—100 állat koncentrációnként

As NEM at neutral pH is a water-soluble SH-inhibitor the K^+-effect can be well examined in a Na^+-free medium. The tonus increases itself already at 5 μM. 8—10 μM in 120 min application evokes closure of 50% of glochidia. In an experiment carried out on 500 glochidia 1 μM NEM does not influence the tonus evoked by 1 mM KCl. 40 μM NEM alone elicits a rhythmic activity of high frequency. The curve of tonic effect is S-shaped *(Fig. 10)*. The last concentration markedly increases the potassium-tonus. The rhythm in the mixture in the first two minutes is pronounced then concerned with the closing it is damped *(Fig. 11)*.

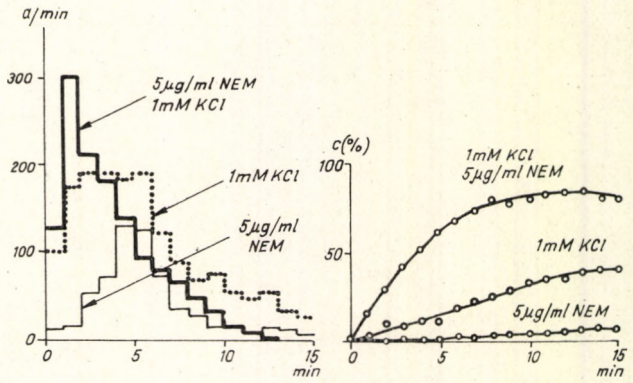

Fig. 11. Potentiation of K^+-effect by NEM. Marks see in *Fig. 9*

11. ábra. A K^+-hatás potencírozása NEM-del. Jelölés mint a *9. ábrán*

7. The effect of D_2O on the Li^+, Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+} and Ba^{2+} response of glochidia

The effect of heavy water was examined in a 50—50% mixture of H_2O and D_2O. This solvent resulted in a tonus ratio considerably smaller than 2% after two hours.

D_2O significantly depresses the action of 1 mM KCl *(Fig. 12)*. Both K^+-rhythm and K^+-tonus is touched by the inhibitory effect. The degree of inhibition depends also on the actual response of the population to 1 mM KCl. Thus the inhibition increased with decreasing KCl reaction. Thus it is evident that the response elicited by a concentration of higher than 3 mM cannot be blocked. Some characteristic data for the rhythm-inhibition: in 10 min at 1 mM KCl 1134 or 600 reversible contractions were produced in natural or heavy water, respectively. The effect of the remaining activator cations (Cs^+, Rb^+, Ba^{2+}) also is inhibited by the D_2O. The time needed to 50% closing is doubled by 0.5 mM RbCl, 1 mM CaCl and 200 μM $BaCl_2$ *(Fig. 12)*.

The change of tonus evoked by 25—100 mM LiCl, NaCl, $MgCl_2$, $CaCl_2$ and $SrCl_2$ in heavy water of 50 per cent is essentially different. Opposite to the inhibition of activator ion-effects a strong potentiation of tonus was observed proceded by inhibition lasting for a short time *(Fig. 13)*. The biphasicity is the most evident in case of LiCl. The surplus of tonus is most pronounced after 20 min.

The K^+-specificity in D_2O is damaged. On the base of the cases demonstrated in *Fig. 13* we estimated the change in the specificity of K^+ as compared to Li^+. Taking into account the changes in tonus ratio at 120 min and the ratios of concentrations we obtained the following informatory "specificity-constants":

H_2O	D_2O
k = 63	k = 30
KLi	KLi

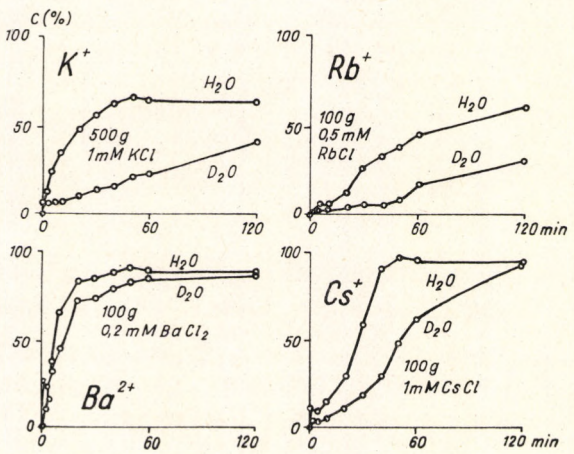

Fig. 12. Effect of D_2O on the K^+, Cs^+, Rb^+ and Ba^{2+}-response. Marks see in *Fig. 9*

12. ábra. D_2O hatása a K^+, Cs^+, Rb^+, és Ba^{2+} válaszra. Jelölés mint a *9. ábrán*

The contrary changes of initial and steady state response observable sometimes are remarkable. Thus, for example, in Li^+ inhibited, in Ba^{2+} promoted initial activations are shown *(Fig. 13)*. Summarizing the phenomena

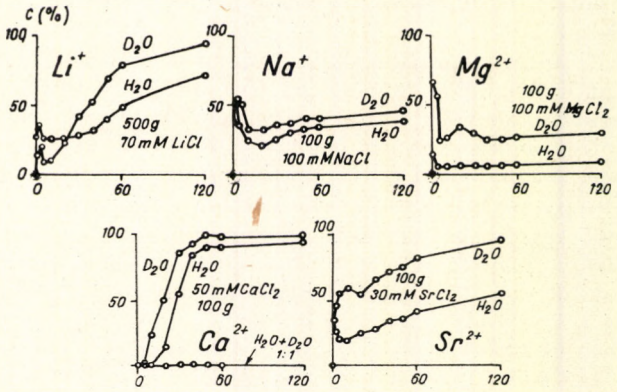

Fig. 13. Effect of D_2O on the Ca^{2+}, Mg^{2+}, Sr^{2+}, Li^+ and Na^+ response. Marks see in *Fig. 9*

13. ábra. D_2O hatása a Ca^{2+}, Mg^{2+}, Sr^{2+}, Li^+ és Na^+ tónusra. Jelölés mint a *9. ábrán*

observed in D_2O it can be stated that the ion-effects producible at most in 1 mM range are inhibited, but those produced with solutions of 10—100 mM are enforced.

8. The action of K^+-sensitors

At 1 mM KCl and CsCl the actions of 100 μg/ml veratrine, 20 μg/ml histamine, 50 μg/ml guanidine were studied. These substances in the used concentrations are well-known sensitors of the vertebrate cross-striated muscle concerning the potassium contracture (Bacq 1947).

Only the veratrine displayed a significant effect. NaSCN, histamine inhibited, guanidine in a small degree have potentiated only the K^+-tonus. 20 μg/ml veratrine alone increased the tonus. However, the time course of the proper effect of veratrine shows clearly that it is not responsible for the excess-tonus.

9. Previous and synchronous applications of tryptamine, serotonin, dopamine, d-TC, TMA, TEA, TnBA. Their effect of K^+-tonus

Earlier we reported the KCl and tryptamine given together can cause an increased tonus (Lábos 1966). The situation is different when the glochidia preincubated and driven with tryptamine were forced to produce tonus. Already 5 min preincubation in 100 μg/ml tryptamine significantly increased the secondary relaxation observed in 30 mM KCl. When we performed preincubation of 40 min in 100 μg/ml tryptamine, serotonin and dopamine the following KCl-incubation of 60 min produced a final inhibition of tonus of 92, 50 and

47%, respectively. The degree of such effect of d-TC is similar to that of dopamine.

The combination of 1 mM KCl with 1 mM TMA, TEA and TnBA showed decreased tonus-ratios: 95—13.5—8.5 and 60% tonus was observed in the control TMA, TEA, TnBA solutions, respectively.

10. Changes and differences in potassium susceptibility

It is an unquestionable experience of some years that under seemingly same conditions significant differences are observable in the K^+, Na^+ and Li-responses of glochidia.

The possible factors which could be responsible for the differences are: ontogenetic stage, season, the finer composition of the medium, keeping conditions, species. The significance of season and in a hidden mood the ontogenesis and a seasonally changing medium was well shown, by a summary of experiments carried out in the years, of 1960—67. Working with lake water it seemed to be clear that the K^+-sensitivity is increasing in autumn, the top is reached in December then a decrease could be observed. Some seasonal shift is possible but the maximum in winter was frequent. The pH of lakewater is varying between 7.8 and 8.4. However, solutions with artificially changed pH could not reproduce the observed differences. The changes in the antagonistic ion-composition may be one of the real factor. The low susceptibility in October may be connected to the early larval stage.

The highest sensitivity to potassium means a detectable reaction sometimes in 100 μM/lit KCl.

The significance of changes in the keeping conditions also cannot be neglected. In an experiment we observed a very marked decrease in the susceptibility after a large and sudden temperature step (nominally: +6 °C $\leftrightarrows$ +37 °C.

11. Ion-effects and electric excitability

The electric excitability of larval adductor was examined with a method described previously (LÁBOS 1964). In the isotonic (20 mM/lit) solutions of $MgCl_2$ and $CaCl_2$ the excitability decreased, then disappeared. In 20 mM NaCl the threshold slowly decreases or is unchanged. In 20 mM LiCl for 10—40 min the excitability decreases or increases a little then suddenly significantly increases. Finally, inexcitability results. The contractions in $MgCl_2$,$CaCl_2$, NaCl and LiCl are very quick and are not complete.

In KCl, after the secondary relaxation there is no excitability. Low, subcritical concentrations of KCl and $BaCl_2$ (0.2—0.05 mM) increase the excitability. The lowest and most constant thresholds were observed in KCl of low concentration and in physiological solution of MARCZYNSKY (1959). In distillated water a real decreased excitability was observed.

Discussion

The problem of alkali metal ion-specificity was elucidated with remarkable results by LING (1960), EISENMANN (1961), and by LING and OCHSENFELD (1966). They give also experimental support to the anion-field and ion-exchange

hypotheses. The sequence of $Rb^+ > K^+ > Cs^+$ is equivalent to the III-rd order of EISENMANN. He explained 11 types of the possible 120 (5 !) lyotropic orders only by the strength differences of anionic field binding the cations. LING and OCHSENFELD (1966) determined association constants on the basis of his association induction hypothesis in frog-sartorius; the obtained sequence of constants was: $Rb^+ > K^+ > Cs^+$. Thus a real significance must be attributed to their discussions, namely for example to the β- and γ-carboxyl groups and to the desolvatation process of cations.

However, the total tonus-sequence ($Na^+ < Li^+ < Cs^+ < K^+ < Rb^+$) and even the rhythm-order Cs^+, $K^+ > Rb^+$, Na^+, Li^+ cannot be explained alone by the changes in the strength of anionic field. That is to say, such sequences are not among those of EISENMANN. We believe other factors to be the causes of the described facts, for example enzyme-activation could play an explicit role in forming such sequences of the rhythmic activity and it is not based alone on the ion-uptake. This is supported by SKOU's finding (1960) on K^+-activated ATP-ase in the presence of Mg^{2+}. He obtained a part of our sequence in question: $Na^+ < Li^+ < K^+$, Rb^+, Cs^+. (This is similar to EISENMANN's Xth sequence.) It is more important to take into account also that EISENMANN' hypothesis is of an equilibrium-type. On the other hand, in our experiments the glochidia are in the transient phase of ion-uptake especially concerning the rhythmic activity which is a typically transient phenomenon. Nevertheless, after the secondary relaxation nearer to the equilibrium the "normal" lyotrop sequence appears ($Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$).

In spite of these a steady-state-discussion similar to that of EISENMANN has proved to be useful in the interpretation of K^+-inhibitory and Li^+-potentiating effect of heavy water (see in Appendix). It must be supposed that D_2O damages the mechanisms responsible for K^+-specificity. It causes this partly by the different changes in the hydration energies of potassium (lithium and) of the counter-ion and partly by the new properties of the D_2O-modified exchange-phase. The possible modified groups because of further causes may be —COOD and —SD.

Another possible explanation of D_2O-effect can be based not on the ion-exchange-type discussion but on the common and coupled diffusion of ions and solvents. Thus, according to ONSAGER's hypothesis (1945) of solvent drag influence the solvent accelerates or retards the movement of ions through the porus according to the direction of its stream. If this is right an opposite net change in the direction of solvent flow must take place in the two groups of ions. KOEFOED-JOHNSEN and USSING (1953) used D_2O succesfully to measure influx of water through biological membrane. However, theories reckoning with equivalent porus in an old sense cannot explain sufficiently all the anomalies of ion cation-sequences. For this reason we incline to give a more general ion exchange model (see Appendix).

In the case of alkaline earth metal ions the sequence for the tonus is a normal HOFFMEISTER-series. In the order for the rhythmic activity there is the anomaly: $Mg^{2+} > Ca^{2+}$; it is probable that its transient character is which is responsible. The Ba^{2+} susceptibility of glochidia similarly to other smooth muscle preparation is about at 10 μM. Therefore the glochidia are suitable for testing spasmolytica. The dose-effect curve does not cover a process of binding of the first order. The dissociation constant is $pK = 4.5$ *(Fig. 4)*. The same process is characteristic also for potassium but $pK = 3$.

The antagonistic and synergetic action effects similarly to K^+-H^+ competition observed by CONWAY and his co-workers (1963) on K^+-uptake of yeast cells in a first approximation can be explained by a binding to the same sites. According to this, if we take the effects as being in direct connection with the uptake (which is plausible with regard to the effect = time diagrams then the antagonisms and synergisms could be considered as competitions on the sites of binding. All the K^+-inhibiting actions applying them alone even also Li^+ decrease the electric excitability, too.

But it is remarkable that there are significant differences in the degree of cooperations. The orders obtained by antagonisms and synergisms are not the same as the above reviewed ones. Thus, for example, the strength of Na^+-inhibition on the activator cations is: $K^+ > Cs^+ > Rb^+$. Likewise, one hardly could explain that the Mg^{2+} causing weak tonus inhibits the K^+-tonus to a less degree than Ca^{2+}. Therefore, it must be supposed that the antagonistic ion effects interfere partly at different sites. The double effect of Mg^{2+} also argues for this mechanism: initial closing and rhythm and inhibition of proper and potassium tonus. The secondary relaxation observable in the case of all the cations also argues for a double effect. The sequence based on its degree is:

$$Ba^{2+} < Cs^+ < Rb^+ < K^+ < Na^+ < Li^+ < Mg^{2+} < Ca^{2+}$$

In the extreme cases (Ba^{2+}, Mg^{2+}, Ca^{2+}) the tonus was not observed (Ca^{2+}) or only for a short time (1—2 min, Mg^{2+}) or the secondary relaxation was missing. Accordingly with the last sequence the quickest and strongest inhibitions were achieved by Ca^{2+}. The order of K^+-inhibition is $Ca^{2+} > Mg^{2+} > Na^+ > Li^+$.

Kinetically at the given conditions the ion-effects are nearer to the non-competetive self-inhibition than to the competetive one. In the first case characteristic plateaus of different extent are observable on the dose-effect curves (ARIENS 1964). Furthermore, it is desirable to observe that these diagrams somewhat resemble to those of the negative adsorption.

The extreme sensitivity to KCl sometimes 10^{-5} M/lit is the threshold calls the attention to a specialized chemoreceptive mechanism, too.

Analysing the effect of metabolic inhibitors we wish to emphasize the tonus-inhibition displayed by KCN and the block of relaxation as well as the own activation effect shown by the SH-inhibitors PCMB and NEM. The tonus increasing effect of specific PCMB and NEM can be contrasted with the tonus-decreasing effect of Cd^{2+} (LÁBOS and co-workers 1964a) being considered as nonspecific SH-inhibitor but can be compared to the closing action of the also nonspecific Zn^{2+} and Hg^{2+} heavy metal ions. Therefore we may conclude the partial and indirect role of SH-groups, beside other groups, in the excitation. Previously we (LÁBOS and co-workers 1964b) supposed the role of SH-groups in the K^+-action. The skeletal muscle myosin contains SH-groups (KIELEY 1965). The potassium outflow of red blood cells are increased by NEM (TOSTESON and JOHNSON 1957). Thus, it does not seem to be unreasonable to suppose about the SH-groups to have an indirect role in the fastening mechanism of K^+-selectivity and the reversibility of contractions.

NaN_3 inhibits the action evoked by K^+ in low concentrations and potentiates the effect displaying in high concentrations. There must be a neutral point in the K^+-concentration. The cyanide block is regarded as a proof for a close but a possible indirect coupling of K^+-action and the terminal electron-

transport. In plant cells and frog skeletal muscle LUNDEGÅRDH (1939, 1960), CONWAY (1951), CONWAY and MULLANEY (1960) suggested their well-known redox-pump model mainly on the base of cyanide-sensitivity of salt-respiration terminal electron transport. In plant cells and frog skeletal muscle the cyanide-sensitive cytochromoxydase at LUNDEGÅRDH (1939) is Cl^- mediator, according to CONWAY is K^+-carrier (1951). In our case the small effective CN^--concentration and the inhibition apparently taking place competetively are remarkable (parallel shift of the curves of *Fig. 7*. The deterioration of parallelism in the equilibrium is taken as a sign of non-competetive connection of K^+ and CN^- effects. As the observed reaction is produced by a group of animals and in such cases the incorrect forming of mean values is a possible source of mistake leading to a gently sloping curve (see ARIËNS 1964, Vol. I. p. 144) for this reason the slope of curves have to be analysed. It is evident that the experimentally observed slope is far greater than the expected one on the base of a reaction of the first order and of the mass-effect law (on the figures they are drawn by dotted lines). For this reason an averaging with the mentioned mistake is not possible. Furthermore, the reaction of apparently higher order may be a sequence of the first order reactions and its nearly threshold-character leads to the steeply sloping curves. This kind of distorsion refers to the curves measured with and without cyanide, too.

The final conclusion is: cyanide acts at the essential point of the complex reaction graph of the potassium-response.

The K^+-tonus inhibiting effect of quaterner and tertier amines, furthermore, the sensibilization evoked by veratrine hardly fits into the metabolism. It is greatly probable that these amines do not act as mediators or as their antagonists, however, the effect of tryptamine is striking. The positively charged amines can modificate the binding or carrying anionic field nonspecifically and as it is known that veratrine can cause K^+-outflow (SZENT-GYÖRGYI and co-workers 1939, BACQ 1947, GOFFART and BACQ 1952). Nevertheless, this mechanism does not lead by any means to a K^+-sensibilization.

In order to obtain a clearer picture of certain double and/or opposite effects we must discuss mainly the N_3^--inhibition and potentiation. It is a good example for the apparently contradictionary effect of the same substance. There is no essential problem for example in Rb^+/K^+ and Cs^+/K^+ synergisms. They can be regarded as competetive agonists. But the K^+/N_3^- "synergism" was observed at other conditions. It is evident that a possible cause of the double azide-effect (opposite action at low and high potassium concentration) is the fact the two K^+-responses can be regarded as two different phenomena. Thus azide would inhibit the tonus and potentiate the true contracture. The D_2O-effect may also be accounted for such a mechanism. It increases the contracture achieved by high cation-concentrations and inhibits those obtained in low concentrations. Unfortunately this wide-spread view (BETHE and FRANKE 1925, FUJIMO and FUJIMO 1964) is disturbed for example by the fact that veratrinesensitization opposite to that of azide can be obtained already at low (1 mM) KCl and CsCl concentrations. It is not probable that the K^+-response observable at low concentrations is a true contracture. The fact that the anion N_3^- and the cation Cd^{2+} show antagonistic effects is a clear proof for the complexity of the reacting system. Consequently, it seems to be a reasonable conclusion that the double response is an attribute of the highly organized system.

Appendix

In the following exposition a simplified possible thermodynamic explanation of the D_2O-inversion is given. Internal energy values are used instead of free energy because the living system is supposed to be open and the direction of reactions is not shown always by an increase of entropy or by a decrease of free energy. From the two groups of ions K^+ and Li^+ are selected as extreme examples. Let us suppose the difference between the internal energy values of X^+ cation to be in B and in isolated state in vacuum (0-state) is ΔU_X^{OB} Let ΔU_X^{OH} and ΔU_X^{OD} be the internal energy of the same cation in normal and heavy water, respectively. Furthermore, let K^+ and for Li^+ be the changes of internal energy attending with a transition from the H_2O and D_2O solvents to the state B by ion-exchange: ΔU_{KX}^{BH}, ΔU_{KX}^{BD}, ΔU_{LiX}^{BH}, ΔU_{LiX}^{BD}.
It can be written

$$\Delta U_{KX}^{BH} = \Delta U_X^{OH} - \Delta U_K^{OH} + \Delta U_K^{OB} - \Delta U_X^{OB}$$

$$\Delta U_{KX}^{BD} = \Delta U_X^{OD} - \Delta U_K^{OD} + \Delta U_K^{OB} - \Delta U_X^{OB}$$

$$\Delta U_{LiX}^{BH} = \Delta U_X^{OH} - \Delta U_{Li}^{OH} + \Delta U_{Li}^{OB} - \Delta U_X^{OB}$$

$$\Delta U_{LiX}^{BE} = \Delta U_X^{OD} - \Delta U_{Li}^{OD} + \Delta U_{Li}^{OB} - \Delta U_X^{OB}.$$

where X^+ is the exchange-cation. Experimentally it has been observed that D_2O inhibits the effect of K^+ and after a transitional block it increases that of Li^+. Let us suppose that the action of a cation is in direct connection with its binding and the effect is more explicit if the change of internal energy accompanying the binding is smaller. In such a case the experimental facts can be written thus

1. $\Delta U_{KX}^{BH} < \Delta U_{KX}^{BE}$
2. $\Delta U_{LiX}^{BH} < \Delta U_{LiX}^{BE}$ then $\Delta U_{LiX}^{BH} > \Delta U_{LiX}^{BD}$

Supposing that the exchange-cation is K^+. In such a case for K^+ and similar ions there is no, or only a small, difference between the internal energies of the hydrated exchanging ions. Therefore the K^+-inhibition is caused, above all, by the change of B-state that is by the modificated exchange phase. This is possible only in that case if the exchange-phase is not absolutely free of water and/or protons therefore it must be inferred a K^+-binding phase of B'-state. For this reason

$$\Delta U_K^{OB} - \Delta U_X^{OB} < \Delta U_K^{OB'} - \Delta U_X^{OB}$$

from where

$$\Delta U_X^{OB} < \Delta U_K^{OB'}$$

If the water were completely excluded from the exchanger-phase the D_2O-effect could not be explained only by an EISENMANN type model (1961). If the K^+ is the exchange ion the effect of water cannot be neglected. In order to explain the firstly similar but finally opposite behaviour observed in the case of Li^+ and related ions let us further suppose that K^+ is the X^+ exchange-cation

which assumption, on the other hand, is an equivalent of the well-known K^+-outflux during a tonic excitation. For this reason and as $X^+ \neq Li^+$

$$(\Delta U_X^{OH} - \Delta U_{Li}^{OH}) - (\Delta U_X^{OD} - \Delta U_{Li}^{OD}) \neq 0$$

In the case of Li^+ the modification of exchange-phase can not be neglected, that is

$$\Delta U_{Li}^{OB} \neq \Delta U_{Li}^{OB'}$$

Thus the inhibitory effects are explained primarily by the alteration of exchanger, the potentiations by the increased difference between the hydration energies of acting and exchanging cations. In the latter case the change of B-phase also must be taken into account. The biphasic change of Li^+-effect clearly shows this postulation.

We desire to emphasize that the binding to the exchange-phase is not an explicit argument for the alteration theory or contra membrane theory of ionic phenomena. Namely, the exchanger could be itself an ion-carrier system into the intracellular phase. Thus the binding would be only transitional but could influence the cation fluxes. Furthermore, there is an alternative exchanger-hypothesis when Ca^{2+} localized into the membrane is the exchange-ion itself. In this case the hydration energy-difference would be responsible for D_2O action displayed on K^+ and related ion effects while the modification of exchange-phase would cause the changes of Ca^{2+} and related cationactions. It is also believed that the ion-exchange model could be compatible with the ONSAGER's solvent drag hypothesis.

Summary

The rhytmic movements and tonic closure of the freshwater clam larvae (*Anodonta*-glochidia) were studied.

1. Anomalous lyotrop order was observed at low concentrations in the tonus-evoking effect:

$$Rb^+ > K^+ > Cs^+ > Li^+ > Na^+$$

which transforms in the course of relaxation into the following order:

$$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$$

Also an irregular sequence can be observed concerning to the frequency of rhythm:

$$K^+,\ Cs^+ > Rb^+,\ Li^+,\ Na^+$$

2. The united sequence of the equipotent concentrations of alkaline earth metal ions is:

$$Ba^{2+} < Rb^+ < K^+ < \underleftarrow{Cs^+} \overrightarrow{<} Sr^{2+} < Ca^{2+} < Li^+ < Na^+ < Mg^{2+}$$

The arrows mark the point of D_2O-effect-inversion (see below).

3. At the same conditions the sequence in K^+-antagonism is:

$$Ca^2 > Mg^{2+} > Na^+ > Li^+$$

Inhibitory effect of Na^+ increases according to this order:

$$Rb^+ < Cs^+ < K^+$$

4. The ions with similar effect act as synergists. The sequence of synergisms: $Rb^+/Cs^+ > K^+/Rb^+ > K^+/Cs^+ > K^+/Ba^{2+}$. Ba^{2+} hardly increases the K^+-effect, moreover the Cs^+ and Rb^+ effect are inhibited.

5. The K^+-effect is not or is a little sensitive to the following metabolic inhibitors: ouabain 2,4-DNP, 2,4-DNPH, NaF. But NaN_3 depending on the ratio of concentrations can increase or depress the K^+, Rb^+ and Cs^+-tonus. KCN in 10 μM/lit can inhibit definitely the effect of 0.1—2 mM KCl. PCMB and NEM potentiate the K-effect. This SH-inhibitors can activate alone, too.

6. In the groups of K^+-sensitors NaSCN, guanidine, histamine are hardly effective but veratrine highly increases the K^+ and Cs^+ effects.

First of all tryptamine, but dopamine and serotonin also strongly block the tonic K^+-response, by a previous application. TMA, TEA and TnBA in 1 mM strongly decrease the K^+-tonus. Surface active agents — digitonin, papaverine, cetylpyridine-bromide, ethanol — in low concentrations inhibit the K^+-tonus, in higher concentrations evoke tonus.

7. In solvents containing 50% D_2O and 50% H_2O the tonus evoked by

$$K^+, Cs^+, Rb^+ \text{ or } Ba^{2+}$$

is inhibited, but the tonus evoked by

$$Na^+, Li^+, Ca^{2+}, Mg^{2+} \text{ and } Sr^{2+}$$

is increased really. In point 2 the given sequence is intersected by a line marking the inversion of D_2O effect; to left inhibition, to right potentiation was observed.

8. Approximately isotonic solutions of Ca^{2+}, Mg^{2+}, Li^+, Na^+ decrease, K^+ increases the electric excitability of larval adductor.

9. Some of the possible factors which could be responsive for the observed seasonal changes in the K^+-susceptibility were studied experimentally. On the base of these experiments the ionic-composition of medium (water from the Lake Balaton), the keeping conditions in laboratory (sudden changes in the temperature), ontogenetic stage are real possibilities; pH changes between 5.5—8.5 are not effective in the influence of tonus at high K^+-concentration.

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ADATOK ALKÁLI FÉM ÉS FÖLDFÉM IONOK AKTIVÁLÓ HATÁSÁNAK MECHANIZMUSÁHOZ ANODONTA LÁRVÁN

Lábos Elemér

Összefoglalás

Édesvízi kagylólárvák*(Anodonta*-glochidiumok) alkáli fém és földfém ionokkal kiváltható ritmusát és tónusát vizsgáltuk,

1. Anomális liotrop sort figyeltünk meg, kis koncentrációknál a tónusokozó hatásban:

$$Rb^+ > K^+ > Cs^+ > Li^+ > Na^+,$$

ami az ionok által kiváltott tartós tónus megszűnésekor átmegy a

$$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+\text{-sorba.}$$

A ritmus frekvenciája szerint szintén anomális a sorrend:

$$K^+, Cs^+ > Rb^+, Li^+, Na^+$$

2. Az alkáli földfémek és alkáli fémek azonos tónust kiváltó koncentrációinak a sorrendje a következő:

$$Ba^{2+} < Rb^+ < K^+ < \overleftarrow{Cs^+ |} \underrightarrow{< Sr^{2+} < Ca^{2+} < Li^+ < Na^+ < Mg^{2+}}$$

3. Azonos feltételek mellett a K^+-hatás antagonizálása tekintetében az alábbi a sorrend:

$$Ca^{2+} > Mg^{2+} > Na^+ > Li^+$$

A Na^+ antagonizáló hatása az alábbi sorrendben erősödik:

$$Rb^+ < Cs^+ < K^+$$

4. Hasonló hatású ionok szinergistaként hatnak. A szinergizmus sorrendje: $Rb^+/Cs^+ > K^+/Rb^+ > K^+/Cs^+ > K^+/Ba^{2+}$. A Ba^{2+} alig potenciroz, sőt a Cs^+ és Rb^+ hatást gátolhatja.

5. A K^+-tónus nem vagy alig érzékeny az alábbi anyagcseregátlókra: ouabain 2,4-DNP, 2,4-DNPH, NaF. NaN_3-dal a koncentrációarányoktól függően a K^+, Cs^+ és Rb^+-tónus gátlása és potencírozása is észlelhető. KCN 10 μM/lit körüli koncentrációkban 0,1—2 mM KCl hatását jelentősen gátolja. A PCMB és NEM potencírozza a K^+-tónust. Ezek az SH-gátlók önmagukban is aktiválnak.

6. K^+-szenzitorok közül alig hatásos a NaSCN, guanidin, hisztamin, míg a veratrin a K^+ és Cs^+ hatást erősen potencírozza. Elsősorban a triptamin, de a dopamin és serotonin előzetes alkalmazása is jelentősen gátolja a K^+-választ. TMA, TEA, TnBA 1 mM koncentrációban gátolják a K^+-tónust. Felületaktív anyagok (digitonin, papaverin, cetilpiridinbromid, etanol) kis koncentrációban gátolják a K^+-tónust, nagyobb koncentrációban általában tónust okoznak.

7. 50%-os D_2O tartalmú vizes oldatokban a K^+, Cs^+, Rb^+, Ba^{2+}-tónus és K^+-ritmus gátlását, a Li^+, Ca^{2+}, Mg^{2+} és Sr^{2+}-tónus valódi potencírozását észleltük. A 2. pontban adott szekvencia jelzett helyétől jobbra fokozza, balra gátolja a nehézvíz az ionhatást.

8. Az anyai limfával közel izotóniás koncentrációban a Ca^{2+}, Mg^{2+}, Li^+, Na^+ csökkenti, K^+ fokozza a lárvális izom elektromos ingerlékenységét.

9. A K^+ érzékenység „szezonális" változásait előidéző lehetséges faktorokat kísérletesen vizsgáltuk. Ennek alapján szóba jöhet a balatonvíz ion-összetétele, az egyedfejlődés, tartási körülmények (hőmérsékletugrások); pH-változások hatása nem valószínű

ДАННЫЕ К МЕХАНИЗМУ АКТИВИРУЮЩЕГО ЭФФЕКТА ИОНОВ ЩЕЛОЧНОГО И ЩЕЛОЧНО-ЗЕМЕЛЬНОГО МЕТАЛЛОВ НА ГЛОХИДИЯХ БЕЗЗУБКИ

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Были изучены ритмическая и тоническая активности глохидиев беззубки, вызванные ионами щелочного и щелочно-земельного металлов.

1. — В тоническом эффекте был обнаружен аномальный лиотропный ряд:

$$Rb^+ > K^+ > Cs^+ > Li^+ > Na^+$$

который при прекращении продолжительного тонуса, вызванного данными ионами переходит в следующий ряд:

$$Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$$

По частоте ритма ряд тоже аномальный:

$$K^+, Cs^+ > Rb^+, Li^+, Na^+$$

2. — Концентрационный ряд щелочного и щелочно-земельного металлов, вызывающих одинаковый тонический эффект был следующим:

$$Ba^{2+} < Rb^{+} < K^{+} \overleftarrow{<} Cs^{+} \mid < \overrightarrow{Sr^{2+}} < Ca^{2+} < Li^{+} < Na^{+} < Mg^{2+}$$

3. — При прочих равных условиях в отношении антагонистического действию эффекта калия был получен следующий ряд: $Ca^{2+} > Mg^{2+} > Na^{+} > Li^{+}$ Антагонистическое воздействие натрия увеличивалось по следующему ряду: $Rb^{+} < Cs^{+} < K^{+}$.

4. — Ионы со сходным влиянием действуют как синергисты по следующему ряду: $Rb^{+}/Cs^{+} > K^{+}/Rb^{+} > K^{+}/Rb^{+} > K^{+}/Cs^{+} > K^{+}/Ba^{2+}$. Ионы бария почти не вызывают усиление, а эффект Cs^{+} и Rb^{+} тормозится при даче этого агента.

5. — Тонический эффект, вызванный калием, почти нечувствителен к следующим ингибиторам обмена веществ: оуабаин, 2,4-ДНФ, 2,4-ДНФХ, NaF, NaN_3 в зависимости от концентрации вызывает или снижение или усиление тонуса, наступающего под влиянием K^{+}, Cs^{+}, Rb^{+}. KCN в концентрации 10 μM/л значительно снижает эффект калия (0,1—2 мМ). ПХМБ и NEM не увеличивают калиевый тонус. Эти ингибиторы SH-групп сами по себе являются активаторами.

6. — Из сензиторов калия NaSCN, гуанидин и гистамин почти неэффективны, а вератрин сильно увеличивает эффект K^{+} и Cs^{+}. Предварительная дача прежде всего триптамина, но и допамина и серотонина тоже значительно снижает калиевый эффект. ТМА, ТЕА и ТВА в концентрации 1 мМ понижают калиевый тонус. Поверхностно-активные вещества (дигитонин, папаверин, цетилпиридинбромид и этанол) в низких концентрациях снижают тонус, вызванный калием, а в больших концентрациях сами вызывают тонус.

7. — В водных растворах 50%-ного D_2O наступало торможение тонуса, вызванного K^{+}, Cs^{+}, Rb, Ba^{2+} и калиевого ритма, а тонус, вызванного Li^{+}, Ca^{2+}, Mg^{2+} и Sr^{2+} увеличивался. В ряде, данном во втором пункте, тяжелая вода вызывает усиление эффекта ионов, расположенных в правой стороне, а в левой—торможение.

8. — В изотонических составах лимфы материнского организма в концентрациях Ca^{2+}, Mg^{2+}, Li^{+} и Na^{+} снижают, а K^{+} увеличивает электрическую возбудимость личиночной мышцы глохидиев.

9. — Изучались факторы, вызывающие «сезональные изменения» калиевого эффекта. Из возможных факторов надо принимать во внимание ионный состав Балатона, индивидуальное развитие и условия придерживания животных (температурные изменения) — изменение pH вероятно не имеет значения.