LATE POTENTIATING EFFECT OF LOW-K RINGER SOLUTION ON THE CONTRACTILITY OF THE BULLFROG VENTRICLE

Tsuguhisa EHARA

Department of Physiology, School of Medicine, Kyushu University, Fukuoka, Japan

Summary 1) The electrical and mechanical activity of bullfrog ventricle was investigated during and after a prolonged exposure to a K-depleted fluid, which was applied for the purpose of increasing the intracellular Na ions in exchange for K ions.

2) There was a gradual increase in twitch tension during immersion in the K-depleted fluid in addition to the immediate potentiation by this solution. The action potential was slightly depressed in height during the immersion.

3) There was also a relative reduction in the rate of relaxation of twitch with the gradual increase in tension, and the twitch pattern became a type of rapid onset and slow relaxation.

4) The muscles treated with cold K-depleted fluid for an extremely long period of time (24 hr) showed a similar augmentation in contractility and a particularly high sensitivity to extracellular Na-depletion for generating a Na-lack contracture.

5) Possible mechanisms relating the intracellular ionic environment to the myocardial contractility were discussed, and attempts were made to interpret the results in terms of Na-Ca exchange mechanism across the cell membrane or Na-Ca competition at the subcellular membrane sites.

When muscle is soaked in a K-depleted fluid, the membrane Na-K ATPase, which is responsible for cellular Na extrusion and K uptake, is inhibited (BONTING, 1970), and Na ions progressively accumulate within the cells in exchange for K ions (DESMEDT, 1953). The effect of such an increase in the intracellular Na concentration on the electrical activity of myocardium was the subject of previous investigations (NIEDERGERKE and ORKAND, 1966; EHARA, 1972), and it was demonstrated that Na-loaded frog myocardium exhibits a characteristic action potential with depressed plateau and reduced overshoot.

On the other hand, it has been well established that Na ions in extracellular

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fluid influence the myocardial contractility (WILBRANDT and KOLLER, 1948; NIEDERGERKE and LÜTTGAU, 1957) presumably by affecting the kinetics of activator Ca in a specific manner (NIEDERGERKE, 1963; REUTER and SEITZ, 1968). Therefore, it is an interesting problem from the view point of excitation-contraction coupling whether the intracellular Na ions as well as the extracellular Na ions play a role in the regulation of myocardial contractility. Thus, in the present experiments, the effects of a prolonged low-K perfusion on the contractility of bull-frog ventricle were studied with particular regards to the dependence of contractility upon the intracellular ionic environment.

A preliminary note of the results has already appeared (EHARA, 1973).

METHODS

Preparations, perfusion bath, and apparatus for stimulation and tension recording were essentially the same as described in the preceding paper (EHARA, 1974). Measurement of the transmembrane potential was made by means of conventional glass microelectrodes filled with 3 M-KCl. The bathing solutions used are listed in Table 1. In a first series of experiments, the preparations set in the perfusion bath were initially immersed in normal Ringer solution for 1–1.5 hr until the twitch tension became steady, and then low-K Ringer solution (solution B, Table 1) was applied to the preparations for 2 hr in order to detect the immediate and late effects of K-depletion on the twitch contraction. According to NIEDERGERKE and ORKAND (1966), the heart muscle cells substantially gain Na ions and lose K ions during this treatment. The rate of stimulation was 10 shocks per min throughout.

In a second series of experiments, the muscles containing a large amount of Na ions were prepared, and their contractility was investigated during the course of recovery. The method for increasing the intracellular Na ions was the same as used in the previous report (EHARA, 1972). The dissected muscle strips were laid on a small plate made of hard rubber and fixed onto it with a few insect pins attached to the ends of the muscles. Then, the strips were incubated for 24 hr at

Table 1. Solutions

	NaCl	KCl	CaCl ₂	MgCl ₂	Na-HCO ₃	Na-gluconate	Na ₂ -SO ₄	Na ₂ -HPO ₄	Glucose	Sucrose
A	70.8	2.50	1.0	1.2	25.0	1.9	2.1	3.0	21.0	—
B	70.8	0.16	1.0	1.2	25.0	1.9	2.1	3.0	25.7	—
C	70.8	0.65	1.0	1.2	25.0	1.9	2.1	3.0	24.7	—
D	70.8	0.16	—	1.2	25.0	1.9	2.1	3.0	28.7	—
E	84.9	0.16	0.2	1.2	25.0	1.9	2.1	3.0	—	—
F	—	2.50	1.0	1.2	25.0	—	—	1.0	21.0	157.7

Concentrations given in mm/liter. All solutions were equilibrated with 95% O₂ + 5% CO₂.

1–3°C in a high-Na solution containing only 0.16 mM-K (solution E). The Ca concentration was also kept low (0.2 mM) in order to diminish the intracellular accumulation of Ca ions which was suggested to occur under these circumstances (THOMAS, 1960). One or two strips were kept in 400 ml of the solution which was stirred continuously by bubbling of 95% O₂ and 5% CO₂ gas mixture. After this treatment, the muscles were allowed to recover in normal Ringer solution at 19–20°C. At the time of starting the recovery of muscles, the strips were removed from the plate and mounted in the perfusion bath in the usual manner. Then, the stimulation was begun and the measurements of tension and membrane potential were made for 2–3 hr thereafter. In some experiments, 25%-Na Ringer solution (solution F) was applied to the Na-loaded muscles for an attempt to see whether Na-lack contracture (NIEDERGERKE and LÜTTGAU, 1957) occurs or not.

RESULTS

Changes in contractility during immersion in K-depleted fluid

Figure 1 shows the simultaneous records of twitch contraction and membrane potential obtained before, during, and after immersion in 0.16 mM-K Ringer solution. The results of similar experiments in 12 muscles are summarized in Fig. 2, in which the changes in several parameters for contraction and relaxation,

Fig. 1. Early and late effects of K-depleted fluid on the twitch contraction of bullfrog ventricle. Record a, in normal Ringer solution; b and c, 20 min and 2 hr after application of 0.16 mM-K Ringer solution; d and e, 15 min and 1 hr after reintroduction of normal Ringer solution, respectively. The differential curve of tension (upper trace) and the membrane potential (middle trace) were simultaneously recorded.

i.e. peak tension (T), maximum rate of rise of tension (V_e), maximum rate of fall of tension (V_r), time to peak tension (t_p), and time to maximum rate of rise of tension (t_m) are illustrated. t_p and t_m were measured from the onset of contraction.

When the muscle was soaked in K-depleted fluid, the twitch tension was immediately strengthened, and this potentiation was mainly due to an increase in rate of rise of tension (Fig. 1b). When the immersion in K-depleted fluid was continued, a delayed effect was found to occur. During 2 hr immersion in this fluid, T gradually increased with increase in V_e , whereas V_r rather decreased. Concomitantly, t_m gradually shortened in spite of a prolongation of t_p . Therefore, the muscle soaked in K-depleted fluid for 2 hr exhibited a characteristic pattern of twitch, rapid onset and slow relaxation (Fig. 1c), in contrast to normal. When normal Ringer solution was reapplied to the muscle thereafter, the twitch tension was immediately weakened and the altered twitch pattern gradually recovered in about an hour, although the final steady values of T , V_e , and V_r were considerably

Fig. 2. Changes in the parameters of twitch contraction of bullfrog ventricle during immersion in K-depleted fluid. T , peak tension; V_e , maximum rate of rise of tension; V_r , maximum rate of fall of tension; t_p , time to peak tension; t_m , time to maximum rate of rise of tension. Each point is the mean ($\pm$ S.D.) of 12 experiments, and is expressed as relative to control. At 0 time, the bathing solution was changed from normal Ringer (2.5 mM-K) to 0.16 mM-K Ringer solution, and at 120 min again to normal Ringer solution.

smaller than the initial control values. This kind of depression in contractility seen after immersion in K-depleted fluid probably reflected some toxic properties of the solution affecting the muscle conditions (DESMEDT, 1953).

On the other hand, overshoot and plateau of the action potential were gradually depressed during the immersion (Fig. 1). These changes are due to the intracellular accumulation of Na ions as reported earlier (NIEDERGERKE and ORKAND, 1966; EHARA, 1972). A common effect of low-K Ringer solution, prolongation of action potential, was also seen so that the relaxation of twitch began during the plateau potential (Fig. 1c). Therefore, the relative reduction in rate of relaxation observed during the soaking in K-depleted fluid might be at least partly due to the prolongation of the action potential. It is interesting, however, that the twitch tension tended to increase in spite of the marked lowering of plateau level.

Retarded recovery of twitch pattern in 0.65 mM-K Ringer

K-depleted fluid seems to have at least two distinctive effects on the twitch contraction of bullfrog ventricle; one, the immediate potentiating effect which was

Fig. 3. A. Retarded recovery of twitch pattern in 0.65 mM-K solution. a, in normal Ringer solution; b, 2 hr after application of 0.16 mM-K Ringer solution; c and d, 20 min and 1 hr after further application of 0.65 mM-K Ringer solution; e, 1 hr after reintroduction of normal Ringer solution. B. Effects of soaking in Ca-free K-depleted fluid on the twitch contraction of bullfrog ventricle. a, in normal Ringer solution; b, 10 min after application of Ca-free 0.16 mM-K Ringer solution. The immersion in this fluid was continued for 2 hr thereafter; d, 10 min after further application of 1 mM-Ca, 0.16 mM-K Ringer solution; e, 1 hr after reintroduction of normal Ringer solution. Record c is a superimposed record of the twitches which were becoming stronger at the beginning of the application of Ca-containing K-depleted fluid. Upper traces are the differential curves of tensions. Upper vertical bar is calibration of 100 mg/sec for A and 150 mg/sec for B, and lower one, that of 40 mg for A and 60 mg for B. Horizontal bar, calibration of 1 sec.

already discussed in the preceding paper (EHARA, 1974), and the other, the delayed potentiating effect which is cumulative and accompanies a considerable alteration in the twitch pattern. They were more clearly distinguished in the following experiment. The muscle, which had been soaked in 0.16 mM-K Ringer solution for 2 hr and hence exhibited the altered twitch pattern (Fig. 3Ab), was initially exposed to 0.65 mM-K Ringer solution in stead of normal Ringer solution (2.5 mM-K). The twitch tension was immediately reduced (Fig. 3Ac), but there was only a slight recovery in the twitch pattern throughout the 1 hr immersion in this fluid (Fig. 3Ad). The final exposure to normal Ringer solution for 1 hr produced a sufficient recovery in the pattern and a further reduction in twitch tension (Fig. 3Ae). Thus, the concentration of 0.65 mM-K was still too low to bring about the recovery of the twitch pattern at least within 1 hr, probably because the membrane Na-pump was activated by 0.65 mM-K Ringer solution only incompletely (see DISCUSSION).

Soaking in K-depleted fluid in the absence of Ca

It has been suggested that a prolonged exposure of myocardium to K-depleted fluid induces not only an intracellular accumulation of Na ions but also that of Ca ions (THOMAS, 1960). Therefore, there remains a possibility that Ca ions so taken up by the muscle cells produced the alterations in the twitch pattern by an unknown mechanism. This point was tested in the following experiment. The muscles were soaked initially in K-depleted fluid in the absence of Ca (solution D) in stead of the ordinary K-depleted fluid which contained 1 mM-Ca. The twitch tension was immediately reduced and was persistently very small throughout the immersion in this solution (Fig. 3Bb). After 2 hr, the ordinary K-depleted fluid was applied to the muscle. The tension immediately increased and reached a steady level within a few minutes (Fig. 3Bd). As can be seen, the twitch of the muscle at this time was already associated with the characteristic pattern of slow relaxation. Figure 3Bc is a superimposed record of twitches obtained during the initial short period (a few min) of the second exposure where the twitch tension was becoming stronger in response to 1 mM-Ca. It can be noticed in this figure that the twitch pattern was already characterized by slow relaxation even in the early small twitches.

Since the preceding incubation was made in the absence of Ca, there must have been no intracellular accumulation of Ca ions. Still, it was shown that the twitch pattern was altered in this situation. Therefore, the changes in the pattern generally observed after immersion in K-depleted fluid could not be attributed to the intracellularly accumulated Ca ions.

In this kind of experiment, the final steady tension in normal Ringer solution (Fig. 3Be) was frequently larger than the initial control (Fig. 3Ba). The improved contractility may probably be due to the 'mechanical rest' during Ca-free perfusion.

Contractility of Na-loaded muscles

In the following experiments, muscle strips containing a large amount of Na ions were prepared by soaking in cold K-depleted fluid for 24 hr, and their contractility was studied during the course of recovery. NIEDERGERKE and ORKAND (1966) showed that the frog ventricle which was soaked in 0.2 mM-K Ringer solution for 20 hr gained about 69 mm/kg cell water of Na and lost 92 mm/kg cell water of K.

When Na-loaded muscles were allowed to recover in normal Ringer solution, they began to generate an action potential and twitch tension in response to electrical stimulation. The twitch tension at first decreased to some extent with time and reached a steady level in 20–30 min. This initial decrement in tension may be of nonspecific nature, and probably represents the development of so-called hypodynamia which is also observable in fresh heart preparations at the beginning of driving after dissection (CLARK, 1913; CHAPMAN and NIEDERGERKE, 1970). Figure 4a shows the record obtained when the tension reached the early steady state. As can be seen, the muscle had an action potential with markedly depressed plateau and overshoot, accompanied by a small twitch tension. As the depressed action potential gradually recovered with time resuming the plateau potential, the twitch tension also became larger. The previous examination (EHARA, 1972) showed that the depression of action potential seen at first is due to the increased

Fig. 4. Changes in the electrical and mechanical activity of Na-loaded bullfrog ventricle during recovery. The muscle had been soaked in cold K-depleted fluid (solution E) for 24 hr before it was transferred to warm normal Ringer solution for recovery. Record a, after 25 min of recovery time; b, 45 min; c, 60 min; d, 80 min; e, 95 min; f, 115 min; g, 130 min. Tension (lower trace), action potential (middle trace) and differential curve of tension (upper trace) were simultaneously recorded.

$[\text{Na}]_i$ and that its subsequent recovery is due to the gradual decrease in $[\text{Na}]_i$ produced by the activity of Na-pump. After about 90 min of recovery time, the tension reached an maximum level (Fig. 4e), and then began to decrease again and reached a final steady level which is about a half of the maximum (Fig. 4g). During this later period of recovery where the tension decreased with time (Fig. 4e-g), there was no parallelism between tension and action potential, which was apparent during the earlier period (Fig. 4a-e).

The biphasic time course of the changes in contractility is graphically shown in Fig. 5 in which the changes in several contraction parameters are illustrated. To build up the graph, which summarizes the results of 18 experiments, a selection of several time points was needed for each preparation with regards to the maximum and minimum of peak tension during the course of experiment, since the exact time course of recovery was a little but not negligibly different depending upon the preparations. The selected time points were as follows; t_1 , the time, the twitch tension reached the early steady level; t_3 , the time, the tension achieved the maximum level; t_5 , the time, the tension reached the final steady level; t_2 and t_4 were the middle points between t_1 and t_3 , and between t_3 and t_5 , respectively. Then, the value of respective contraction parameter was measured at each time point in each preparation, and was converted to the value relative to the value at t_5 which

Fig. 5. Changes in the parameters of twitch contraction of Na-loaded bullfrog ventricle during recovery. The muscles which had been soaked in cold K-depleted fluid (solution E) for 24 hr were transferred to warm normal Ringer solution for recovery. Closed circles, peak tension (T); open circles, maximum rate of rise of tension (V_c); crosses, maximum rate of fall of tension (V_r); open triangles, time to peak tension (t_p); closed triangles, time to maximum rate of rise of tension (t_m). Each point is the mean of 18 experiments and is expressed as relative to the final steady values. Further explanations are in text.

was regarded as the control in this experiment. Thus, the data obtained from 18 muscles were collected and averaged in respect to each parameter and each time point (Fig. 5). When the exact time of t_1 , t_3 , and t_5 was averaged among 18 preparations, 32 ± 12 min, 97 ± 20 min, and 160 ± 29 min were obtained, respectively.

As can be seen in the figure, t_p increased markedly during the period from t_1 to t_3 . Therefore, the increase in T observed therein could be attributed not only to the increase in V_e but also to that in t_p . These changes could be explained by the increase in the degree and duration of muscle activation, since this early period was the time during which the action potential progressively became large in height and duration (Fig. 4). On the other hand, at t_3 when T attained to the maximum, V_r was relatively smaller than V_e ($P < 0.05$). It was also found that t_m was significantly shorter than control ($P < 0.05$) at this time, although t_p already recovered nearly to the control level. Therefore, the twitch pattern at t_3 was characterized by slower relaxation and shorter t_m , compared with the final pattern at t_5 . Considering that there was rather a finishing improvement in the shape of the action potential during the later recovery period (from t_3 to t_5), it is obvious that these changes in mechanical and electrical activity of muscles occurring during this time were quite similar but reverse to those observed when the fresh preparations were exposed to K-depleted fluid for 2 hr in the preceding study (Fig. 1).

Na-lacking contracture in Na-loaded muscle

It is known that a moderate Na-depletion in external fluid potentiates the twitch contraction of myocardium and that an extreme Na-depletion beyond a

Fig. 6. Na-lack contracture examined in Na-loaded bullfrog ventricle. The muscle which had been soaked in cold K-depleted fluid (solution E) for 24 hr was transferred to warm normal Ringer solution for recovery. After 20 min of recovery time (record a), stimulation was ceased and 25%-Na Ringer solution (solution F) was applied to the muscle for a short time indicated by a pair of arrows. A contracture was seen. Record b, a similar experiment after 50 min of recovery time. No contracture was elicited in this case. Upper vertical bar is calibration of 200 mg/sec, lower one, that of 50 mg. Horizontal bar is calibration of 1 min.

certain threshold concentration elicits a contracture in this tissue (NIEDERGERKE and LÜTTGAU, 1957). In the present experiments, it was found that Na-lacking contracture was easily produced in Na-loaded bullfrog ventricle but became less detectable in the same muscle as the time of recovery went by. Figure 6 shows these behaviours of a Na-loaded muscle. Similar results were also obtained from other muscles. Generally, contracture by 25%-Na Ringer solution was producible in Na-loaded muscles only during the first 40–60 min of recovery time.

Since preliminary experiments showed that, in fresh muscles, 25%-Na Ringer solution increased only the twitch tension but never elicited a contracture, it was concluded that Na-loaded muscles had higher sensitivity to Na-depletion for generating a contracture tension compared with fresh muscles but that the sensitivity gradually declined as the muscles recovered from the state of Na-loading.

DISCUSSION

It has been well known for some time (RINGER, 1883) that a reduction of K concentration in external fluid immediately potentiates the twitch tension of myocardium. In the present investigation, it was found that the contractility of bullfrog ventricle was further strengthened by a prolonged exposure to this solution, in addition to the initial positive inotropic response. As to the changes in the membrane electrical activity, the plateau of the action potential was rather depressed during the exposure to K-depleted fluid, and there seemed to be no simple relationship between electrical activity and mechanical activity of the muscle explaining the delayed potentiation.

It is known that, when soaked in K-depleted fluid, heart muscle cells, as well as skeletal muscle cells, gain Na ions and lose K ions because the membrane Na-pump is inhibited under K-deficient conditions, and that Na-loaded muscle cells, when immersed in a solution containing a proper amount of K, extrude Na and absorb K against their concentration gradient by the Na-pump (BONTING, 1970). KEENAN and NIEDERGERKE (1967) showed that, after 2 hr immersion in K-depleted fluid, $[Na]_i$ and $[K]_i$ of frog ventricle changed from 6 to 46 and from 163 to 108 mm/kg cell water, respectively. Similar results have been reported in skeletal muscle (DESMEDT, 1953). The muscle cells of bullfrog ventricle used in the present experiments, therefore, are considered to have gained Na ions and lost K ions appreciably during the exposure to K-depleted fluid, though accurate estimation was not possible. The previous and present observations that the plateau of the action potential was gradually depressed during the immersion are explainable from the increased $[Na]_i$ (EHARA, 1972).

With these considerations, it was tentatively concluded that the delayed changes in contractile properties of the muscle observed during the exposure to K-depleted fluid was due to the intracellular Na accumulation and/or K loss which were brought about by the inhibition of Na-pump, and that the reintroduction of

normal Ringer solution activated the Na-pump and restored the intracellular ionic conditions, thereby producing a recovery of twitch pattern. It must be mentioned that the intracellular Ca accumulation which might have occurred under K-deficient condition (THOMAS, 1960) played no appreciable role in producing these effects, as discussed in connection with Fig. 3B.

The role of the intracellular Na and/or K was further indicated by the experiments on the muscles which were made to accumulate a large quantity of Na ions in exchange for K ions through an overnight treatment with K-depleted fluid. At the time when the twitch tension attained to the maximum during the course of recovery, the twitch pattern was characterized by slower relaxation and shorter t_m which was very similar to that observed in the first series of experiments on the fresh preparations. Furthermore, the subsequent decline of tension accompanied a relative increase in rate of relaxation and an increase in t_m , while there was no corresponding alteration in the action potential configuration (Fig. 4). The train of events can be explained as follows. The Na-loaded (and K-deficient) muscles had high contractility, their twitch pattern being a type of slow relaxation and short t_m . The contractility decreased with time during the immersion in normal Ringer solution because the activation of membrane Na-pump by this solution restored the intracellular ionic conditions. The reduced twitch tension at the initial recovery period was simply due to the depressed membrane activation, *i.e.* small action potentials, which were also related to the increased intracellular Na ions (EHARA, 1972).

That the Na-loaded muscles had high contractility was also shown in the contracture experiments where the Na-loaded muscles generated contracture tension in response to 25%-Na Ringer solution which could not produce a contracture in fresh preparations.

For interpretations of the present findings, possible mechanisms relating the intracellular ionic condition to the myocardial contractility have to be considered. REUTER and SEITZ (1968) proposed a Na-Ca exchange mechanism in which Ca efflux across the cell membrane is linked with Na influx. GLITSCH *et al.* (1970) expanded the idea for the coupling of Ca influx and Na efflux and suggested a possible role of the intracellular Na ions in the regulation of the intracellular free Ca concentration. According to their model, it is possible that an increase in $[Na]_i$ promotes Ca influx during an action potential and hence brings about a potentiation of twitch contraction.

The present results showed that an increase in $[Na]_i$ not only strengthened the twitch tension of bullfrog ventricle but also changed the twitch pattern into a type of slower relaxation and shorter t_m . These effects are similar to those of the reduction of $[Na]_o$ (GOTO *et al.*, 1972a; EHARA, 1974). It was also shown presently that Na-lacking contracture was more easily elicited in Na-loaded muscles. These findings, in accord with the theory of Na-Ca exchange mechanism mentioned above, seem to make it possible that the internal and external Na ions have a

reverse action on the myocardial contractility or that the concentration gradient of Na ions across the muscle membrane is an important factor for controlling the contractility. Thus the tension and the rate of relaxation of twitch in myocardium may depend upon the concentrations of both external and internal Na ions. Recently, GOTO *et al.* (1972b) have studied on this point in bullfrog atrium with voltage clamp technique and suggested that the electrochemical potential gradient of Na ions across the membrane regulates the rate of relaxation of twitch and also plays a specific role in generation of tonic tension in this tissue.

On the other hand, certain interactions between Na ions and Ca ions at the subcellular sites could explain the increased contractility and slower relaxation of Na-rich bullfrog ventricle observed in the present experiments. There have been reported several *in vitro* evidences suggesting that the ability to accumulate Ca ions of the cardiac subcellular fractions isolated from mammalian heart is influenced by other alkali-metal ions. KATZ and REPKE (1967) showed that the rate and extent of Ca uptake by cardiac microsome is greater when K instead of Na is present in the incubation medium. PALMER and POSEY (1967) revealed that Ca uptake of cardiac sarcoplasmic reticulum is inhibited when Na concentration is high. DRANSFELD *et al.* (1969) found a similar inhibitory effect of Na and a facilitatory effect of K on Ca uptake by cardiac mitochondria and pointed out that the Na-K ratio in the incubation medium is the important factor determining the rate and amount of Ca uptake. Although it is known that sarcoplasmic reticulum is poorly developed in frog heart (STALEY and BENSON, 1968; SOMMER and JOHNSON, 1969), other subcellular membrane systems in this tissue including much extending sarcolemma or mitochondria may act as a Ca-releasing and Ca-sequestering system, while they may have certain sites for Na-Ca competition (VASSORT, 1973).

It is clear, therefore, that there are at least two possible mechanisms by which the intracellular Na ions affect the kinetics of the activator Ca, and hence modulate the myocardial contractility. One is Na-Ca exchange mechanism across the muscle membrane and the other, Na-Ca competition at certain subcellular sites. Which is more relevant to the present findings is still a difficult problem because of insufficiency of reliable data. In addition, it must be mentioned that there is at present little evidence even to rule out a role of intracellular K ions, the amount of which usually changes concomitantly with that of Na ions. A change in $[K]_i$, as that in $[K]_o$ (EHARA, 1974), might affect the mechanism of myocardial excitation-contraction coupling in an unknown manner.

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