# EFFECTS OF LANTHANUM AND ALKALINE EARTH METAL IONS ON THE POTASSIUM CONTRACTURE OF THE HELIX HEART

L. Erdélyi

*Department of Animal Physiology, Attila József University, Szeged*

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It is known that the heart muscle of molluscs differs in several respects from that of vertebrates. It will suffice to refer from among these to the structure of the heart muscle, the differences demonstrated in its biochemical composition, the presence of a diffuse pacemaker, the different responses to electric stimulation, the difference in excitatory mediators, as well as to the different characters of some ion effects. At the same time, in other relations, some similar features can be recognized, too. Worthy of note, from this point of view, are the myogenic automatism, the inhibiting effect of ACH, the presence of a Na—Ca-dependent action potential, the depolarizing effect of potassium and the contracture connected with this.

On the basis of all this, it is justified to raise the question of the regularities of structure and functioning of the excitation-contraction coupling. From this point of view, data connected with the problem of the potassium contracture and its ion-dependence, have been published by Ozeki (1964), and Hill, Greenberg, Irisawa, Nomura (1970) with regard to the pharynx retractor or radula protractor muscle of two snail species, and by Nomura (1965) and Burton and Loudon (1972) with regard to the heart of *Dolabella* and *Helix*. It can be established from these investigations that the K-depolarization of molluscan muscles and the associated contracture, and also the Ca-dependence of the phenomenon are similar to those reported in vertebrate organs and particularly in the heart of the frog (Niedergerke, 1956; 1963; Lamb and McGuigain, 1966).

The ion-dependence $(Ca) : (Na)^2$, known from the works of Wilbrandt and Koller (1948), Lüttgau and Niedergerke (1958) on the contractility of frog heart, is not valid for the molluscan heart (Nomura, 1965; Burton and Loudon, 1972). According to the investigations of the authors cited, the effect of magnesium on the molluscan heart is similar to that of sodium on the frog heart.

In the calcium-dependent potassium contracture the problem of the replacement of calcium by various cations seems to be rather contradictory in the literature. It is sufficient in this connection to refer to two data. Frank (1962) found many bi- and trivalent cations to be calcium-substitutive in the potassium contracture in the skeletal muscle of the frog. At the same time, Gainer (1968)

could not substitute essentially similar cations for the specific role of calcium in the muscle of the lobster.

It is known, mainly from investigations on vertebrate muscles, that lanthanum can interact with the $Ca^{++}$-binding sites or stores on the membrane surface, in this way being able to inhibit the calcium-dependent potassium contracture (SANBORN and LANGER, 1970; GOODMAN and WEISS, 1971; and others).

In this paper a study is made, in connection with the potassium contracture, of the problem of calcium substitution, and of the effect of lanthanum on the excitation-contraction coupling of the edible snail heart.

## Materials and Methods

Physiological experiments: Recordings were made of the isotonic contractions of the isolated *Heltix pomatia* hearts, hung up in an organ vessel and extended with a 1,3 g weight, on a smoked kymograph drum. Solutions of the following composition were used:

*Helix—Ringer (according to Jullien—Ripplinger)* NaCl 111,1 mM, KCl 1,87 mM, CaCl₂ 1,08 mM, NaHCO₃ 2,39 mM pH = 7,4

*K—Ringer*

| KCl | 10 | 15 | 20 | 30 | 50 | 100 | mM |
|---|---|---|---|---|---|---|---|
| NaCl | 101,1 | 96,1 | 91,1 | 81,1 | 61,1 | 11,1 | mM |
| CaCl₂ | 1,08 | 1,08 | 1,08 | 1,08 | 1,08 | 1,08 | mM |
| NaHCO₃ | 2,39 | 2,39 | 2,39 | 2,39 | 2,39 | 2,39 | mM |

For the investigation of the calcium-dependence of the potassium contracture, as well as the possibility of calcium-substitution, the contracture elicited by 50 mM K-Ringer was considered. Not more than 5—10% difference was obtained in the height of the contractions, even after eliciting contractures with solution three times successively in the same heart (taking into consideration a ten-minute washing interval).

In the course of the experiments, the snail hearts were equilibrated in Helix—Ringer under oxigenated conditions for 20 minutes, and then control contraction was recorded in 5O mM K-Ringer. After washing, the hearts were pretreated for 5 to 30 minutes in a solution (changed several times) containing the appropriate concentration (0—1 mM) of calcium or calcium-substitute, and a contraction was again elicited with a solution containing calcium the same concentration as the incubating solution or calcium-substitute and 50 mM KCl. The relation of the two contractions was evaluated. In one heart, always only one control and one experimental reaction were recorded. Generally the average of at least three parallel experiments was taken into consideration. In the course of the experiments solutions of the following composition were used:

*Ca-Ringer*

| CaCl₂ | 1 | 0,8 | 0,5 | 0,4 | 0,3 | 0,2 | 0,1 | 0 | mM |
|---|---|---|---|---|---|---|---|---|---|
| NaCl | 111,1 | 111,1 | 111,1 | 111,1 | 111,1 | 111,1 | 111,1 | 111,1 | mM |
| KCl | 1,87 | 1,87 | 1,87 | 1,87 | 1,87 | 1,87 | 1,87 | 1,87 | mM |
| NaHCO₃ | 2,39 | 2,39 | 2,39 | 2,39 | 2,39 | 2,39 | 2,39 | 2,39 | mM |
| EDTA | — | — | — | — | — | — | — | 1,5 | mM |

The composition of the Ca-50 mM K-Ringer always conforms to the calcium content of the corresponding Ringer solution, but the concentration of NaCl was only 61,1 mM.

The compositions of the 1, 0,8, 0,5, 0,4, 0,3, 0,2, 0,1 mM barium, strontium, magnesium Ringers, as well as that of the alkaline earth metal ion-50 mM K-Ringer of the corresponding concentration were formed in a completely analogous way as described for calcium. In the case of strontium NaHCO₃ was not used.

In the experiments carried out with LaCl₃, the compositions of the 1 mM Ca-Ringer and 1 mM Ca-50 mM K-Ringer were taken into consideration, with the difference that LaCl₃ was also added to the solutions, in a quantity of 0,005, 0,02, 0,2, 0,5 or 1 mM and NaHCO₃ was not used. The pretreatment in the La-1 mM Ca-Ringer lasted for five minutes.

Isotope experiments: A study was made, palallel with the physiological experiments, of the $^{140}$Ba uptake of *Helix* hearts and the washing out of the isotope. The isotope experiments were performed in the same way as the physiological investigations, in 30 ml 0.3 or 0.1 mM inactive Ba-Ringer, mixed with about 0.6 $\mu$Ci/ml $^{140}$Ba (pH = 7.4, 22 °C). The isotope uptake of the hearts and the depolarizing effect of 50 mM KCl on the development of the uptake were then examined. The uptake is given as a percentage of the incubating solution, referred to 1 mg dry weight.

After a 45-min. uptake the hearts were washed in 30 ml Ca-free Ringer, for $3 \times 1$ min. The washing out of the isotope in 30 ml Ca-free Ringer was evaluated on the basis of the change in activity (count/minute/ml). The effect of Ca$^{++}$ on the $^{140}$Ba washing out was investigated. $^{140}$Ba was detected via its gamma-radiation, in 0.1 or 0.2 ml organ-bath samples.

Electron-microscopic investigations: The electron-microscopic procedure was made in the same way as described in an earlier publication, after being fixed in glutaraldehyde-osmium as usual (ERDÉLYI—HALÁSZ, 1972).

## Results

In the first series of experiments we worked with animals of 3 cm shell diameter, investigating the effect of K-Ringers of various concentrations on the contracture of the heart muscle. The results obtained are shown in Fig. 1, taking into consideration the average of five different hearts. In the graph the height of contracture is shown measured in mm, after a contracture period of 1 minute, as a function of the extracellular potassium concentration indicated on the logarithmic scale. The results, in spite of the different Helix—Ringer compositions and the other system of recording, can be compared well with the data achieved by BURTON and LOUDON (1972) for membrane depolarization and potassium contracture.

The contracture observed in the 50 mK K-Ringer was also found by us to be rather of a phasic character, in contrast with the barium contracture described in our earlier publications, that proved to be of a markedly tonic character (ERDÉLYI, 1971). There is also a considerable difference in that the spontaneous contraction generally undergoes a pause during the potassium contracture, whereas in the case of barium contracture it may change but in continues nonetheless. The contracture elicited by 50 mK K-Ringer attains its peak value in about 30–50 sec and, after a short plateau, begins a spontaneous ralaxation after the end of the second minute. This fast decreasing section of the initial phase turns at 50% relaxation into a relaxation of slowly decreasing tone. As compared to the fast period, the slow one lasts 5–10 times longer, reaching the base level only very slowly. Such a high insentivity then develops that, after a ten-minute washing, no contraction can be elicited in a new 50 mM K-Ringer, or only a contraction of very low degree. The spontaneous decrease in the potassium contracture was analysed by NIEDERGERKE (1965) in the heart of the frog. It is probable that similar factors may play a part in inducing the phenomenon here too.

After a fluid exchange within the two-minute contraction period, and after the insertion of a ten-minute washing pause the potassium insensitivity is not manifested. Under these conditions, there is no more than a 5–10% decrease in the height of the contraction elicited subsequently three-four times.

In the next experimental series we investigated the calcium-dependence of the potassium contracture and the problem of the possibility of substituting alkaline earth metal ions for calcium in the process. In this experimental series

we worked with animals of 4.5 cm shell diameter. The results obtained are
shown in Fig. 2. The decrease in the extracellular calcium concentration is
followed by a decrease in the potassium contracture to 1–0 mM and by its
complete cessation, corresponding to the Ca-dependence of the phenomenon.
At the various potassium concentrations in the heart of the frog and snail, as
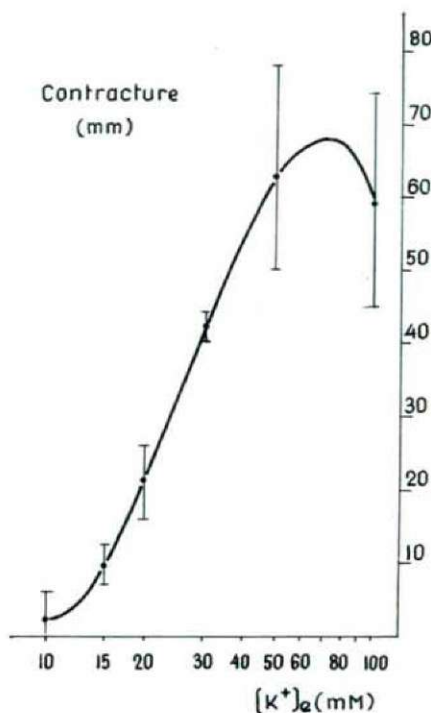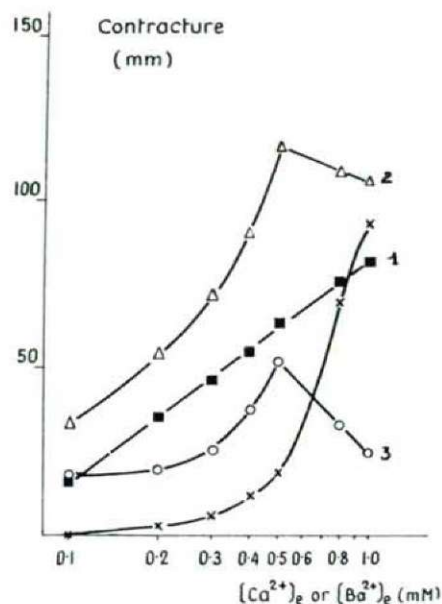well as in the radula protractor muscle of *Busycon,* a contraction curve of



Fig. 1                                                    Fig. 2

Fig. 1. Effect of the extracellular potassium concentration on the contracture of the *Helix* heart
muscle. Every point is a mean value of contractions measured on five different hearts.
The vertical lines indicate the height of the minimum and maximum contractures
obtained in five measurements.

Fig. 2. Change in the contracture elicitable by 50 mM K-Ringer, plotted against the con-
centration $(Ca^{2+})e$, and $(Ba^{2+})e$. X = change in the contracture as a function of the
extracellular calcium concentration. o = change in the contracture as a function of the
extracellular barium concentration, i.e. the difference of the two latter curves. □ = level
of tonic contracture in Ba-Ringer of different concentrations, at the end of the 30-minute
pre-incubation. △ = level of contracture, in the corresponding Ba-50 mM K-Ringer.
Every measurement is the mean value of the contractions obtained in at least three
different hearts. The shell diameter of the animals used in the experimental series was
4.5 cm. The size of the contraction in the 1 mM Ca-50 mM K-Ringer, considered as
control, was obtained from the average of 10 measurements as 92 mm, S.D. ± 13.

Fig. 3. Myofiber of the *Helix* heart ventricle in longitudinal (A, B) and transverse (C)
sections. The electron micrograph shows the structure of the sarcotubular system.
M = mitochondrium, SR = transverse and longitudinal tubules of the sarcoplasmic
reticulum, S = sarcolemmal infoldings, T = dilated subsarcolemmal vacuoles, touching
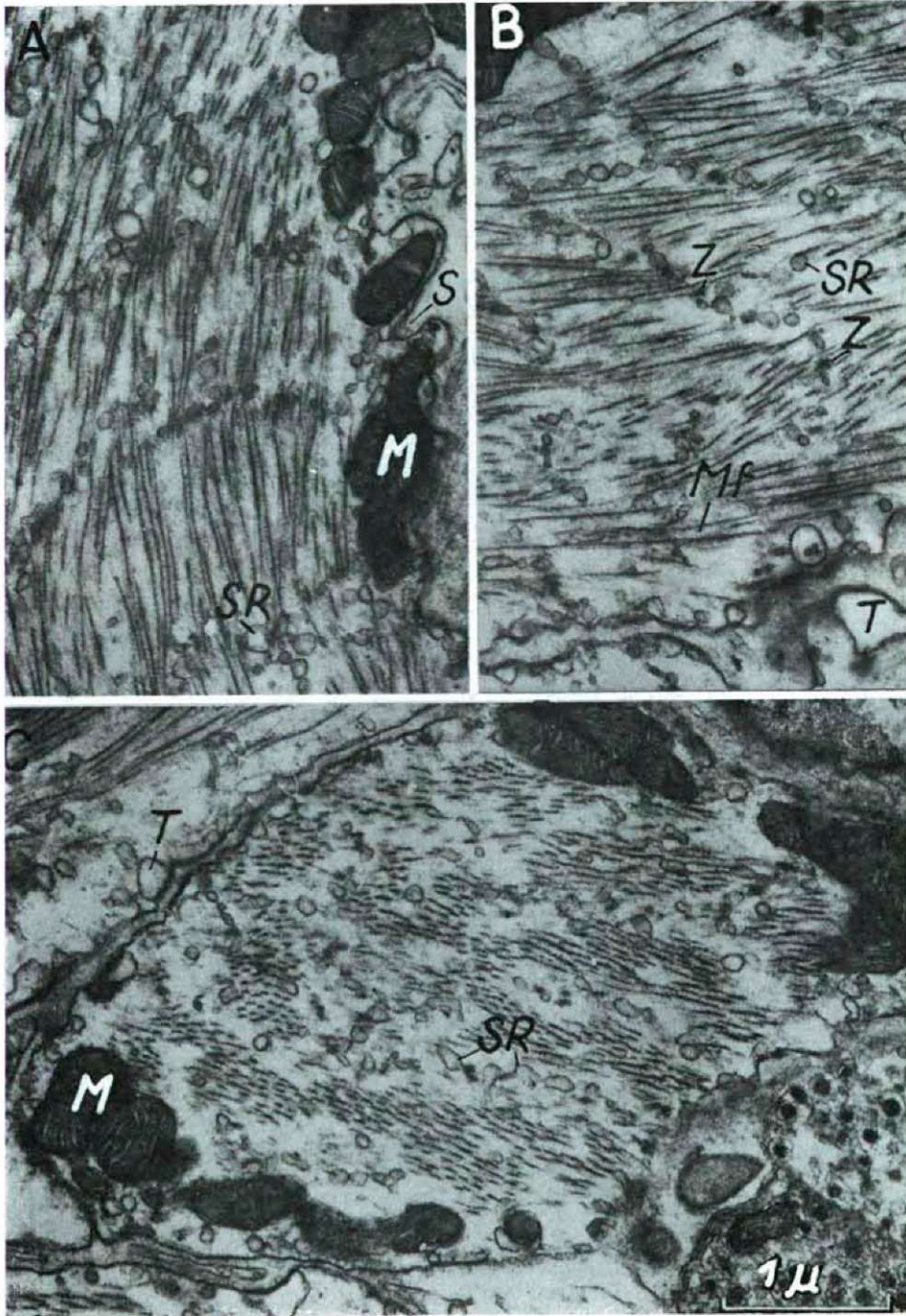the membrane surface, Z = Z-material, mf = myofilaments. Glutaraldehyde-osmium.

Fig. 3

similar type may be recorded as a function of the extracellular calcium con-
centration (LAMB and McGUIGAIN, 1966; HILL, GREENBERG, IRISAWA, NOMURA,
1970; BURTON and LOUDON, 1972). All this shows the possibility of a similar
mechanism from the point of view of the phenomenon. According to the ge-
nerally accepted opinion, the rise of the ionic calcium level within the muscle
fibre is the contraction-inducing key factor. On the other hand, the release of
the sarcoplasmic calcium is influenced by the state of the $Ca^{++}$-binding sites
on the outer surface of the membrane (GAINER, 1968 and others). The morpho-
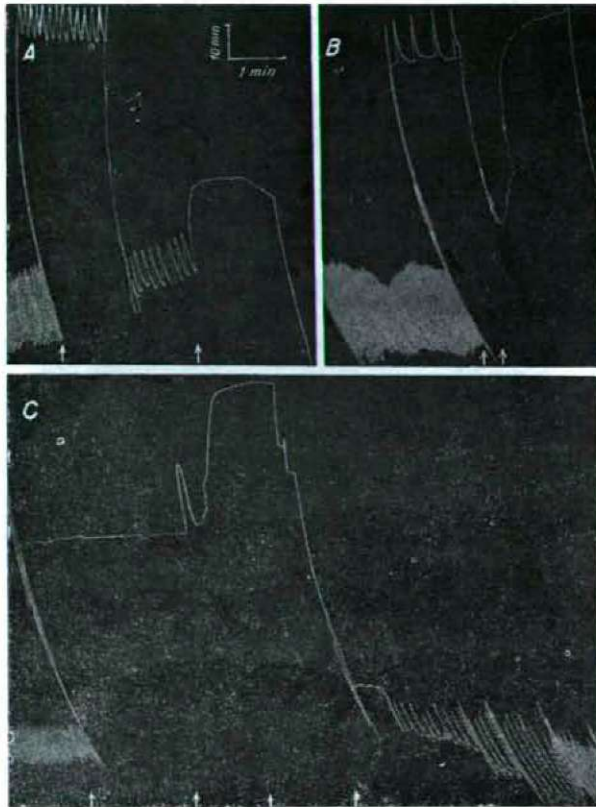logical basis for the function of both systems in the vertebrate skeletal and



Fig. 4. Change in the contracture elicitable by 50 mM KCl, plotted against the extracellular
barium concentration.
    A. First arrow: pretreatment in 1 mM Ba-Ringer for 30 minutes. At the end of the
thirtieth minute, during recording of the spontaneous contraction, the level of the pen
arm was artifically lowered. Second arrow: 1 mM Ba-50 mM K-Ringer.
    B. First arrow: pretreatment in 0.5 mM Ba-Ringer for 30 minutes. At the end of the
spontaneous contractions recorded from the thirtieth minute the level of the pen arm
was artifically lowered. Second arrow: 0.5 mM Ba-50 mM K-Ringer.
    C. First arrow: pretreatment in 0.3 mM Ba-Ringer for 30 minutes. Second arrow:
0.3 mM Ba-50 K-Ringer. Third arrow: beginning of washing in Ca-free Ringer. Fourth
arrow: washing in 4 mM Ca-Ringer. Parallel with the physiological experiments the
[140]Ba uptake of the heart and the washing out of the isotope taken up were investigated,
on the basis of the activity change in the 0.1, and 0.2 ml samples of the bath.

heart muscles is provided by the T-system, ensuring the connection with the sarcolemma, and by the sarcoplasmic reticulum (Phyiol. Symp. 1965, PHILPOTT and GOLDSTEIN, 1967, and others). It is known from NORTH's (1963) publication that the myocardium of *Helix aspersa* has a very rich sarcotubular system. Practically the same is shown by the electron-micrographs in Fig. 3, as well as by SCHLOTE's (1964) Figures with regard to the heart of *Helix pomatia*, too. In the longitudinal and transverse sections reported, the transversal and longitudinal arrangement ot the very richly developed sarcotubular system can be well seen. The connection of the sarcotubular system with the sarcolemma is very conspicuous here too. Sarcolemmal infoldings associated with more dilated subsarcolemmal vesicles can be observed sporadically. In some places, a connection between the characteristic Z-granules and the sarcotubular system can similarly be observed. On the basis of all this, it may be stated that in the heart of the *Helix*, in harmony with the physiological results, the organization of the muscle membrane and the sarcotubular system may be compared, in a less differentiated form, to those described in the vertebrate skaletal and heart muscles. There is, therefore, in essence a suitable morphological basis for comparing the similar physiological phenomena.
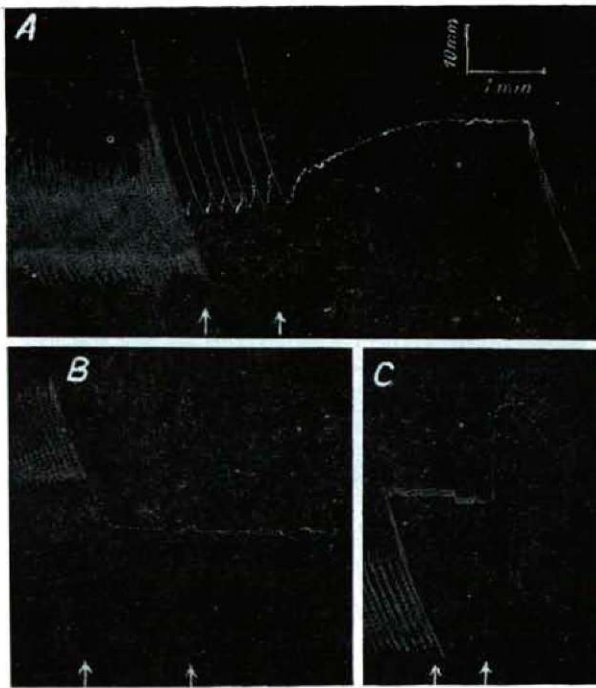


Fig. 5. Effects of strontium, barium, magnesium as calcium-substitutes on the potassium contracture.
A. First arrow: pretreatment in 0.1 mM Ba-Ringer for 30 minutes. Second arrow: 0.1 mM Ba-50 mM K-Ringer.
B. First arrow: pretreatment in 1 mM Mg-Ringer for 20 minutes. Second arrow: 1 mM Mg-50 mM K-Ringer.
C. First arrow: pretreatment in 1 mM Sr-Ringer for 10 minutes. Second arrow: 1 mM Sr-50 mM K-Ringer.

From among the possible calcium-substitutes, we have investigated in detail the effects of the alkaline earth metal ions as these can be considered to be in interaction in several objects with various known calcium effects. The surface membrane binding sites must be common and, according to the investigations of PAPAHADJOPOULOS (1968), HAFEMANN (1969), and others, they are probably phospholipids, and oxigen ligands, or more exactly $PO_4^{3-}$ groups.

In the graph shown in Fig. 2, plotted against extracellular barium concentration, three different affects are presented. It can be seen from the first curve that the contracture rises linearly with the logarithm of the barium concentration (between 1–0.1 mM), according to the data measured at the end of the 30-minute pretreatment (cf. the mechanograms shown in Fig. 4 too). This tonic contracture level till 0.5 mM Ba-Ringer, as seen from the second curve, can be raised to an increasing degree by the 50 mM KCl applied. From 0.5 mM extracellular barium concentration, however, a decrease takes place in the KCl-induced contracture. The third curve shows the difference of the two previous ones, that may be compared with the data obtained for the various calcium concentrations. It can be seen from comparison of the two curves that the potassium contracture till 0.5 mM $(Ca^{2+})e$ or $(Ba^{2+})e$ exhibits a similar slope but in a barium-containing medium the contracture level is more raised. From 0.5 mM, in the Ca-Ringer, a steep rise follows in the contracture as a result of K-depolarization, while in the Ba-Ringer a strong decrease can be observed (Fig. 4).

In the Sr-Ringer a lesser rise in tone takes place as compared with the Ba-Ringer. The height of the contracture obtained in 1 mM Sr-50 mM K-Ringer rather resembles that of barium, and is about 20 per cent of the response measured in 1 mM Ca-50 mM K-Ringer (control).

In 1 mM Mg-Ringer the tone falls and after a pretreatment for 20 minutes the contracture cannot be elicited generally by 50 mM KCl (Fig. 5). We have not investigated the Mg-effect in other relations as it is treated in detail in the paper of BURTON and LOUDON (1972) in connection with calcium antagonism.

It can be seen from the investigations carried out that in the excitation-contraction coupling of the potassium contracture barium and strontium can be substituted partially for calcium. Until 0.5 mM this replacement is very striking, following the sequence of the reciprocal hydrated ion-radius: Ba $\geqslant$ Sr > Ca $\gg$ Mg. At a higher concentration, there is only a partial replacement, calcium coming into prominence and the sequence changing to Ca $\gg$ Ba > Sr $\gg$ Mg. Both variants belong to the seven sequences actually observed from among the twenty-four possible sequences (WRIGHT and DIAMOND, 1968). To clarify the cause of the change in sequence, further investigations are needed. The reason of the change may be that, on proceeding towards higher concentrations, the independent contracture-eliciting effect of the barium ion prevails more and more, supposing a mechanism that is different from its calcium-substituting part in the potassium contracture and inducing an interaction at the expense of the latter. The sequence based on the reciprocal hydrated ion-radius valid at 0.5 mM extracellular ion concentration resembles the result of PAPPANO (1970), who found the same ionic sequence to be valid in the re-establishment tendency on the action potential of the K-depolarized guinea pig atrial muscle. At the same time, it differs from the data published by FRANK (1962) for the

toe muscle of *Rana pipiens* and by GAINER (1968) for the muscles of the lobster.

For barium or strontium ions to replace calcium in analogous processes, their uptake in the membrane binding sites must be assumed and, owing to the K-depolarization of the membrane, a change is to be expected in the uptake. Experimental investigations were made of the barium ion uptake and the possibility of washing out the isotope taken up. The isotope experiments were carried out under the same conditions as the physiological ones, in 0.3 or 0.1 mM Ba-Ringer with 30 ml total volume (pH = 7.4, 22°C). $^{140}$Ba was added to the inactive carrier in a quantity of about 0.6 $\mu$Ci/ml. The uptake was plotted against time, as a percentage of the incubating solution, referred to 1 mg dry weight. The results are shown in Fig. 6. It can be seen well that till the thirtieth minute, in the period of pre-incubation, in the probably two-compartment systems, the uptake for both concentrations (0.3 and 0.1 mM BaCl$_2$) approximately attained the saturation value. Corresponding to the more increased rise in tone, the uptake is stronger at the higher Ba-concentration (cf. also Figs. 4. C and 5. A). The depolarization elicited by 50 mM KCl increases the $^{140}$Ba uptake, corresponding to the stronger contracture, in 0.3 mM Ba-Ringer to a greater extent. In the last section, the decrease in the uptake coincides with a spontaneous decrease in the potassium contracture.

Fig. 7 shows the washing out of $^{140}$Ba plotted against the time, in the
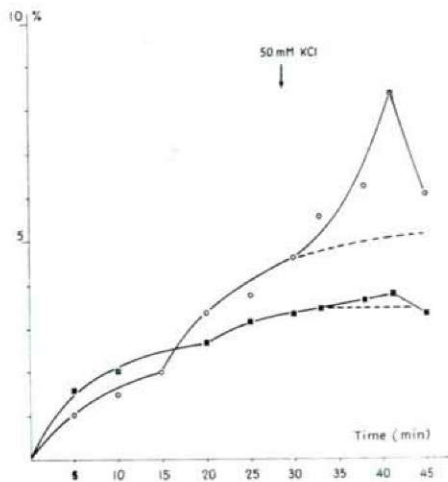


Fig. 6.        Fig. 7
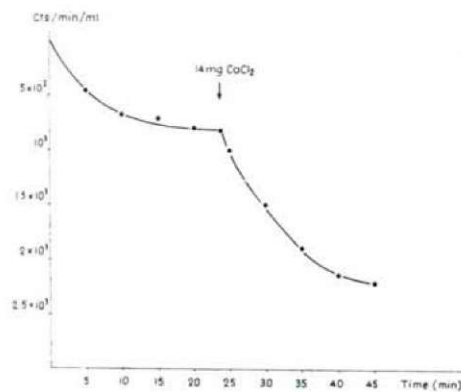
Fig. 6. Change in the $^{140}$Ba uptake of the Helix hearts plotted against time. 0 = in 0.3 mM Ba-Ringer, □ = in 0.1 mM Ba-Ringer. At the point of time marked with an arrow, 50 mM KCl was put into the organ bath. Every point is the mean value of three parallel experiments. The incubating solution contained about 0.6 $\mu$Ci/ml $^{140}$Ba (pH = 7.4, 22 °C).

Fig. 7. $^{140}$Ba wash-out curve plotted against time. After uptake for 45 minutes, the washing out began in a Ca-free Ringer. At the point of time marked with an arrow, 14 mg CaCl$_2$ was mixed with 30 ml organ bath, corresponding to 4 mM Ca-Ringer. Every point is the mean value of three parallel experiments (pH = 7.4, 22°C).

Ringer of 0.3 mM BaCl₂ content, after an uptake for 45 minutes. Washing out
took place in 30 ml Ca-free Ringer, after being rinsed three times for one
minute each. At the point of time marked with an arrow 14 mg CaCl₂ was
added to the Ca-free Ringer, corresponding to 4 mM Ca-Ringer. According to
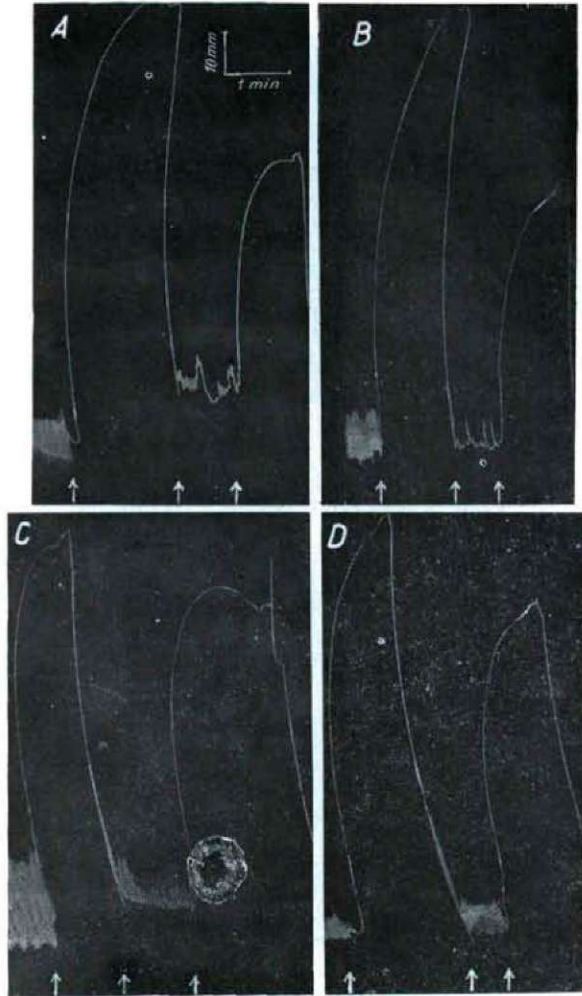the antagonism of the two ions demonstrated earlier (ERDÉLYI, 1968), the loss



Fig. 8. Effect of change in extracellular lathanum concentration on the potassium contracture.
A. First arrow: control contracture in 1 mM Ca-50 mM K-Ringer. Then a 10-minute
washing period followed. Second arrow: 5-minute pretreatment in 0.5 mM La-1 mM
Ca-Ringer. Third arrow: 0.5 mM La-1 mM Ca-50 mM K-Ringer.
B. First arrow: control contracture, as under „A". Second arrow: 5-minute pretreatment
in 0.2 mM La-1 mM Ca-Ringer. Third arrow: 0.2 mM La-1 mM Ca-50 mM K-Ringer.
C. First arrow: control contracture, as under „A". Second arrow: 5-minute pretreatment
in 0.02 mM La-1 mM Ca-Ringer. Third arrow: 0.02 mM La-1 mM Ca-50 mM K-Ringer.
D. First arrow: Control contracture, as under „A". Second arrow; 5-minute pretreatment
in 0.005 mM La-1 mM Ca-Ringer. Third arrow: 0.005 mM La-1 mM Ca-50 mM
K-Ringer.

of $^{140}$Ba increases, and the tone in the mechanogram decreases. Parallel with the advance in time of the assumed $Ba^{2+}–Ca^{2+}$ exchange in the binding sites, besides the decrease in tone, the spontaneous activity of the heart is increasingly restored too (Fig. 4, C).

In the further experiments, in 1 mM Ca-50 mM K-Ringer, from 0.005 till 1 mM, the effect of the extracellular lanthanum concentration on the potassium contracture was investigated. It is known that lanthanum, the ionic radius of which is close to that of calcium, and which forms stronger bonds, can be bound to the surface binding sites of the membrane, in this way interacting with calcium in the excitation-contraction coupling (SANBORN and LANGER, 1970; GOODMAN and WEISS, 1971).

Fig. 8 shows the effects of different $(La^{3+})_e$ concentrations in the heart of the edible snail. The first curve is always the control contracture, in 1 mM Ca-50 mM K-Ringer. The second curve shows the contracture obtained with 50 mM KCl, after 10 min washing and 5 min lanthanum-pretreatment in a Ringer of appropriate lanthanum content. In each of the hearts, here too, only the effect of one lanthanum concentration was investigated, in at least three parallel experiments. The data obtained are illustrated graphically in Fig. 9. The Figure shows the change in the contracture as a percentage of the control.
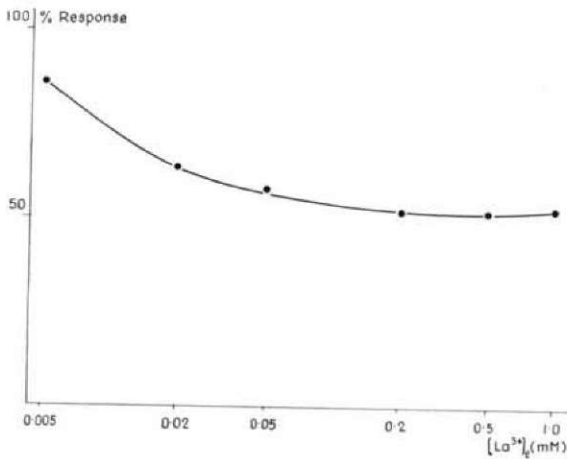


Fig. 9. Effect of the external lanthanum concentration on the contracture elicited in 1 mM Ca-50 mM K-Ringer. The results are expressed as a percentage of the control. Every point is the mean value of three experiments.

plotted against 0.005–1 mM extracellular lanthanum concentration. It can be seen in the graph that an inhibition higher than 50% was not obtained in the contracture elicited by 50 mM KCl, even in the case of the largest lanthanum concentration applied. In spite of the different KCl concentrations, these data show a fairly good agreement with the results of GOODMAN and WEISS (1971) for mammalian vascular smooth muscles and other smooth muscles. The only difference is that, up to 1 mM lanthanum concentration the authors cited observed a stronger inhibition in the objects investigated than we did in the *Helix* heart. According to the Figure, in the *Helix* heart essentially two sections

can be distinguished in the potassium contracture, plotted against the extra-cellular lanthanum concentration. One of the sections lasts till 0.2 mM lantha-num concentration. Here a considerable decrease in the contracture can be observed. Later, towards higher concentrations the slope of the curve is reduced, the process of inhibition becomes slower and there is no stronger inhibition than 50% even in a domain of higher than 1 mM concentration. It is shown by all this that the *Helix* heart is less sensitive to the influence of this ion than the vertebrate muscles are, and that in this object the lanthanum ion is in direct interaction with only few $Ca^{2+}$-binding sites.

## Discussion

It can be considered as a physiological phenomenon of general validity that if any muscle is put in a medium of excess potassium, after the membrane depolarization a contraction follows, as a function of the extracellular potassium concentration. Similarly to the data published for the vertebrate muscles, this is essentially valid for the various molluscan muscles, too (OZEKI, 1964; NOMURA, 1965; HILL, GREENBERG, IRISAWA, NOMURA, 1970; BURTON and LOUDON, 1972). In bringing about the contraction the extracellular potassium concentration plays a definite part. This is shown by the experiments that analyse the change in potassium contracture as a function of the $(Ca^{2+})_e$ con-centration. In this relation there can also be established a considerable resemb-lanse in the hearts of the animals belonging to various branches. The similarity of the physiological phenomena supposes the possibility of identical mechanisms in the excitation-contraction coupling. In this relation, the regularly arranged sarcotubular system, demonstrated from the heart of the *Helix pomatia*, may play the intermediary part which must be assumed on the basis of the investi-gations carried out on vertebrate muscles (Physiol. Symp. 1965, PHILIPOTT and GOLDSTEIN, 1967; HOFMANN, 1969; JOHNSON and LIEBERMAN, 1971; and others). It seems that in bringing about the contracture, that is in releasing the intracellular ionic calcium, apart from the sarcotubular system, the $Ca^{2+}$-binding sites localized on the membrane surface also play a considerable part. It has not yet been cleared up completely as to how these two mechanisms co-operate. At any rate it is probable that a possibility of activating and inactivating me-chanisms must be supposed here too (BEELER and REUTER, 1970, and others). Taking into consideration the nature of the surface $Ca^{2+}$-binding sites or stores, the difference established between the hearts of frogs and snails, as well as in the case of the lobster muscle, in the relation of the antagonistic actions of Ca-Na, Ca-Mg, or Ca-K in the competition for the identical binding sites, is noteworthy (NOMURA, 1965; GAINER, 1968; BURTON and LOUDON, 1972).

It is shown by our experiments on the potassium contracture that these important $Ca^{2+}$-binding sites or stores may interact with each of the alkaline earth metal ions. But the connection between the alkaline earth metal ion and the surface membrane binding site does not induce favourable conditions to an equal extent for bringing about the excitation-contraction coupling. In this re-lation, both the cation configuration and the force of the binding site created may be a determining factor. With each alkaline earth metal ion an interaction can take place that is similar to that with the calcium ion. This conclusion can

be drawn from the isotope experiments with the barium ion, in so far as the [140]Ba uptake was increased by the K-depolarization while the washing out of the isotope taken up was accelerated by the increased extracellular calcium concentration, corresponding to the antagonism of the two ions. Different concentrations of the various alkaline earth metal ions must be regarded as optimum from the point of view of the function of the excitation-contraction coupling. This may be concluded from the change in ion sequence taken as a function of the extracellular concentration. Up to 0.5 mM, the ionic sequence based on the reciprocal hydrated ion radius prevailed, that is $Ba \geqslant Sr > Ca \gg Mg$. From 0.5 mM this changed in favour of the calcium ion, and the sequence $Ca \gg Ba \geqslant Sr \gg Mg$ prevailed, with a strong decrease of the calcium-substituting role of the other alkaline earth metal ions. As several effects of the alkaline earth metal ions on biological systems are known including the action potential, the influence exerted on the membrane permeability, membrane resistance, metabolism, etc., the phenomenon produced is more complicated than the interaction brought about with the surface $Ca^{2+}$-binding sites or stores. It seems to be probable that the lanthanum ion is associated with essentially the same calcium-sensitive membrane binding sites or stores through which the extracellular calcium ion exerts its regulative effect upon the excitation-contraction coupling (GOODMAN and WEISS, 1971). By means of its stronger binding, the trivalent lanthanum ion may fix these membrane binding sites in a configuration established so firmly that they cannot completely fulfil their physiological part in the mechanism of releasing the intracellular calcium ion. This phenomenon may be brought into connection with the effect of the lanthanum ion in inhibiting the potassium contracture, that is similarly to a certain extent a function of the extracellular lanthanum ion concentration. It seems that the mammalian muscles are more sensitive to lanthanum, for the lanthanum ion concentration that resulted in a 100% inhibition of the potassium contracture in the mammalian muscle, could not inhibit the contractility of the heart by more than 50% in the *Helix* heart. All this indicates the supposed differences as regards the density of the binding sites and the sensitivity of the binding site populations.

## Summary

A study was made of the effect of the extracellular potassium concentration on the *Helix* heart contracture, and the calcium-dependence of the phenomenon. As regards the potassium contracture, the calcium-substituting capacity of the alkaline earth metal ions was examined. In this respect, a concentration-dependence was ascertained in the case of barium and strontium. Up to 0.5 mM, bot alkaline earth metal ions were found to be effective calcium-substitutes. At 1 mM, however, only a partial calcium replacement of a strongly reduced degree is found. On the basis of data measured at a 0.5 mM alkaline earth metal ion concentration, the effect exerted on the potassium contracture follows the sequence of the reciprocal hydrated ion radius: $Ba \geqslant Sr > Ca \gg Mg$. At 1 mM this sequence changes, the calcium ion coming strongly into prominence: $Ca \gg Ba > Sr \gg Mg$. In the excitation-contraction coupling the magnesium ion is practically unable to play the part of a calcium substitute.

The barium effect is probably associated with the surface membrane $Ca^{2+}$-binding sites. This can be concluded from the isotope experiments in so far as the K-depolarization, like calcium, can increase the $^{140}Ba$ uptake, while the washing out of the isotope taken up is considerably increased by raising the extracellular calcium concentration, corresponding to the antagonism of the two ions.

The lanthanum ion (0.005–1 mM) could inhibit the potassium contracture, in an interaction with the surface membrane $Ca^{2+}$-binding sites or stores, but to a lower degree than expected, up to a maximum 50%. This points to the lower sensitivity of the Helix heart, as compared to vertebrate muscles.

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Address of the author:

Dr. L. ERDÉLYI

Department of Animal Physiology

A. J. University,

H — 6701 Szeged P.O. Box 428,

Hungary