THE DECREASE IN THE AUTOMATISM OF THE PURKINJE PACEMAKER FIBERS PROVOKED BY HIGH FREQUENCIES OF STIMULATION

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It has been shown previously in the dog heart that the activation of either its A. V. node¹⁷ or its false tendon⁸⁹ at frequencies higher than the spontaneous, produces a decrease in the automatism of these structures. The magnitude of this decrease was closely related to the frequency and to the duration of the period of stimulation. An increment in either of these two parameters provoked a large decrease in the automatism. This fact suggested that some accumulative changes could be responsible of the decrease in the automatism. If these changes were of ionic nature, the alteration in ionic concentration would in turn modify the characteristics of the cell membrane and therefore reduce the spontaneous activity. To explore this possibility we have inserted micropipettes into Purkinje fibers of a false tendon of the dog heart and recorded the action potentials generated under different experimental conditions.

METHODS

The experiments were performed on false tendons from the right ventricle of a dog heart. The right ventricle was opened and a papillary muscle with its attached false tendon was excised and fixed in a small chamber (20 ml) filled with flowing Tyrode solution at 37°C. The composition of the Tyrode solution (pH 7.2) was as follows: NaCl 137 mM, KCl 2.7 mM, CaCl₂ 1.8 mM, MgCl₂ · 6H₂O 0.5 mM, NaH₂PO₄ · H₂O 0.4 mM, NaHCO₃ 12.0 mM, dextrose 5.5 mM. The gas mixture aerating the solution was 95 per cent O₂ and 5 per cent CO₂.

When the concentration of either KCl or NaCl of the Tyrode solution was modified, the osmolarity was maintained by equimolar amounts of dextrose. In the experiments in which the effects of KCl and NaCl were studied, the replacement of the normal Tyrode solution was accomplished rapidly.

The false tendon was stimulated either through fine platinum wires or glass micropipettes, both connected to a square pulse generator. The strength of the stimuli ranged from 1.5 to 2 times the threshold value.

The transmembrane potentials from the Purkinje fibers were recorded with glass micropipettes filled with 3M KCl, made according to the techniques of LING and GER-

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ARD¹⁴) and NASTUK and HODGKIN¹⁶). The recording microelectrodes (10-25 Megohms) were connected to one end of a cathode follower with a grid current less than 10^{-13} amps; the other end was grounded through the solution of the chamber. The action potentials were amplified by means of d.c. preamplifiers (P6 Grass) and recorded in a double-beam cathode-ray oscilloscope (Tektronix 565).

RESULTS

Simultaneous recordings from the two ends of an excised false tendon showed that in general, the spontaneous impulses were generated in the thicker segment of the false tendon. The potentials recorded from this segment preceded in time the action potentials recorded from any other site of the false tendon. After the tendon was cut between the two recording electrodes, the automatism of both portions became different; the frequency recorded from the thicker segment was higher than that of the other segment.

The two types of Purkinje fibers from a false tendon. When simultaneous

FIG. 1. The two types of Purkinje cell transmembrane potentials from a false tendon. Simultaneous records from two different Purkinje fibers separated each other by 8 mm. The upper trace corresponds to the electrical activity of a pacemaker fiber and the lower to the response of a fiber being activated by the impulse generated in the pacemaker. Note that the pacemaker potential has a steeper slope of diastolic depolarization and a longer plateau as compared to the non-pacemaker action potential. The records in B are the same as in A, but with higher gain to show that both action potentials have different slopes during diastole. Observe in A that the action potentials from the two Purkinje cells have similar amplitude. Calibrations, 250 msec; 25 mV in A and 12.5 mV in B.

records were taken from two Purkinje cells 5 to 10 mm apart, it was usually found that their transmembrane potentials differed in shape (FIG. 1). One of the cells generated potentials with characteristics similar to those of pacemaker-like action potentials. The main features were a steep slope of the diastolic depolarization and a pronounced plateau that lengthened the repolarization phase. The other cell generated potentials which had an extremely slow diastolic depolarization and a shorter plateau. The two types of action potentials had a similar amplitude.

Changes in the automatism of the pacemaker Purkinje fibers. When a false tendon, with spontaneous activity, was stimulated during brief periods (5-40 sec) with progressively increasing frequencies, its automatism decreased

FIG. 2. Reduction in the automatism of Purkinje pacemaker fibers provoked by a brief period of high frequency stimulation. Spontaneous activity from two different Purkinje fibers (A). Through one of the recording microelectrodes, square pulses of progressively increasing frequencies (up to 6 per sec) were applied and the corresponding responses recorded through the other micropipette (B). After 15 sec of stimulation the responses from the stimulated cell were recorded again (C). Note the reduction in the spontaneous activity and in the amplitude of the action potentials which have in addition, a slight diastolic depolarization. After a short time (25-30 sec) the frequency, the amplitude and the diastolic depolarization of the spontaneous responses gradually recovered (D). Calibrations, 1 sec and 50 mV.

immediately after the period of stimulation. This phenomenon is illustrated in FIG. 2, in which two Purkinje fibers were impaled and their spontaneous activity was recorded (FIG. 2A). One of the micropipettes was chosen for stimulation of a pacemaker Purkinje fiber. The depolarizing square pulses applied were twice the threshold, 1 msec duration and the frequency was gradually increased up to 5 times that of the spontaneous activity (FIG. 2B). The corresponding responses to the applied stimuli were recorded from the other Purkinje fiber in order to know if the false tendon was following such a frequency. In every experiment the frequency of stimulation was lower (5-6 per sec) than the maximal frequency followed by the Purkinje cells (8-9

FIG. 3. Relationship between the characteristics of the stimulation and the magnitude of the reduction in the automatism. In the ordinates (A and B) the magnitude of the reduction in the spontaneous activity in times fold of the control frequency. Abscissae in A, different frequencies of stimulation. The period of stimulation for each frequency lasted 30 sec. Observe that the higher the frequency larger the reduction. Abscissae in B, duration of the period of electrical stimulation. The same frequency of stimulation (6 per sec) was applied during different periods of time. Note that the longer the period of stimulation greater the reduction in the automatism.

per sec). Immediately after the period of 15 seconds of stimulation the spontaneous activity of both fibers was considerably reduced (FIG. 2C). The magnitude of this reduction varied from one experiment to another but in every case the automatism recovered gradually until the intervals between the spontaneous responses were similar to those occurring before the period of stimulation (FIG. 2D).

After the period of high frequency stimulation the corresponding transmembrane potentials of the first and subsequent spontaneous responses, had a slighter slope of their diastolic depolarization (FIG. 2C) as compared to that of the spontaneous control responses (FIG. 2A). The reduced diastolic depolarization gradually became steeper until it reached the slope previous to the period of stimulations. Similar observations were made for the amplitude of action potentials which was also reduced during the gradual recovery of the spontaneity (FIG. 2A and C).

The magnitude of the reduction in the automatism was determined by several factors, three of which are as follows. a) There was a relationship between some of the parameters of the stimuli and the magnitude of the reduction. This relationship is illustrated in FIG. 3A in which for a given period of stimulation, as higher the frequency, the time for the appearance of the first spontaneous response was longer. For a given frequency of stimulation (FIG. 3B) the longer the period of stimulation, the larger was the magnitude of the decrease in the automatism. b) An increase in the external KCl concentration (25 or 50 per cent) produced a larger reduction in the

FIG. 4. Effect of high extracellular potassium concentration on the reduction in the automatism produced by high frequency stimulation. In A, simultaneous records of the spontaneous activity from two Purkinje fibers. Observe that in B, under normal Tyrode solution, a small reduction appeared after stimulating (3 per sec) during 15 sec, while in D under high KCl (3.4 mM), the reduction in the automatism is greater. The records in C were taken after 15 min of exposure to high KCl. Note in C that the rate of the diastolic depolarization of the spontaneous action potentials was reduced by the high KCl. Calibrations, 1.5 sec; 50 mV.

automatism than that obtained under normal KCl concentration. The experiment illustrated in FIG. 4 shows that for a given frequency and period of stimulation, the decrease in the automatism of Purkinje fibers was greater when the false tendon was immersed in a high KCl concentration (3.4 mM) Tyrode solution (FIG. 4C and D). When the preparation was maintained under high potassium for longer times (40–50 min) this effect was enhanced. With higher concentrations of extracellular KCl (4.1 mM) the reduction was even more accentuated and occurred within a shorter period of time (Fig. 6A). The opposite effect was observed when the KCl concentration of the Tyrode solution was lowered from 2.7 mM to 1.9 or 1.3 mM. These latter concentrations reduced, and in some experiments abolished, the effect produced by high frequency stimulation on the spontaneous activity. c) A decrease in the external NaCl concentration (12, 20 or 25 per cent) provoked changes in the automatism of the Purkinje fibers (FIG. 5) similar to those described for high KCl concentration. The magnitude of the reduction in the automatism pro-

FIG. 5. Effects of low extracellular sodium concentration on the reduction in the automatism of Purkinje fibers. A, spontaneous responses. In B, a pacemaker Purkinje fiber was stimulated at 5 per sec during 10 sec and the corresponding responses recorded from another Purkinje cell. Immediately after the cessation of the stimuli, the frequency of the spontaneous activity decreased. After several seconds the automatism gradually recovered. The amplitude of the first responses was slightly diminished. In C, the external NaCl concentration was decreased (103 mM). The records in C were taken at 24 minutes of exposure to low NaCl. The magnitude of the reduction in the automatism was greater (C) than under normal Tyrode solution (B). Observe that the first responses appearing after the period of stimulation have an extremely slow diastolic depolarization. Calibrations, 2 sec; 50 mV.

duced by the high frequency stimulation was proportional to the decrease in concentration of sodium chloride. The reduction in the automatism occurring after the stimulation was greater during exposure of the tendon to a lower sodium concentration (FIG. 6B).

FIG. 6. Effects of sodium and potassium ions on the reduction in the automatism of Purkinje fibers produced by a period of high frequency stimulation. Ordinates, decrease of the automatism in time fold of the interval between spontaneous responses previous to the period of stimulation. Abscissae, time in minutes of exposure of the tissue to the modified Tyrode solution. In A, the external KCl concentration was increased in 25 and 50 per cent (3.4 and 4.1 mM). Note that for higher potassium concentration, the reduction in the automatism is greater and appeared within a shorter time. In B, the external NaCl concentration was reduced in 12, 20 and 25 per cent (120.5, 109.6 and 102.7 mM). Observe that the magnitude of the reduction in the automatism was closely related to the magnitude of the change in NaCl concentration.

Effects of potassium and sodium ions on the slope of the diastolic depolarization of the pacemaker Purkinje action potentials. The spontaneous activity of Purkinje fibers was substantially reduced by the change in external KCl or NaCl concentration. An increase of 25 to 50 per cent in the external KCl concentration produced, concomitantly with the reduction in the frequency of the automatism, a decrease in the rate of diastolic depo-

FIG. 7. Changes in the slope of diastolic depolarization and in the frequency of automatic responses of pacemaker Purkinje fibers produced by high KCl and low NaCl. Spontaneous activity of two Purkinje cells from a false tendon. In A, the preparation was immersed in normal Tyrode solution and in B, after 15 min of perfusion with high KCl (4.1 mM). Note that the rate of diastolic depolarization was decreased and the interval between two spontaneous responses was longer. A similar effect was produced in D by the reduction in 20 per cent in external NaCl as compared with the control potentials in C. Calibrations, 500 msec and 25 mV.

larization (FIG. 7A and B). Similar effects were observed during the diminution (20 per cent) in external NaCl concentration. As it is shown in FIG. 7, the diastolic depolarization of Purkinje fiber transmembrane potentials became slower after 10 minutes of exposure to low sodium (FIG. 7C and D).

Modification of the Purkinje fibers resting membrane potential after the period of high frequency stimulation. When the resting membrane potential

was measured from 4 to 5 seconds after the cessation of the period of high frequency stimulation, it was found that the membrane was partially depolarized and concomitantly a reduction in the frequency of spontaneous activity was observed. There was a relationship between the percentage of the decrease in membrane potential and the magnitude of the reduction in automatism. As it can be seen in TABLE 1, the greater the changes in membrane potential, the larger the diminution in spontaneous activity.

TABLE 1.
Relationship between the percentage of the decrease in membrane potential, occurring after high frequency stimulation, and the magnitude of the reduction in the automatism.

Membrane potential			Magnitude of the reduction in automatism in time fold of the control
Control	Decrease during reduction in automatism		
(mV)	(mV)	(per cent)	
89.0	83.5	6.1	1.9
81.5	70.5	13.5	3.3
81.5	72.3	11.0	3.7
89.0	70.5	20.8	4.0
81.5	70.5	13.5	4.5
81.5	73.4	10.0	5.1
74.2	64.8	12.8	5.9
89.0	72.3	18.8	7.0
96.2	66.7	30.8	7.3
89.0	66.7	25.0	8.3
89.0	68.6	22.9	9.0
96.3	55.6	42.3	15.8

Non-propagated potentials appearing during the reduction in the automatism.

In some experiments after the period of high frequency stimulation, small rhythmic potentials with slow rate of rise appeared during the abolition of the automatism. The amplitude of these potentials augmented gradually and when they reached a certain value, an active spontaneous response occurred (FIG. 8). The magnitude of these small potentials ranged from 1 to 30 mV. The frequency of their appearance varied from one experiment to another, but it was always slower than that of the spontaneous propagated responses. These small potentials were also observed under high KCl (3.4 mM and 4.1 mM) specially when the reduction in the automatism, elicited by the period of high frequency stimulation was of a greater magnitude. The non-propagated nature of the small and rhythmic potentials can be observed in FIG. 8A. In

this case the prepotential was generated in a pacemaker Purkinje fiber but it did not depolarize the non-pacemaker cell.

FIG. 8. Non-propagated potentials appearing during the reduction in the automatism produced by high frequency stimulation. In A, a small potential (upper beam) was recorded from the Purkinje fiber which had been stimulated at a high frequency. Note that the other Purkinje fiber did not show any depolarization. When the spontaneity recovered the amplitude of the small potential increased and propagated to the other cell. Observe that its action potential (lower beam) is not preceded by a slow depolarization, indicating that the cell is a non-pacemaker. Calibrations, 500 msec and 50 mV. Two different experiments (B and C) which show that the small potentials may appear simultaneously in two pacemaker Purkinje cells separated each other by 2 mm and that they gradually grew in amplitude until propagated responses are produced. Note in C that the frequency of the rhythmic small potentials is slower than that of the propagated responses (due to the high gain, the action potentials are not seen). Calibrations, for B, 500 msec and 25 mV; for C, 1.2 sec and 25 mV.

DISCUSSION

The existence, in an excised false tendon, of two different types of trans-membrane potentials, suggests that apparently there are two groups of Purkinje fibers with different membrane characteristics. One of the types can be considered as pacemaker, because of the slow diastolic depolarization²¹⁻²⁴. The other type possesses a rapid upstroke, and was activated by the pacemaker cells. As the false tendon from a dog heart also contains myocardial fibers, it is possible that the action potentials with a rapid upstroke were

generated in a myocardial cell rather than in the Purkinje fiber. This was not the case, since the action potentials from the myocardial cells have a faster repolarization than those of the Purkinje fibers. Although the two types of Purkinje potentials described above (FIG. 1) differ also in the duration of their plateaus, this difference is negligible if compared with that existing between typical myocardial and Purkinje fibers. In addition, both types of Purkinje action potentials appeared almost simultaneously in contrast to the latency observed between the activation of Purkinje and myocardial fibers. This latency, as has been shown by ALANIS *et al.*^{1,2)} is of considerable magnitude (10 to 32 msec) and is always present between these two different fibers even when they are a few microns apart.

Although the two types of Purkinje cells seem to be arranged at random all along the false tendon, there is a segment of the tendon with higher rate of spontaneous activity. In other words, there is a gradient in automatism from the thicker segment, which in general has the highest frequency, to the thinner parts and branches of the false tendon. This gradient is also observed for the threshold values when driving the preparation by electrical stimuli, i.e. the excitability is higher in the thicker segment. This is in agreement with some observations reported by TRAUTWEIN²⁰⁾ regarding the possibility of two areas in the same false tendon with independent pacemaker activity.

When a Purkinje cell, with pacemaker activity was stimulated at a frequency higher than the spontaneous, the corresponding transmembrane potential changed its shape. The slow diastolic depolarization was considerably reduced in such a way that the pacemaker-like potential became a "non pacemaker" (FIG. 9). In other experiments the action potential from a driven Purkinje cell, with no diastolic depolarization, was transformed into a pacemaker-like response when the propagated impulse from the pacemaker region was blocked by various procedures. These facts suggest that all the Purkinje fibers from a false tendon are capable of becoming pacemaker when disconnected from the cells which were driving them.

The experimental conditions in which the false tendons have been studied, i.e. excised and perfused, might suggest that the Purkinje fibers are not in good physiological conditions. The reduction appearing after high frequency stimulation could be due to either a decrease in the electrical excitability or to an exhaustion of metabolic processes. None of these possibilities seem to be involved in the present experiments, since the Purkinje fibers were always stimulated at frequencies lower than the maximal rate that they could followed. In addition, the application of an electrical pulse immediately after the period of stimulation, elicited the corresponding response at the time when the spontaneous activity was considerable reduced or abolished.

We have no definite experimental evidence to explain the reduction in the automatism. However, the observed relationship between the parameters of

FIG. 9. Changes in the shape of the pacemaker action potential produced by electrical stimulation. The two first potentials correspond to the spontaneous activity of a pacemaker Purkinje cell. When the false tendon was stimulated at a frequency higher than the spontaneous (at the arrow) the corresponding transmembrane potentials from the same cell changed their shapes. Note that the responses to the applied stimuli have a rapid upstroke. Calibrations, 500 msec and 50 mV.

the applied stimuli, frequency and duration of the period of stimulation, and the magnitude of the reduction in the automatism (FIG. 3A, B) would suggest that accumulative changes of ionic nature might be responsible for this phenomenon.

It is well known that sodium and potassium ions play, according to the ionic theory¹¹⁾, an important role in the generation of electrical activity in nerve fibers. The slope of the slow diastolic depolarization of potentials from cardiac structures is highly dependent, among other factors, on the external ion concentrations. As it was suggested by ARVANITAKI³⁾, the spontaneity of the heart muscle is related to the existence of a slow depolarization during diastole. DRAPER and WEIDMANN⁶⁾ found that the rate of the slow depolarization was approximately proportional to the extracellular sodium concentration and TRAUTWEIN²⁰⁾ suggested that the slow diastolic depolarization could be explained if the conductance to sodium (g_{Na}) increases relative to conductance to potassium (g_K) either by an increase in g_{Na} or by a reduction in g_K .

According to the above mentioned facts and results, the spontaneous activity of the Purkinje fibers occurring after high frequency stimulation, could be interpreted as being due probably to the interaction of sodium and potassium ions. In fact, the reduced slope of the diastolic depolarization, the decrease in resting membrane potential appearing during the depression or

abolition of the automatism, and the diminution of the amplitude of the first spontaneous responses can be considered as produced by an increase in potassium efflux.

The present results show in addition, that an increase in extracellular potassium concentration diminished the slope of the slow diastolic depolarization, in such a way that it takes a longer time to reach the firing level for the action potential, reducing in consequence the frequency of the automatic activity.

Although no determinations of either extracellular or intracellular concentrations of potassium were made in the present experiments, we are inclined to explain the reduction in the automatism, provoked by high frequency stimulation, by an exaggerated efflux of potassium ions. This hypothesis is based on the following fact. During the activation of cardiac structures at high frequencies, the movement of potassium ions from the inside to the outside of the membrane is substantially increased. HUMPHREY et al.¹²⁾ reported, in an isolated rabbit heart, that an increase in heart rate from 100 to 200 per minute, produced an increase in the total flux of potassium, from 42.0 to 49.0 pmoles/cm². HAJDU⁹⁾ has shown that during the contraction of frog ventricle there is a release of potassium ions. This author calculated the value of the loss of potassium per contraction to be 20 $\mu\mu$ Moles/cm². Working on perfused strips of frog ventricles, LORBER et al.¹⁵⁾ have observed an increase in the outward movement of potassium occurring during the action potential and of approximately the same duration as the electrical transient. These results and the observations made in the present paper, i.e. a reduction in the slope of the diastolic depolarization and a decrease in the membrane potential after the period of high frequency stimulation, suggest that potassium ions are moving outwards during the high frequency activation of cardiac tissues. A similar phenomenon has been described by FREYGANG et al.⁷⁾ in skeletal muscle. These authors postulated that the negative after-potential appearing after a train of impulses was due to an accumulation of potassium in an intermediary space located between the major portion of the sarcoplasm and the external fluid.

According to the results of SCHREIBER in heart muscle¹⁸⁾, HODGKIN et al. in skeletal muscle¹¹⁾ and TASAKI et al. in nerve¹⁹⁾, the increase in extracellular potassium augments the g_K and on the contrary, a decrease in external potassium concentration will produce a diminution in g_K ^{15,18)}. From these observations it can be assumed that the larger the external concentration of potassium, the greater the reduction in the frequency of automatic response. In fact, in our experiments 25 or 50 per cent increase in external KCl decreased the spontaneous activity of Purkinje fibers and potentiated the reduction produced after high frequency stimulation. A decrease in the extracellular KCl concentration provoked the opposite effects.

There is an agreement in the literature concerning the role played by the sodium ions on the slow diastolic depolarization of the cardiac pacemaker action potentials^{5,6,21}. It is accepted, in general, that the reduction in the extracellular sodium concentration slowed the pacemaker activity and thereby retards spontaneity. The reduction in the automatism of the Purkinje fibers produced by high frequency stimulation could be due, according to this observation, to a diminution in the electro-chemical potential of sodium between the intra- and extracellular phases.

The appearance of small and rhythmic depolarizations (FIG. 8) which occurred during the abolition of the automatism might be interpreted as a limited entry of sodium ions. The progressively growing amplitude of these small potentials would suggest that sodium entry is also in process of recovery. The experimental observation that a decrease in the extracellular sodium concentration produced a greater reduction in the automatism (FIG. 5) points towards the same conclusion conferring a partial influence of sodium ions on the phenomenon under study.

Since both, the increase in extracellular potassium or the diminution of external sodium produced modifications similar to those observed after the period of high frequency stimulation, it is likely, according to the present knowledge, that both cations are operating.

When the Tyrode solution was modified either by increasing the KCl or diminishing the NaCl, not only the cations were altered but also the anion, i.e. the chloride. The modifications of chloride could be of importance for the reduction in spontaneity. Since the same final effect was obtained under increase or decrease of chloride concentration, the role played by this anion could be discarded. This does not mean that greater changes in chloride concentrations than those made in the present experiments could modify the pacemaker activity, as it has been shown by HUTTER and NOBLE¹³.

The reduction in the automatism produced by the gradual increase in frequency of stimulation is of importance from the functional point of view, since it has been observed¹⁷ in the spontaneous activity of the atrio-ventricular node of the "in situ" dog heart. This would mean that all cardiac cells with pacemaker-like action potentials have the possibility of showing the above mentioned reduction in their automatism.

SUMMARY

The experiments were performed on the false tendon from the right ventricle of a dog heart. The transmembrane potentials from the Purkinje fibers were recorded through glass micropipettes.

The Purkinje action potentials may appear with a steep slope of the diastolic depolarization (pacemaker fibers) or with a slight diastolic depolarization

(non-pacemaker fibers). Under different experimental conditions one type of action potential may be transformed into the other.

When the false tendon, with spontaneous activity, was stimulated during brief periods with progressively increasing frequencies, its automatism decreased immediately after the period of stimulation. The magnitude of the reduction in spontaneity varied according to the parameters of the stimuli, but in every case the automatism recovered gradually.

After the period of high frequency stimulation, the corresponding transmembrane potentials of the first and subsequent spontaneous responses, had a slighter slope of their diastolic depolarization as compared to the spontaneous control responses. The amplitude of the action potentials and the resting membrane potential were also reduced during the recovery of the spontaneity.

After the period of high frequency stimulation, small rhythmic potentials with slow rate of rise appeared during the abolition of the automatism. The amplitude of those potentials augmented gradually and when they reached a certain value, an active response occurred.

An increase in the external KCl concentration (3.4 or 4.1 mM) produced a larger reduction in the automatism than that obtained under normal KCl concentration. The opposite effect was observed when the KCl concentration of the Tyrode solution was lowered. A decrease in the external NaCl concentration (120.5, 109.6 or 102.7 mM) provoked changes in the automatism of the Purkinje fibers similar to those described for the high KCl concentrations.

During spontaneous activity the increase in the external KCl concentration produced, concomitantly with the reduction in the frequency of the automatism, a decrease in the rate of diastolic depolarization. Similar effects were observed during the diminution in external NaCl concentration.

The reduction in automatism produced by high frequency stimulation is discussed in terms of the ionic theory and tentatively explained as due to an increased efflux of potassium combined with a reduced sodium ions influx.

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