Acta Biologica Szeged 17 (1—4), pp. 171—184 (1971)

EFFECT OF ION MILIEU AND BARIUM IONS ON THE VENTRICULAR ELECTROCARDIOGRAM OF THE EDIBLE SNAIL (HELIX POMATIA L.)

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(Received December 31, 1970)

The ECG of the heart of edible snail was described already by EVANS in 1912. He established, too, that, the barium ions exerting their effect in a physiological solution cause cardiac arrest and stop electric activity. Since then the literature in this field has become richer by the toxicological investigations of AKVANITAKI and CARDOT (1933) and the publication of SMIRNOV and TURPAEV (1948). There are also numerous publications treating of the effect exerted by the barium ions on the electric activity of various nerve and muscle structures. It is practically impossible to sum up these works completely.

The depolarizing effect of barium ions on various muscles is emphasized by the publications of BÜLBRING and KURIYAMA (1963), SPERELAKIS and LEHMKUHL (1968), ZETT and KÜCHLER (1969).

Fatt and GINSBORG (1958), SPERELAKIS and LEHMKUHL (1966), GERASIMOV and AKOEV (1967), GOMAA (1969) quote the effect of barium ions on different nerve and muscle tissues as initiating spontaneous activity and emphasize their capacity for lengthening the duration of action potential.

On the lobster axon and in respect of the stimulating effect on the release of acetylcholine DOUGLAS, LYWOOD and STRAUB (1961), BLAUSTEIN and GOLDMAN (1968), HAFEMANN (1969) consider the barium ions as substitutes for calcium while on other objects others deny this readiness (ZETT and KÜCHLER 1969).

Fatt and GINSBORG (1958) mention barium and strontium to be very permeable on the muscle membrane. Finally, the comprehensive publication of DIAMOND and WRIGHT (1969) discusses the selectivity rules of alkali-earth metals observed on the biological mebranes.

In earlier publications the present author has also dealt with the effects exerted by the barium ion on the functioning of the isolated hearts of edible snails (ERDÉLYI, 1965, 1967, 1968). The most characteristic of its effect was a strong tonic contracture observed both on smooth muscles and on the myocardium.

Recently I have investigated barium ions in respect to their effect on the electric activity under various ionic conditions in systems containing cations of one, two and more types.

Materials and Methods

In present work, the effects of barium ions on the ventricular action potential of the isolated edible snail (*Helix pomatia* L.) were investigated, as compared with other one-, bi-, or trivalent cations in an ion milieu of more and more complicated composition.

Electrical recording. An extracellular lead took place from the aortic end of the isolated heart ventricle with unipolar direct method, by use of non-polarizable Ag/AgCl electrodes. The leading off electrode was in contact with the arorta while the indifferent one sank into the bath and was connected to the amplifier through the common earth-point. The isolated heart ventricle was fixed at the end of the leading electrode, tightened with a weight of 1 g. at the atrial end cut asunder, and kept it in RIPPLINGER's (1957) oxygenated physiological solution. The monophasic action potentials were observed by means of a DC- amplifier of a DISA Universal Indicator and recorded with COSSOR camera.

The heart ventricle was allowed to beat in oxygenated physiological solution for 20 minutes after preparation. Then the ventricle was raised with its upper third part above the surface of the bathing fluid, the oxygenation was temporarily interrupted and the spontaneous electrical activity were lead off. In JULLIEN-RIPPLINGER's physiological solution (22 °C, pH: 7,4) there could be observed only a minor change in the form of the action potential in summer and autuum, as recorded by this method, even after 24 hours. The system is less sensitive to changes in pH from 4 to 8, but change in temperature causes considerable variation in the appearance of the action potential. The physiological solution was exchanged for further investigations by repeated washing for isotonic NaCl or sodium acetate (116.4 mM) and the effect of some of the following solutions was tested at 22 °C.

Ionic composition of the solutions used;

(1) Calcium-free solution: 112.4 mM NaCl, 4 mM KCl.

(2) Potassium-free solution: 112.4 mM NaCl, 4 mM $CaCl_2$.

(3) Potassium-free solution in which barium ions replace calcium ions:

a) 114.4 mM NaCl, 2 mM $BaCl_2$,

b) 112.4 mM NaCl, 4 mM $BaCl_2$,

c) 108,4 mM NaCl, 8 mM $BaCl_2$.

(4) Sodium-free solution: 112.4 mM Sucrose, 4 mM $BaCl_2$.

(5) Solution containing three kinds of cations:

a) 105,4 mM NaCl, 5 mM KCl, 4 mM $BaCl_2$,

b) 105.4 mM NaCl, 5 mM $CaCl_2$, 4 mM $BaCl_2$.

(6) Solution containing four kinds of cations: 111.4 mM NaCl, 1.87 mM KCl, 1.08 mM $CaCl_2$, 2,39 mM $Na(HCO_3)$, 4 mM $BaCl_2$.

(7) Chloride-free solution: 112.4 mM Na— acetate, 4 mM bariumacetate.

(8) Solution containing 50 percent chloride and 50 percent acetate 58.2 mM Na— acetate, 54.2 mM NaCl, 4 mM $BaCl_2$.

Organic ions (Tris, Cholinchlorid, TEA) were not used at all because the spontaneous electrical activity is strongly inhibited by them.

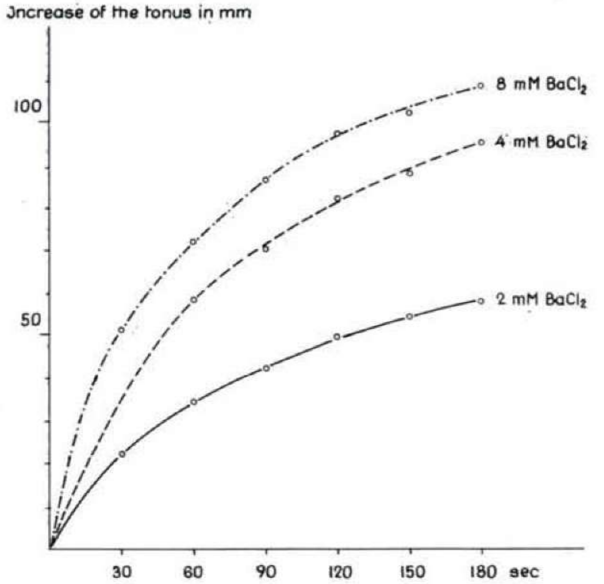
Investigations carried out by use of Ba—140 included the examination of Ba—140 uptake in a system containing 20 μC isotope, NaCl and as a carrier, 4 or 8 mM inactive $BaCl_2$ (at 22 °C,pH = 4,5, total volume: 100 ml).

The was-out curve was plotted by elution in a physiological solution (22 °C, pH = 7,4) after 4 min. uptake period, on the basis of activity measurements of subsequent samples of 1 ml. The hearts examined were put into the eluent after rinsing for three times half minutes. The total volume of the eluent was 100 ml in this case too.

The hearts suspended on a small stand and loaded with a weight were treated a way identical with the control (physiological) experiments. At the investigation of the uptake each of the analysed samples contained four hearts in two parallel systems. In wash-out experiments four hearts were allowed to function in 100 ml physiological solution. The uptake and elution of the isotope were followed on the basis of the gamma radiation of Ba—140.

Results

The action potential obtained from the aortic end of the isolated heart ventricle in unipolar, ectracellular lead is monophasic and can readily be identified with the known biopotential types of the hearts of other molluscs and invertebrates (NOMURA 1965, EBARA 1969, MCCANN and SANGER 1969). The observed cardiogram shows the characteristics of the atrial or pacemaker potentials of the vertebrate heart. As to the time course this electrical activity is readily comparable with the potentials of spontaneusly beating frog heart fragments (DOUARIN, RENAUD, LIGNON and NANOT 1968). The prepotential (diastolic depolari-



Graph. 1. Hypertonic effect of 2, 4, and 8 mM $BaCl_2$, as expressed in the function of time.

zation), with a duration of 500—1000 msec as depending upon the frequency of heart beat (at 22°C), can readily be indentified on the action potentials of the spontaneously functioning heart ventricle loaded with a weight of 1 g. Maximum depolarization of the prepotential is 2 to 4 mV. The duration of the spike potential is 200—500 msec, the peak potential is 12—20 mV in amplitude. The slow repolarization period shows a uniform decay. Sometimes, however a little negative or positive afterpotential may be observed, as well (Fig. 1).

The replacement of the physiological solution by isotonic NaCl results in characteristic changes of the ventricular electric activity. In isotonic NaCl solution the total duration of the potential increases and the spike period merges into the flattening action potential (Fig. 2, I. A., B). This process manifests itself still better in a calcium-free solution, as a result of the depolarizing effect of the potassium ions. In figures C of Fig. 2, the decreasing tendency of amplitudes can be well observed in the 2nd, 3rd, and 5th minutes. In a Na-free solution (isotonic cholinchlorid) the electric and mechanical activity is entirely arrested. If the heart is transferred from the isotonic NaCl into a potassium-free medium, the spike generation recovers, the spike amplitude and duration at-

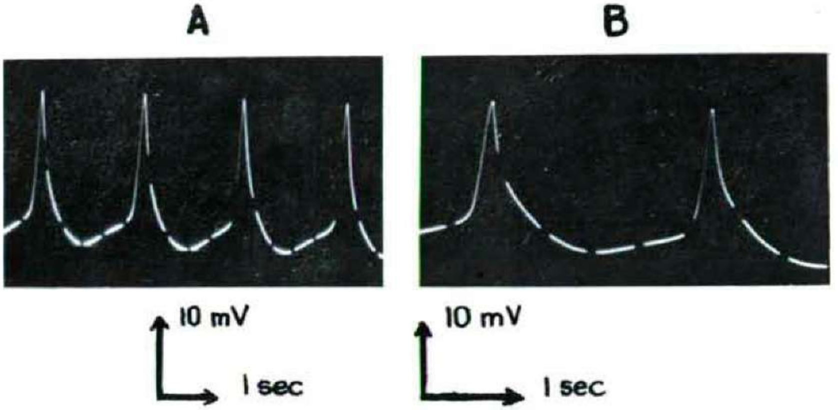


Fig. 1. Action potentials recorded from the aortic end of an isolated heart ventricle in JULLIEN-RIPPLINGER's physiological solution (22 °C, pH = 7,4). Recorded with A = 145 sec/m, B = 96 sec/m film speed.

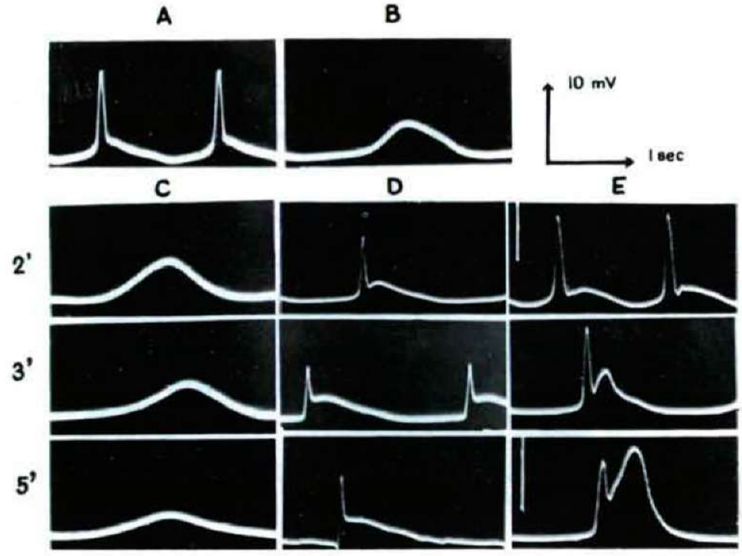
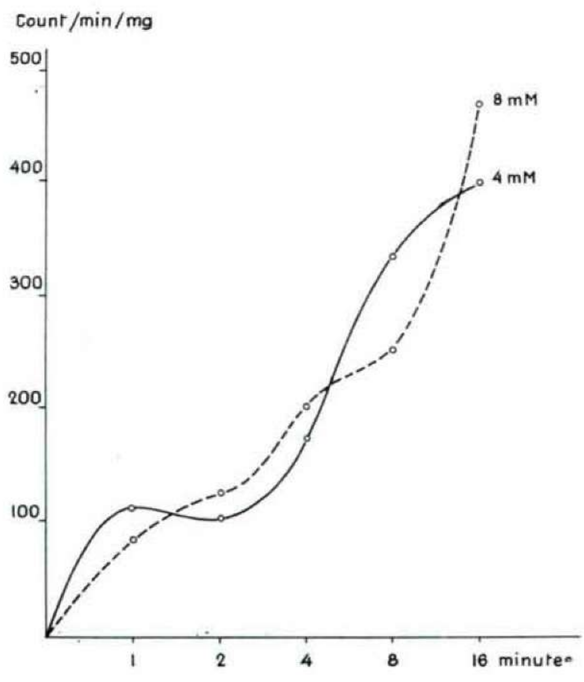


Fig. 2. Action potentials recorded in a medium containing one and two cations. A = control registered in a physiological solution, B = action potentials obtained in isotonic NaCl in the fifth minute, C = in a medium containing 112.4 mM NaCl and 4 mM KCl, in the second, third, and fifth minutes of the effects, D = in a medium containing 112.4 mM NaCl, 4 mM $CaCl_2$, in the second, third and fifth minutes of the effect, E = in a solution containing 112.4 mM NaCl, 4 mM $BaCl_2$, in the second, third and fifth minutes.

tains values observable in the physiological solution. At the same time, the prepotential period is modified, the slow period of repolarization becomes longer and a negativ afterpotential appears. Finally an ordinary spike generation restores with slower heart beat (Fig. 2., D).

Other experiments were directed to explore whether barium ions can substitute for calcium ions in respect of the spike generation. The action potentials seen in Figs. E of Table I were recorded in a medium where in a potassium-free solution barium ions substituted for calcium ions. It can be seen in the figures that the spike generation is unimpaired in the presence of barium ions, the pre potential is, however, less conspicuous.

The most striking change is, anyway, the increase in duration and the appearance of a strong negative afterpotential in the period of repolarization. The effect of barium ions was further analysed by investigating the doses of 2, 4 and 8 mM $BaCl_2$. In graph I, the tonic contracture-inducing effect of these three concentrations can be seen as the function of time, on the basis of several experiments in each case. It is obvious from the curves that the three barium ion doses, until reaching a saturation value, are able to bring the contractile system into a state of tonic contracture of different level, although the time course of contracture is similar in all the three cases.



Graph. 2. Uptake of Ba—140, expressed as fuction of time, from a medium containing 20 μC Ba—140, NaCl, and 4 mM or 8 mM $BaCl_2$. pH = 4.5, temperature 22 °C.

This may be explained on the basis of the concentration dependance of the uptake and supposed localization of barium ions. The experiments dealing with the uptake of Ba—140 actually show differences in respect to the intensity of ion-uptake, in systems containing 4 and 8 mM barium ions. The difference is however is not expressed enough in the concentration range exerting almost maximal physiological effect (graph 2).

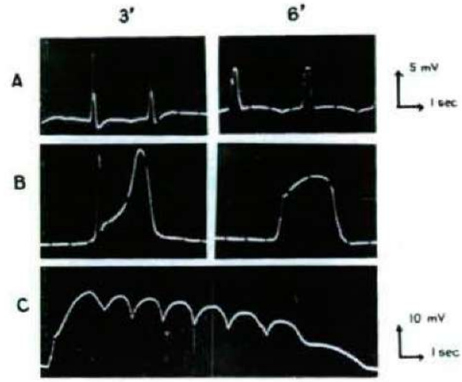


Fig. 3. Effect of 2, 4, and 8 mM $BaCl_2$ on the generation of the action potential. A = Modification of the action potential in a solution containing 114.4 mM NaCl, 2 mM $BaCl_2$, in the third and sixth minutes, B = in a medium containing 112.4 mM NaCl and 4 mM $BaCl_2$, in the third and sixth minutes, C = in a solution containing 108.4 mM NaCl and 8 mM $BaCl_2$, in the sixth minute of the effect.

It is probable that in this phase the course of uptake is disturbed by the exchange diffusion, releasing the isotope already received. So the effect of barium ions resulting in modifications of the action potential, proved to be more interesting. In Fig. 3 the effect of $BaCl_2$ (2 mM A., 4 mM B., 8 mM C) on the spontaneous action potential is demonstrated in the 3rd and 6th minutes after its application. As shown by the figures, the time course of the action potential is prolonged pari passu with the increasing barium concentration, first of all because of lengthening the repolarization phase. In addition, an afterpotential that is proportional to the dose, or rhythmical afterpotential-series are generated.

Other experiments were made to clarify whether the anions present modify the specific effect of barium ions. The presence or abesence of sodium ions has not any major influence on the form of the action potential because in the solution containing 112,4 mM sucrose and 4 mM $BaCl_2$ it is very similar to that observable in presence of sodium ions. In examining the role of anions, chloride ions were firstly substituted by acetate ions wich are considered to be unpermeable on muscle membranes (McCann, 1964). Graph 3 demonstrates the effect of 4 mM $BaCl_2$ or barium acetate in media containing 100 percent chloride, 50 percent chloride-50 percent acetate, and 100 percent acetate, respectively it is apparent from the graph that the tonic contracture of the muscular system is the least in an acetate medium and the highest in a chloride medium, showing that a medium containing the highly permeable chloride anions may bring about more favourable conditions for the development of the hypertonic effect of the barium ions than that containing acetate ions which have rather low permeability. In further experiment the effect on the action potential was investigated. The results of these experiments are shown in the physiological Fig. 4 A of Fig. 4, shows the action potentials observed in the physiological solution, Fig. B illustrates those observed in isotonic sodium acetate. Fig. C demonstrates the effect of 4 mM $BaCl_2$ or barium acetate in a medium containing 100 percent acetate, Fig. D in that containing 50 percent chloride-50 percent acetate, and Fig. E in that containing 100 percent chloride, in the 3rd and 6th minutes after application.

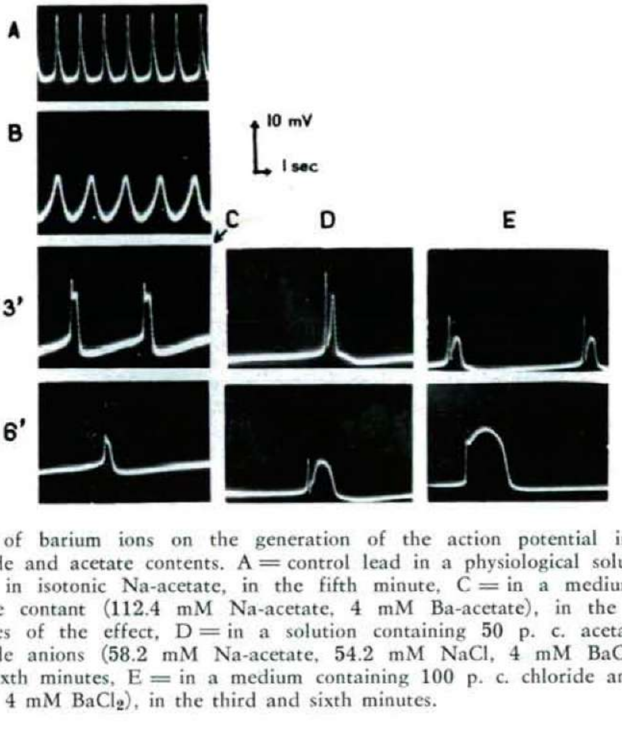
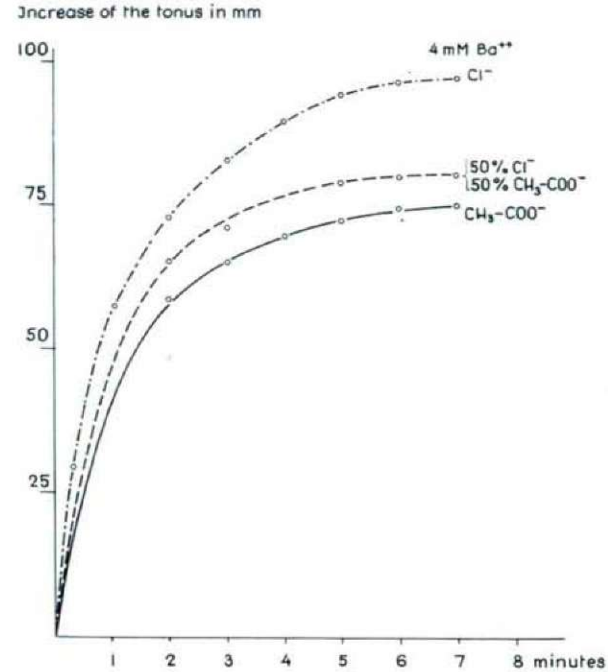


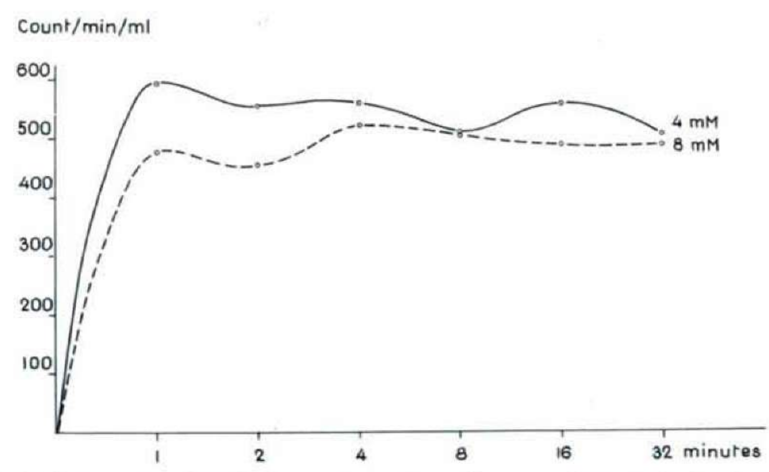
Fig. 4. Effect of barium ions on the generation of the action potential in a medium of chloride and acetate contents. A = control lead in a physiological solution, B = record made in isotonic Na-acetate, in the fifth minute, C = in a medium of 100 p. c. acetate contant (112.4 mM Na-acetate, 4 mM Ba-acetate), in the third and sixth minutes of the effect, D = in a solution containing 50 p. c. acetate — 50 p. c. chloride anions (58.2 mM Na-acetate, 54.2 mM NaCl, 4 mM $BaCl_2$) in the third and sixth minutes, E = in a medium containing 100 p. c. chloride anions (112.4 mM NaCl, 4 mM $BaCl_2$), in the third and sixth minutes.

In an acetate-type medium, in contrast to the medium with chloride content, the decrease of the potential duration is striking, and the negativ afterpotential is also depressed. The changes in the potential duration and in the tonic contracture proved to be entirely unequivocal.

The fact that calcium ions can be substituted for by barium ions in view of spike generation and furthermore that the contracture provoking effect of barium ions can be prevented by calcium ions administered in excess (ERDÉLYI, 1968), suggests that the two ions may be attacked to the same sites and, if both ions are present, there may be a competition for obtaining the same sites of binding. According to HAFEMANN's investigations, the sites of attackment are



Graph. 3. Hypertonic effect of barium ions in media containg 100 p. c. chloride, 100 p. c. acetate, 50 p. c. chloride and 50 p. c. acetate, respectively, plotted as a function of time.



Graph. 4. Wash-out curve plotted after a four min. taking up of Ba—140. Uptake took place from a system containing 20 μC Ba—140, NaCl, and 4 mM or 8 mM $BaCl_2$. Elution was performed in physiological solution: 22 °C, pH = 7.4.

likely also here the O_2-ligands (HAFEMANN, 1969). In this process — according to BLAUSTEIN and GOLDMAN (1968) — barium is more active than calcium. The strong barium-ion binding capacity of the system is shown by the wash-out curves, as well. Graph 4 shows the wash-out curve (in a normal physiological solution) of an isotope taken up for four minutes from a system containing NaCl, 4 or 8 mM $BaCl_2$, as well as 20 μC Ba—140.

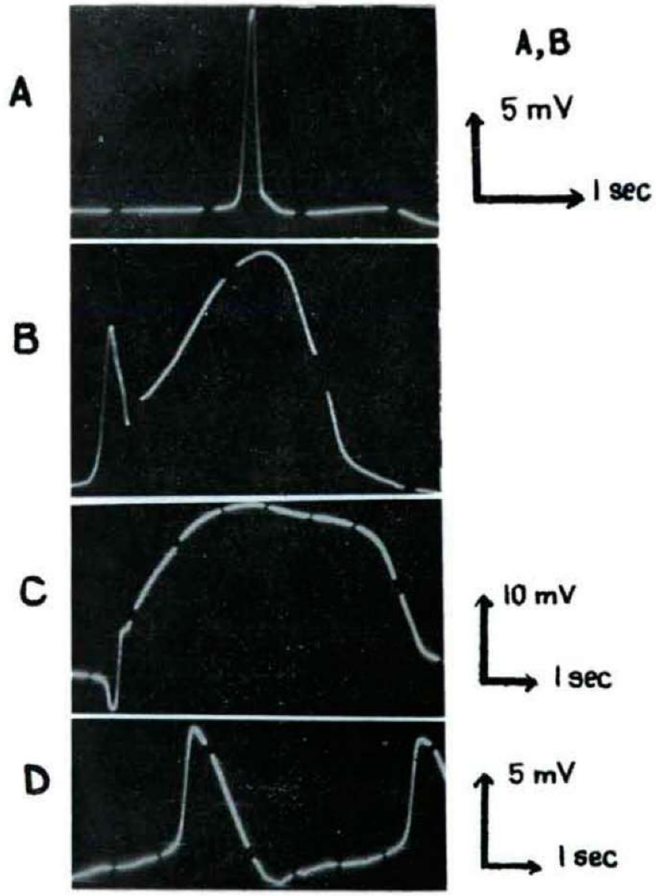


Fig. 5. Effect of barium ions on the action potential of an isolated heart ventricle. A = action potential lead in a physiological solution, B = effect of 4 mM $BaCl_2$ in the third minute, C = in the sixth minute, and D = action potentials lead in a physiological solution changed three times, after being eluted for six hours.

As it is shown by the curves, the delivery of barium ions linked loosely is already completed in the first minute while the firmly linked ions can hardly be released during the 32 minutes of the wash-out. At the end of washing, 53 percent of the Ba—140 taken up from the medium containing 4 mM $BaCl_2$, and

78 percent of that taken up from the medium containing 8 mM $BaCl_2$ were still bound.

A similar firm binding has to be supposed on the basis of the physiological events. Fig. 5 shows the results of an experiment demonstrating the action potentials. Record A of Fig. 5 was made about the heart kept in a physiological solution, record B in the third minute of the effect of 4 mM $BaCl_2$, record C in the sixth minute of that effect. The action potential seen in D was recorded after being washed out for six hours altogether in a physiological solution changed three times. In the course of washing, the gradual decrease in the afterpotential can be observed, the duration becomes shorter, as well, without reaching the original value even at the end of the washing.

Later on, the effect of barium ions was investigated in a milieu where the number of kinds of cations was increased, bringing about in that way more and more complicated conditions on the outer side of the membrane. The results obtained are shown in Fig. 6 A demonstrates here, too, the action potentials recorded in the physiological solution, and Fig. B those in NaCl. The action potentials of Fig. C developed in a medium containing 105.4 mM NaCl, 5 mM KCl, and 4 mM $BaCl_2$, in the second, fourth, resp. sixth minutes of the effect.

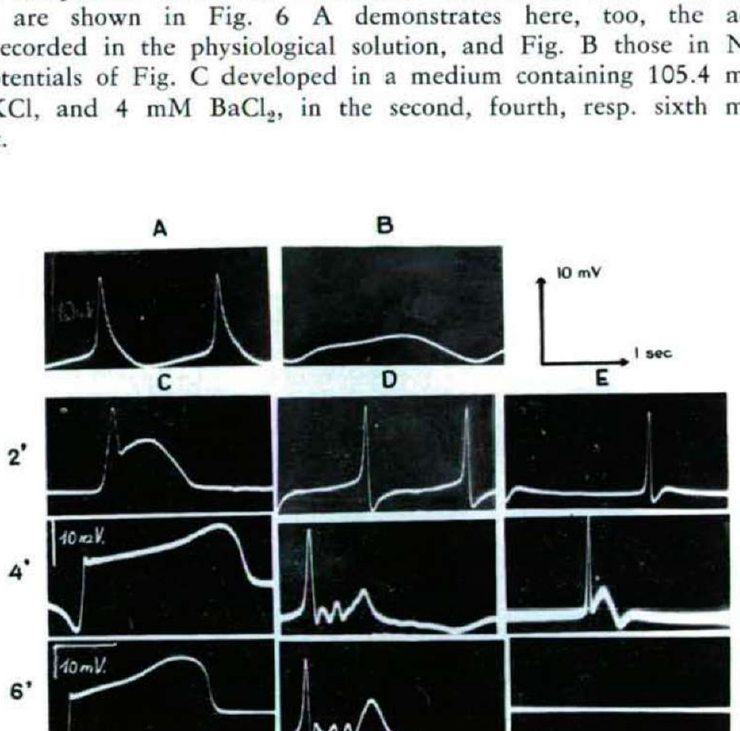


Fig. 6. Effect of barium ions on the generation the action potential in a medium containing three and four kinds of cations. A = control lead in a physiological solution, B = in isotonic NaCl in the sixth minute, C = in a solution containing 105.4 mM NaCl, 5 mM KCl, and 4mM $BaCl_2$, in the second, fourth, and sixth minutes, D = in a medium containing 105.4 mM NaCl, 5 mM $CaCl_2$ and 4 mM $BaCl_2$, in the second fourth, and sixth minutes, E = in a medium containing 111.4 mM NaCl, 1.87 mM KCl, 1.08 mM $CaCl_2$, 2.39 mM $Na(HCO_3)$, 4 mM $BaCl_2$, in the second, fourth, and sixth minutes of the effect.

It is obvious in the Figures that the presence of potassium ions exerts potentiating effect on the action of the barium ions increasing the potential duration, whereas the generation of after potential remains unchanged. In the prepotential and spike phases some changes can be observed, as well. The Fiugres D were obtained in a system containing 105.4 mM NaCl, 5 mM $CaCl_2$, and 4 mM $BaCl_2$. The prepotential is well developed, the spike generation is quite normal. In this experiment, the effect of barium ions is compensated by the calcium ions on the basis of the antagonism of the two ions.

The generation of afterpotentials continues here too. The joint presence of calcium and barium ions results in a membrane oscillation generating positive and negative afterpotentials. Figures E were made in a medium containing four kinds of cations (sodium, potassium, calcium, and barium). Here, probably because of the presence of potassium ions, the generation of positive and negative afterpotentials is depressed, the oscillation is driven back, the duration of prepotential, however, increases; then, from the fifth minute, a total electric silence ensues supposedly as a consequence of the complex interrelations of the four kinds of cations and of the disturbed equilibrium.

Discussion

The action potentials lead off extracellularly from the aortic end of the isolated heart ventricle of the edible snail are of the pacemaker type. From the changes experienced in different ion milieus, the conclusion may be drawn that the myocarium generates an action potential of mixed type, depending on Na—Ca ions. In this respect, we can observe some similarity to the results obtained in the giant neurons of *Aplysia,* as well as in the myocardium of *Mytilus* and the oyster *(Junge,* 1967; *Irisawa, Irisawa* and *Shigeto,* 1969). It seems that in the heart ventricle of the edible snail it is again the spike generation, that is particularly calcium-dependent.

According to our experiments, in this process calcium can be substituted by barium ions too. BÜLBRING and TOMITA (1968) have similarly observed the recovery of action potential in the smooth muscle taenia coli of a guinea-pig in a calcium-free solution in presence of barium ions. Some authors emphasize, however, that in other muscles the barium ions are not able to replace effectively the membrane-stabilizing capacity of calcium ions (ZETT and KÜCHLER, 1969).

The capacity of barium ions for increasing the duration of potentials and generating a negative afterpontential manifests itself in the heart ventricle of the edible snail, too, similarly to the results obtained by FATT and GINSBORG (1958) in lobster muscles, by GREENGARD and STRAUB (1959) in the B, C fibres of the rabbit, by BÜLBRING and TOMITA (1968) in the taenia coli smooth muscle of the guinea-pig. GREENGARD and STRAUB (1959) bring the retarded repolarization effect into connection with the repression of the inactivation of the sodium-carrier system. However, the matter is probably more complicated. This conclusion may be drawn from the publication of SPERELAKIS and LEHMKUHL (1968), as well, who observed an increase of the membrane resistance in a chicken heart culture as a result of barium ions, supposedly because of a decrease in the conductivity of potassium, followed also by a partial depolarization.

It is ascertained by isotopic experiments carried out with Ba—140 that the heart ventricle takes up barium ions. The influx of barium ions may take place along the ascending branch of the action potential. In accordance with the findings of FATT and GINSBORG (1958) in a lobster muscle, the snail heart ventricle can also be said to be highly permeable for barium ions. A considerable part of the ions taken up are bound very firmly and evoke the tonic contracture of the contractile system with an efficiency known in the smooth muscles. Barium is described also by LÁBOS (1967) in the glochidium closing muscle as an ion causing tonic contracture. This effect of barium ions is but hardly affected by the presence of sodium ions. The exchange of chloride however, for acetate ions, that are permeable only in a negligible degree, induces a considerable change both in the development of tonic contracture and in the retardation of the repolarization phase of action potential. The effect inducing tonic contracture may be due to an increase in potential duration by some unknown mechanism.

This is explanable by means of the connection between stimulus and contaction in the way that the barium ions entering the muscle extrude calcium ions thus making the latter ones cause a contracture. According to HAFEMANN's investigations (1969), namely, the excitability of lobster axon is also brought about by the calcium and barium ions throguh a binding site considered as a joint O_2-ligand. At the same time, the barium ions are not able to substitute directly for the specific part of calcium in the contraction. This is demonstrated also by the investigations of GAINER (1968) on a lobster muscle and those of ZETT and KÜCHLER (1969) on a frog muscle.

The increase in the number of kinds of cations in the medium induces more and more complicated conditions on the outer side of the membrane, that also influences the efficiency of barium ions. In the medium containing three cations (sodium, potassium, and barium) potassium ions have potentiating effect upon the action of barium ions increasing the duration of repolarization, correspondence to the tendency of both ions for systolization. The calcium ions, on the other hand, in accordance to the antagonism of the two ions, prevent the effect of barium ions on the duration of potentials. The generation of afterpotential, however remains and, owing to the diastolizing effect of the calcium ions, in the presence of barium ions, the membrane shows oscillations with a rhythmic potential generation.

After all, it seems that the barium ions exert their effect frist of all through the calcium-sensitive functional groups, ether in the way that they can substitute for the latter ions in some calcium-dependent process or by occupying the specifically calcium-sensitive sites, inducing changes in this way in the linked processes of the system. This seems to be supported also by the data of ZETT and KÜCHLER (1969) who found changes in frog's sartorius kept in a medium containing barium, first of all in the calcium content, while the sodium and potassium contents proved to be unchanged.

Summary

The effect of barium ions on the ventricular electrocardiogram of the edible snail (*Helix pomatia* L.) was investigated in different ion milieus and the following results were obtained:

1. The extracellularly recorded ventricular action potentials are of pacemaker type. The action potential is sodium- and calcium-dependent.

2. The spike generation is calcium-dependent but in that process barium ions can efficiently be substituted for the calcium ions.

3. The effect of barium ions exerted on the action potential is similar in a solution of sodium content and in that free of sodium (sucrose). The retardation of repolarization and the appearance of a negative afterpotential or afterpotential-series is characteristic.

4. The exchange of the chloride content of the investigated solution for impermeable acetate moderates the effect of barium ions causing tonic contracture and a retardation of repolarization.

5. In a system containing more kinds of cations the potassium ions potentiate the readiness of barium ions to increase the duration of potentials, while with calcium ions the action potential can be replaced to the physiological level, followed by a afterpotential-series with membrane oscillation.

6. Linked to similar sites, the barium ions exert their physiological and pharmacological effect primarily in the course of the calcium processes.

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