Shifts in Pacemaker Dominance within the Sinoatrial Region of Cat and Rabbit Hearts Resulting from Increase of Extracellular Potassium

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ABSTRACT

Simultaneous recording of action potentials from cells in different parts of sinoatrial preparations from cat and rabbit hearts confirms the suggestion by Meek and Eyster that the dominant pacemaker site shifts during treatment with high [K⁺]. The direction of the shift can be either upward or downward depending upon the location of the original dominant pacemaker site. In the majority of cases, the original dominant site is located in the upper part of the preparation, consequently the shift is from above downward. In environments with high [K⁺], both true and latent pacemaker cell action potentials show a decrease in both maximal diastolic potential and the slope of the prepotential but these changes seem to be more marked in the true pacemaker cell. It is suggested that the shift of dominant pacemaker site in response to high [K⁺] is due to the difference in sensitivity to K⁺ between these two types of pacemaker cells.

ADDITIONAL KEY WORDS

potassium effect on SA pacemaker extracellular potassium transformation of pacemaker action potential automaticity true and latent pacemakers transection of sinoatrial region

It is well known that the sinoatrial node region of the heart is composed of a large number of cells which possess various degrees of automaticity. Of these cells only a few at a time can be the true or dominant pacemakers which set the rhythm for the heart (1). It has been noticed that the dominant pacemaker site is not fixed but shifts under certain circumstances (1, 2). As early as 1914, with the aid of a pair of surface electrodes and a string galvanometer, Meek and Eyster (3) were able to detect a shift of the point of initial negativity from the upper to the lower part of the sinus node as the potassium concentration in the blood was raised. This shift of the initial negativity was interpreted as the result of a shift of the dominant pacemaker site.

With the development of modern electrophysiological techniques, new information concerning the effect of potassium on the sinoatrial nodal cell activity has been obtained. It is now known that the sinoatrial nodal cell is highly resistant to the suppressive effect of increased potassium (4, 5). The sinoatrial node still dominates action of the ventricle when extracellular potassium concentration has been raised to a level high enough to inactivate the atrial myocardium (6). As a matter of fact the pacemaker activity of the sinoatrial node is accelerated by raising the extracellular K⁺ concentration within limits (7). This new information may be related to the old finding of the pacemaker shift in response to high [K⁺] but the relation has not as yet been clarified. First of all, no direct evidence of such a pacemaker shift has been provided. Secondly, the question of...
whether or not there is a difference in sensitivity to K⁺ between cells in different parts of the node, as suggested by such a shift of the pacemaker site, has not been answered. My experiments were designed to investigate these problems by a multiple microelectrode recording technique.

**Methods**

Sinoatrial node preparations were obtained from adult cat and rabbit hearts. Under pentobarbital anesthesia, the heart was removed from the animal through an intercostal incision. A piece of tissue, which lies between the interatrial septum and the lateral margin of the crista terminalis and between the root of the superior vena cava and the coronary sinus (Fig. 1), was dissected out of the heart and immobilized, endocardial side up, in a tissue chamber by a plastic tissue holder. The chamber (5 ml capacity) was perfused with an oxygenated (O₂ 95%; CO₂ 5%) Tyrode solution at a constant rate of 2 ml/min. The temperature in the chamber was maintained at 37±0.1°C. Conventional Ling-Gerard (8) type microelectrodes filled with 3M KCl were used for transmembrane potential recordings. The activities of two cells, normally one in the upper part of the preparation and the other in the lower part, were recorded simultaneously. Action potentials were monitored on a dual beam Tektronix oscilloscope through a NF 1 Bioelectric amplifier and then photographed by a Grass camera. Suitable speeds of the oscilloscope beam sweep were selected to permit recordings of the rate of firing, the shape of the action potential, and the sequence of excitation of these two cells. After control recordings had been taken, the Tyrode solution, which had a composition of NaCl, 137 mM; KCl, 2.7 mM; CaCl₂, 1.8 mM; NaH₂PO₄, 0.4 mM; NaHCO₃, 12 mM; MgCl₂, 0.5 mM and dextrose 5.5 mM, was replaced by new solutions with the same composition except for different KCl concentrations. The effects of new solutions on the cell activity were determined in a period between 10 to 15 minutes after the beginning of the perfusion. Each test was preceded by a 20-minute perfusion with the normal Tyrode solution and the record taken at the end of this 20-minute perfusion was used as a control. When separation of the upper and the lower parts of the preparation was desired, a complete transverse section along the midline of the preparation was made; no continuity of tissue connections remained (Fig. 1).

**Results**

All sinoatrial node preparations from 17 cat and 8 rabbit hearts beat regularly. Cellular action potentials showing various degrees of spontaneous diastolic depolarization were recorded from almost all parts of the preparation. But true pacemaker-type action potentials, characterized by a smooth transition from the prepotential to the upstroke of the action potential, were normally found within a small area of only 2 to 3 mm in diameter. The exact position of this area varied somewhat from animal to animal, but was located in the upper portion of the sinoatrial region in 15 out of the 17 cat and in 6 out of the 8 rabbit preparations (Fig. 1). Action potentials recorded from cells within this area always appeared earlier than those recorded elsewhere. When the upper and the lower parts of the tissue strips were separated, the rhythm of the part containing this true pacemaker area usually remained the same as that before transection. The other part usually stabilized at a slower rate after an initial period of irregularity, which lasted from a few seconds to several minutes. After transection, independent but true primary pacemaker-type action potentials were recordable from both parts (Fig. 2). The latent pacemaker, when more...
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True (top tracing) and latent (bottom tracing) pacemaker action potentials recorded before (A1, A2) and after (B) separation. A1: slow sweep recordings showing the difference in shape of the action potentials; B: fast sweep recordings showing the time course relationship between these two action potentials; B: action potentials recorded from same areas as in A but probably not from same cells. Note that (top tracing) activity in the original dominant cell is not much affected by transection of the strip. In the area (bottom tracing) originally giving only subsidiary pacemaker action potentials, an independent automaticity has developed; this pacemaker has a different intrinsic cycle length.

The effect of K+ on the pacemaker activity was similar in both cat and rabbit preparations. Within a range of K+ concentrations between 1.35 mM (50% of that in Tyrode’s solution) and 8.1 mM (300% of that in Tyrode’s solution), the intrinsic rate increased as the [K+] in the perfusate was increased. An increase of [K+] from 2.7 mM to 5.4 mM and in another series from 2.7 mM to 8.1 mM caused an increase in rate of firing by 4.9% (average of ten cat preparations; P < 0.05) and 7.1% (average of eight cat preparations; P < 0.05), respectively. Above 8.1 mM, the effect of increased K+ was variable. Only in six out of nine cat preparations and the average decrease was 4.1% (P > 0.1).

According to the result obtained from two cat sinoatrial cells which gave constant records for longer than 2 hours and permitted repeated testing of the effect of and the complete recovery from [K+] changes, the acceleration in rate caused by increases in extracellular [K+] seemed to be mainly due to a reduction of the diastolic potential level. The steepness of the spontaneous diastolic depolarization actually decreased when extracellular [K+] was raised. Whether or not there is a change in the threshold potential during the acceleratory state was difficult to determine because a shift of pacemaker site invariably occurred as evidenced by the loss of the smoothness in transition from the prepotential to the upstroke of the action potential as seen in Figure 3, a.

The deceleratory effect of low [K+] was mainly the result of a decrease in the speed of spontaneous depolarization. Neither maximal diastolic potential nor threshold potential seemed to be significantly affected as shown in Figure 3, c. In these two cells, 2.7 mM seemed to be the optimal [K+] for the

FIGURE 2

True (top tracing) and latent (bottom tracing) pacemaker action potentials recorded before (A1, A2) and after (B) separation. A1: slow sweep recordings showing the difference in shape of the action potentials; B: fast sweep recordings showing the time course relationship between these two action potentials; B: action potentials recorded from same areas as in A but probably not from same cells. Note that (top tracing) activity in the original dominant cell is not much affected by transection of the strip. In the area (bottom tracing) originally giving only subsidiary pacemaker action potentials, an independent automaticity has developed; this pacemaker has a different intrinsic cycle length.

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FIGURE 3

Effect of extracellular potassium concentration on cycle length, maximal diastolic potential and speed of spontaneous diastolic depolarization of the same cell. a, [K+] = 5.4 mM; b, [K+] = 2.7 mM; c, [K+] = 1.35 mM. Two groups of tracings from same cell, magnification difference between these two sets of records is shown by voltage calibration, 10 mv.
diastolic depolarization development (Fig. 3, b). When exposed to very high concentrations of $[K^+]_o$, both diastolic and threshold potentials did shift significantly (Fig. 4).

As far as the maximal diastolic potential is concerned, both true and latent pacemaker cells were affected by $K^+$ in the same manner; except that the true pacemaker cell was affected to a greater degree as shown in Figure 4.

When time courses of the two simultaneously recorded action potentials were compared, the time interval between the excitation of the two cells, one within the true pacemaker area and the other in the other end of the preparation, was found to be shorter as the extracellular $[K^+]_o$ was raised. In four out of the eight cat and in two out of the three rabbit preparations tested, the normal sequence of excitation actually reversed when the $[K^+]_o$ was raised to 10.8 mM. There was no definite relationship between the rate change and the occurrence of the reversal of excitation sequence, although the reversal of excitation sequence occurred more often when the intrinsic rate was decreased by a very high $[K^+]_o$.

The exact location of the new dominant pacemaker during treatment with high $[K^+]_o$ was not fixed. The shift was not always from above downward as suggested by Meek and Eyster (3). In one cat and one rabbit preparation, in which the original true pacemaker area was in the lower part of the preparation, the direction of shift of the pacemaker site was from below upward. However, in all cases a pacemaker shift away from the originally dominant cell did occur, indicating a difference in sensitivity to potassium ion. Figure 5 shows the result observed in one preparation from the cat heart. While

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**FIGURE 4**

*Difference in sensitivity to extracellular $[K^+]_o$ changes between true (top tracing) and latent (bottom tracing) pacemaker action potentials. A: control, $[K^+]_o = 2.7$ mM; B: 10 minutes after $[K^+]_o$ had been raised to 13.5 mM; C: 5 minutes after $[K^+]_o$ had been raised to 27 mM; D: 10 minutes after washing with normal Tyrode's solution.***

**FIGURE 5**

*Shifts of dominant pacemaker site during treatment with high $[K^+]_o$. A: $[K^+]_o = 2.7$ mM; B: $[K^+]_o = 8.1$ mM; C: $[K^+]_o = 10.8$ mM; A, B, and C are from the same cell. For detail see text. Time calibration, 100 msec.***
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one microelectrode remained in the original dominant cell (top tracings), the other microelectrode was used to search for the new dominant site. When extracellular [K⁺] was increased from 2.7 mM (A₁, 2) to 8.1 mM (B₁, 2), the dominant pacemaker (bottom tracing of B₁, 2) was located 1.8 mm below the original dominant cell which now had become a subsidiary pacemaker. When extracellular K⁺ was increased to 10.8 mM (C₁, 2), the new dominant pacemaker (bottom tracing of C₁, 2) was located 4.5 mm below the original dominant cell.

When the upper and lower parts of the preparation were separated, the acceleratory effect of K⁺ was found more marked and the suppressive effect less marked on the part which did not contain the original true pacemaker area. When the [K⁺] was gradually increased, the cells in the part containing the original true pacemaker area usually stopped beating before cells in the other portion of the preparation lost their automaticity.

Discussion

The results obtained from this study agrees with Meek and Eyster's suggestion that the dominant pacemaker site shifts during potassium treatment (3). In the majority of cases, the direction of the shift is from above downward as described by Meek and Eyster in the intact dog heart. Shifts in a reverse direction from below upward can also occur provided the dominant pacemaker site is originally located in the lower portion of the preparation. Whether this pacemaker shift occurs only within the anatomically defined sinoatrial node or may go beyond this anatomical structure is not clear from results of this study. All one can see here is that, in both cat and rabbit sinoatrial preparations, the area which consists of cells showing spontaneous diastolic depolarization is much larger than the area normally considered to be occupied by the node (9-11). It is true that most of these cells are only subsidiary or latent pacemaker-type cells, but a subsidiary or latent pacemaker cell can be transformed into a dominant pacemaker when cells with higher automaticity have been suppressed or removed as shown in Figure 2. It is apparent from this figure that after transection, a true pacemaker-type action potential can be recorded in an area which previously consisted of cells showing latent pacemaker-type action potentials only. This indicated that some of the latent pacemaker cells, if not all, are capable of independent pacemaker action if they have opportunity to develop their intrinsic rhythmicity.

Because of the difficulty in maintaining the tip of the electrode in a specific cell while the tissue was cut, action potentials shown in A and B of Figure 2 are recorded only from the same areas but probably not from the same cells. Therefore, it is hard to interpret whether the difference in shapes between action potentials in A₁ and B is created by the transection or is due to cellular variation. There is no doubt that cutting the tissue might cause a membrane potential drop in the damaged tissue which might electrotonically affect the membrane potential recorded from adjacent cells. This might explain why the initial spontaneous diastolic depolarization in the bottom tracing of B is steeper after transection. However, if this were the case, the continuous "pulling" of the membrane potential toward a less negative level should affect not only the rate of the spontaneous diastolic depolarization but also the resting or the maximal diastolic potential. In other words, one would expect the resting membrane potential to be lower. Since this was not observed, a significant electrotonic effect seems to be unlikely.

The shift of pacemaker site is, no doubt, the result of a loss of dominance by the true pacemaker cells to the originally latent pacemaker cells. Judging from the fact that the pacemaker shift can occur in both acceleratory and deceleratory states of treatment with high [K⁺], the shift could be the result of either a suppression of the true pacemaker cells, or an acceleration of the latent pacemaker cells, or both.

It is the general concept that the activity or the frequency of discharge of a pacemaker cell...
is determined by three factors, namely, the steepness of the spontaneous depolarization, the level of the resting potential, and the level of the threshold potential (1). Any situation which causes an increase in the steepness of the spontaneous depolarization, a decrease (less negative) in resting potential or an increase (more negative) in threshold potential will lead to an increase in the frequency of discharge.

Whether or not there is a change in the threshold or take-off potential in response to a moderate increase of extracellular [K+] is not clear because of the change in the shape of the action potential caused by the shift of the pacemaker site. The steepness of the spontaneous diastolic depolarization and the maximal diastolic potential are definitely affected by a change of the extracellular [K+].

It has been reported that the rate of depolarization during phase 4 is increased as the frequency of firing of a latent pacemaker cell is accelerated by raising the extracellular [K+] from 2.7 to 13.5 mEq/l (5). My study, however, failed to reproduce this effect but showed consistently that the speed of the spontaneous depolarization is decreased as the [K+] in the perfusate is increased to above 2.7 mEq/l. The same decrease of the speed of spontaneous depolarization caused by high [K+] has been observed by others in both rabbit sinoatrial node cells (1) and in sheep Purkinje fibers (12). In Purkinje fibers, this suppressive effect of high [K+] is believed to be due to an increase in the membrane permeability to K+ (12).

Although a quantitative comparison of the effect of high [K+] on the rate of spontaneous diastolic depolarization in different types of pacemaker cell action potential has not been performed, qualitatively it seems that the suppressive effect of high [K+] on the spontaneous depolarization is more marked in the true than in the latent pacemaker cell as shown in Figure 4. This might be responsible, at least in part, for the shift of dominance from the originally true pacemaker cell to latent pacemaker cells.

It has been known for many years that potassium depolarizes excitable tissue (13). In both atrial (14, 15) and Purkinje fibers (16), the resting potential varies as a linear function of the log of the extracellular [K+] within limits. A similar depolarizing effect of high [K+] is also observed in the sinoatrial nodal cells (17). It seems obvious that this depolarizing effect of K+ is the main factor responsible for the acceleration of the sinoatrial nodal cell activity during perfusion with high [K+]. For reasons to be described later, this acceleratory effect is probably more important for the latent pacemaker cell, which has a higher resting potential, than the true pacemaker cells.

In addition to the acceleratory effect, lowering of the resting potential by high [K+] should also have a suppressive effect on cells which have an original low resting potential. According to Hodgkin and Huxley (18) and to Weidmann (19), the excitation of the membrane, both in squid giant axon and in Purkinje fiber, depends upon an increase in Na+ conductance and Na+ influx. The Na+ conductance increase, as indicated by the maximal rate of depolarization during excitation, is determined by the amount of a postulated Na+ carrier available for activation. The amount of Na+ carrier available for activation is believed to be related to the membrane potential level before excitation; the lower the resting potential level, the more carrier becomes inactivated and less resting carrier is available for activation.

It is well known that true pacemaker cells have a very low resting potential. According to West (20), the first one to record such action potentials in mammal, the average value for the rabbit sinoatrial node cell is 56 mv. In measurements from 50 true pacemaker cells in 10 cat preparations, we found the maximal diastolic potential to be between 54 and 71 mv with an average of 61 mv. This figure is a little higher than the resting potential measured by West in the rabbit, but is still much lower than the maximal diastolic potential of the latent pacemaker cell which is normally in the vicinity of 80 mv. At the low level of membrane potential found in a true
pacemaker cell, most of the Na\(^+\) carrier should have been inactivated according to the figures given by Hodgkin and Huxley for the squid axon (18) and by Weidmann for the Purkinje fiber (19). A further depolarization of the membrane should inactivate all the Na\(^+\) carrier and cause the membrane to become inexcitable.

It is true, however, that sinoatrial nodal cell action potentials are less affected by both extracellular [Na\(^+\)] change (1, 17) and tetrodotoxin treatment (21), suggesting less dependence of the cell upon Na\(^+\) fluxes for excitation. The finding that the sinoatrial nodal cell is highly sensitive to the suppressive effect of manganese suggests an involvement of a Ca\(^++\) flux in the development of the prepotential and generation of the action potential (22). No matter how different the mechanism of pacemaker action may be in the sinoatrial node cell and the Purkinje fiber, it is still reasonable to assume that an inward flow of cations is an important factor in the spontaneous depolarization. The decrease in the slope of diastolic depolarization in the pacemaker-type cell during treatment with high [K\(^+\)] may have at least three reasons: (a) an increased K\(^+\) conductance of the surface membrane as a direct consequence of increased [K\(^+\)] (12); (b) a decreased rate of fall of K\(^+\) conductance as a consequence of the lowering of maximal diastolic potential (23); or (c) a decrease of background inward current, whatever its nature. Why the slope of prepotential or spontaneous diastolic depolarization is more [K\(^+\)]-sensitive in the true pacemaker cell than in the latent pacemaker cell cannot be answered at present.

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