Role of the Sympathetic Nervous System in the Sinus Node Resistance to High Potassium
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ABSTRACT
The resistance of sinus node automaticity to high potassium (K) levels was studied in anesthetized dogs by perfusing the sinus node artery with oxygenated Tyrode's solution containing different K concentrations. The following results were obtained.
(1) Sinus node automaticity persisted at a K concentration of 21.6 mM, although the pacemaker site tended to shift in and out of the node at this concentration. (2) Sinus node dominance at high K concentrations was progressively lost as sympathetic influences were progressively eliminated. (3) Sinus node pacemaker activity was suppressed at a K concentration lower than 21.6 mM when calcium was omitted from the perfusion fluid. (4) The pacemaker site shifted consistently from the sinus node towards the atrioventricular node at high K concentrations after the elimination of sympathetic activity. (5) The transient sinus tachycardia caused by high K concentrations persisted after bilateral vagotomy. It is concluded that catecholamines participate in the resistance of the sinus node to K, suggesting that the difference in resistance to high K concentrations between the sinus node and Purkinje fibers may, in part, result from the more abundant innervation of the sinus node.

KEY WORDS catecholamines reserpine sympathectomy propranolol calcium dog

Ventricular Purkinje fibers perfused in vitro usually become quiescent when the potassium (K) content of Tyrode's solution is increased from 2.7 mM to 5.4 mM (1). In contrast, the sinus node continues to discharge spontaneously when K is increased to a concentration at which atrial fibers become inexcitable (2-6). The reason for the resistance of sinus node pacemaker activity to high K concentrations is not understood. It seems likely that this resistance to K reflects membrane characteristics peculiar to sinus node cells, but other factors cannot be excluded. One of these factors could be sympathetic innervation, since it has been shown that catecholamines counteract the depressant effect of K on cardiac excitability (4, 7). It does not seem unreasonable, therefore, to propose that catecholamines also counteract the depressant effect of K on automaticity. Sympathetic innervation of ventricular Purkinje fibers is sparse and that of the sinus node abundant (8); this arrangement reinforces the possibility that catecholamines play an important role in the difference in sensitivity between atrial and ventricular pacemakers to K.

The aim of the present investigation was to study the effect of the sympathetic nervous system on the sinus node resistance to high K concentrations by perfusing the sinus node artery with a solution in which K content was varied. Autonomic denervation, catecholamine depletion, and adrenergic receptor blockade were carried out. The results of these and other procedures suggest that part of the resistance of sinus node pacemaker activity to high K concentrations is, indeed, related to catecholamine action.

Methods
A total of 27 mongrel dogs of either sex weighing 26-47 kg were anesthetized with morphine sulfate (5 mg/kg, im) and chloralose (75 mg/kg, iv). The chest was opened through a median sternotomy, and a cradle was made of the pericardial sac. The sinus node was perfused using a technique similar to that developed by James and Nadeau (9). The right coronary artery was dissected free and ligated about 1 cm before and 3-5 mm after the branching of the sinus node artery. All the branches of the ligated section were tied with the exception of the sinus node artery. The ligated section was cannulated with a polyethylene catheter with an external tip diameter of 0.7 mm. The catheter was connected proximally via a three-way cannula to two other catheters: one led to a 50-ml syringe mounted...
on a dual infusion-withdrawal pump (Harvard Apparatus Company model 942) and the other to a pressure transducer (Statham model P23Db) for monitoring pressure in the perfusion system. The perfusing solution was warmed to 38°C in tubing coiled in a constant-temperature bath (Fisher Company).

The dogs were ventilated with an Enstrom respirator (MIVAB, Stockholm, model 200) attached to an endotracheal tube. The temperature of the dogs was monitored with a thermistor probe introduced into the esophagus and connected to a meter (Telethermometer model 41 TF, Yellow Springs Instrument Company). A thermostatic pump (Therm-O-Rite Products Company) circulated water at a selected temperature through a mattress underneath the dog. The right external jugular vein was cannulated, and a solution of 5% glucose in normal saline was infused. Arterial blood samples were withdrawn periodically from the left brachial artery, and pH, Po2, and Pco2 were checked with a Radiometer apparatus. Any degree of acidosis was corrected by the administration of appropriate amounts of sodium bicarbonate. Analyses of plasma K concentrations were carried out with a Baird flame photometer with a lithium internal standard.

Bipolar silver electrodes were usually sutured onto the epicardial surface overlying the sinus node area, the right atrial appendage, and the base of the right atrium. The electrode at the base of the atrium was sutured onto an area delimited by the sinus node artery, the right atrial appendage, and the base of the right atrium. The electrode at the base of the atrium was sutured onto an area delimited by the sinus node artery, the inferior vena cava, and the atrioventricular groove. The sutures for the sinus node electrode were placed so as to avoid interference with the blood supply. In some experiments an electrode was also sutured inside the right atrium near the atrioventricular node area. This procedure was done under inflow occlusion, and the position of the electrode was checked at autopsy. The dogs subjected to inflow occlusion were given blood to compensate for the measured blood loss. Bipolar atriograms, tachometer trace, aortic blood pressure, sinus node artery infusion pressure, and a lead II electrocardiogram were recorded with an Electronics-for-Medicine DR 8 recorder.

The vagi were cut in the neck in the course of the experiment. In some dogs acute sympathectomy was accomplished by severing the stellate ganglia and the next five thoracic ganglia bilaterally. Chronic sympathectomy was carried out through a lateral thoracotomy; about 1 week elapsed between the operations on each side. Propranolol (Inderal) was also used in this study.1

The sinus node was perfused with Tyrode's solution of the following millimolar composition: NaCl 137, KCl 2.7, CaCl2 2.7, MgCl2 1.05, NaHCO3 11.9, NaH2PO4 0.45, and glucose 5.5. K concentration was varied by adding solid KCl to achieve concentrations of 5.4, 10.8, and 21.6 mM. When KCl was increased to 10.8 and 21.6 mM, the tonicity of the solutions was maintained constant by reducing NaCl by appropriate amounts. In some experiments, calcium (Ca) was omitted from Tyrode's solution. All solutions were bubbled with a mixture of 97% O2-3% CO2 and heparinized (500 units/liter). The flow of the pump was 12.6 ml/min during experimental procedures, but it was reduced to 0.1 ml/min in between. The duration of the perfusion during an experimental procedure varied between 4 and 8 minutes. Often, the dog's own blood was allowed to perfuse the sinus node artery through the collateral circulation between experimental procedures.

The advantage of using Tyrode's solution at a constant flow is that the concentration of K outside the sinus node cells is then easily and rapidly changeable to the desired value. The approximate extent of the tissue perfused with Tyrode's solution was readily identified, for, as soon as perfusion with Tyrode's solution was started, the tissue assumed a whitish discoloration.

Results

HEART RATE AND PACEMAKER LOCATION

The effect of different K concentrations on heart rate and pacemaker location is illustrated in Figure 1. The bipolar electrogram from the sinus node area preceded all other electrical deflections in the cardiac cycle, including the P wave. As K concentration was increased, the interval between

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1Ayerst Company donated the propranolol.
the sinus node and the atrial base complexes increased, but the P-R interval remained the same (compare the records for 5.4 and 10.8 mM K). The atrial appendage, of course, was perfused by the dog's own blood. With 21.6 mM K, the initial complex still originated in the sinus node, but the morphology was different. Also, the P-R interval did not increase in spite of the slower sinus rate, suggesting an increment in conduction velocity (10), a shift of the pacemaker site toward the lower end of the sinus node (11), or both. As in most instances, the perfusion pressure increased when the K concentration was increased to 21.6 mM. This increase was possibly due to vasoconstriction, since, after a variable period in the recovery solution, the perfusion pressure usually fell to the original value. The recovery, as yet incomplete, is shown in the last section of the figure.

The pacemaker site during the K infusions was determined in different experiments by the shape, timing, and relationship of electrical deflections. In all instances (ten dogs), the pacemaker remained localized in the sinus node as long as the K concentration did not exceed 10.8 mM. At 21.6 mM K, the pacemaker remained in the sinus node in six experiments, but in four others the pacemaker shifted back and forth between the sinus node and other atrial pacemaker sites.

Typically, as K concentration was increased, the rate transiently increased before settling to a steady value. The average steady-state value of the sinus rate for each K concentration is plotted in Figure 2 (curve labeled control). It should be noted that the curve is essentially flat due to the fact that the sinus rate at higher K concentrations changed inconsistently and was either higher or lower than the rate at 2.7 mM K. In no instance were changes found in the electrocardiogram which could be attributed to an increase in systemic plasma K levels. Chemical determination of plasma K concentrations gave a mean value of 4.84 ± 0.24 mEq/liter at the beginning, 5.05 ± 0.23 mEq/liter during, and 5.03 ± 0.27 mEq/liter at the end of 15 experiments.

A decrease in the tonic inhibitory action of acetylcholine on the sinus node by high external K concentrations could be a contributory factor in the sinus node resistance to K. For this reason, perfusion of the sinus node with different K solutions was repeated after vagotomy. As expected, vagotomy resulted in an increase in sinus node rate to an average of 176 ± 16 beats/min (Fig. 2, vagot.). On increasing K to 10.8 mM, the sinus rate rose to 195 ± 13 beats/min. At 21.6 mM K, the rate fell in seven of nine dogs to an average of 150 ± 11 beats/min. Vagotomy did not influence the pacemaker location at different K concentrations. The results suggest that changes in the tonic vagal discharge play little role in sinus node resistance to high K concentrations.

**ACUTE SYMPATHECTOMY**

Usually, sympathectomy was carried out after bilateral vagotomy but on occasion the order was reversed. After sympathectomy, the average sinus rate fell to 141 ± 27 beats/min (Fig. 2, acute symp.) from the value it had after vagotomy. Little or no change occurred when K concentration was doubled to 5.4 mM, but the sinus rate generally fell as the K concentration was increased to 10.8 and 21.6 mM. Furthermore, when K concentration was increased to 21.6 mM, the pacemaker shifted from the sinus node to other atrial sites in five of the nine dogs tested, suggesting that in the absence of tonic sympathetic discharge the sinus cells are more susceptible to high K concentrations.

**CHRONIC SYMPATHECTOMY**

In an acutely denervated animal, the tonic sympathetic discharge is abolished, but a high K concentration can still release catecholamines in the sinus node by acting on the sympathetic endings.
High K concentrations depolarize cell membranes, and this depolarization might be large enough to cause catecholamine release (12, 13). For this reason, experiments were carried out in dogs subjected to chronic sympathectomy, usually 1 week after the sympathectomy had been completed. The results from one such experiment are illustrated in Figure 3. In the top of the figure, sinus node activity still persisted at 10.8 mM K. At 21.6 mM K, the origin of the heart beat clearly moved away from the sinus node: the activity recorded at the base of the atrium preceded the activity recorded from the other two electrodes on the sinus node and the right atrial appendage (compare the recordings within the two enclosed areas). Also, the recording at 21.6 mM K shows one of the limitations of the standard electrocardiogram as a source of information on pacemaker localization. If conclusions were drawn only from the electrocardiogram, the pacemaker shift at 21.6 mM K might have been missed, since the P wave had the same polarity and configuration as it did at 10.8 mM K, when the pacemaker was located in the sinus node. The electrocardiogram revealed only some shortening of the P-R interval. The bottom of Figure 3 was recorded shortly after the beginning of 21.6 mM K infusion and shows that the initial tachycardia, often observed in the control dogs, was also present in the chronically sympathectomized dogs. The tachycardia was followed by a brief slowing and then cardiac asystole. Shortly thereafter, another pacemaker took over.

In five of six chronically denervated dogs, pacemaker activity was no longer localized in the sinus node at 21.6 mM K. The only dog not conforming to this pattern was tested 3 weeks after the completion of sympathectomy. It is possible that sinus node hypersensitivity to circulating catecholamines might have developed.

**RESERPINE ADMINISTRATION**

Chronic sympathectomy does not eliminate extraneural catecholamine stores in the heart. Reserpine was, therefore, used in five nonsympathectomized dogs to deplete neural and extraneural catecholamine stores. The shift of the pacemaker from the sinus node to other sites occurred in every dog at a K concentration of 21.6 mM. Such a shift during 21.6 mM K perfusion is illustrated in one reserpine-ized dog (Fig. 4). At the time the tracings shown in Figure 4 were recorded, the dog had also received propranolol (see below). In the left section of the figure, the first, third, and fourth beats were of sinus origin since the sinus node and the right atrial appendage traces had the same configuration as they did at lower K concentrations and the sinus node complex preceded the P wave. The second beat shows simultaneous sinus node and right atrial appendage complexes, indicating that the two sites were excited simultaneously. Furthermore, the right atrial appendage complex shows reversal of the polarity. The loss of sinus node dominance is confirmed by the finding shown in the center section of the figure. Here the first three complexes show beats originating from the ectopic pacemaker. The upward shift of the blood pressure reference line marks the beginning of electrical stimulation through the electrode sutured on the sinus node area. During electrical stimulation, the sinus node complex was distorted by the stimulus artifact, but the right atrial appendage complex showed the control morphology and polarity. On cessation of
Pacemaker shifts as a function of high K concentration and electrical stimulation. Explanation of the labeling and the blood pressure calibration is the same as it is for Figure 1.

Left: Recorded during 21.6 mM K infusion and shows spontaneous shifts in pacemaker site. The two vertical lines were drawn on the record to facilitate the evaluation of temporal relationships among electrical events. Center: Shows the change in morphology of the atriograms when the sinus node was stimulated electrically through the sinus node electrode. Electrical stimulation (120/min) was begun at the upward shift of the zero reference line for the blood pressure. The first stimulus excited the sinus but failed to propagate to the atrium. A spontaneous beat is interpolated between the first and the second electrical stimulus. Right: Shows the events following the last two driven beats.

results, for in five of seven dogs the sinus dominance was lost at 21.6 mM K and occasionally at 10.8 mM K. The latter finding occurred only after sympathetic suppression.

One example of pacemaker shift in a dog subjected to atriotomy and propranolol administration is illustrated in Figure 5. The normal sequence of atrial activation during perfusion with 10.8 mM K is shown in the enclosed area of the control recording. After propranolol administration, increasing the K concentration to 10.8 mM caused a shift in the temporal relationships of the atrial complexes (enclosed area, propranolol recording). The activity of the atrioventricular node electrode was first and that of the sinus node last. The P wave disappeared from the electrocardiogram and only the atrioventricular node deflection preceded the QRS complex. To test the temporal relationships of the atriograms when it was certain that the activation proceeded from the atrioventricular node upward, electrical stimuli were applied to the right ventricle (R.V. STIM.). The atrioventricular node complex appeared first with respect to the atrial activity of the other leads. If, instead, the stimulus

electrical stimulation, the ectopic rhythm returned. Again, the P waves changed very little with pacemaker shifts.

PROPRANOLOL ADMINISTRATION

The adrenal medulla is more resistant than the sympathetic nerves to the depleting action of reserpine (14). Therefore, catecholamines from chromaffin cells in the sinus node area might be available to counteract the depressant action of K even in reserpinized animals. To test the role of these catecholamine stores, beta receptors were blocked with propranolol. In six dogs, sinus node activity was consistently suppressed with 21.6 mM K infusion. The average rates in 5.4, 10.8, and 21.6 mM K were 145 ± 16, 146 ± 36, and 72 ± 13 beats/min, respectively (Fig. 2). The effectiveness of sympathetic beta-receptor blockade by propranolol was tested by stimulating the right stellate ganglion and, in one dog, by administration of norepinephrine into the sinus node artery. No sinus acceleration occurred with either procedure.

To determine the areas where the pacemaker shifted during high K perfusion, an extra electrode was sutured near the atrioventricular node area. The implantation of the intra-atrial electrode involved atriotomy and inflow occlusion procedures. It was found that these procedures influenced the

FIGURE 5

Effect of propranolol on pacemaker shifts induced by high K concentrations. Explanation of the labeling is the same as it is for Figure 1 except that AV indicates an atriogram from the atrioventricular node area. The number at the bottom of each section of the figure shows the K concentration at which the traces were recorded. CONTROL traces were recorded during 10.8 mM K infusion prior to propranolol administration. PROPRANOLOL traces show pacemaker shifts induced by K after propranolol administration. R.V. STIM. traces were recorded during electrical stimulation of the epicardial surface of the right ventricle. S.A. STIM. traces were obtained during electrical stimulation of the sinus node area. RECOVERY traces were recorded during 2.7 mM K infusion. The rectangles enclosing the atriograms were drawn to facilitate the comparison of temporal relationships among electrical events in the atrium. Pressure and time calibration are the same as they are for Figure 1.
was applied through the sinus node electrode, the atrioograms followed the sinus node activation as in the control (S.A. STIM.). The recovery in normal K is also shown. After propranolol administration to dogs subjected to atrioventricular node electrode implantation, the pacemaker shifted toward the atrioventricular node under the influence of high K concentrations in five of six experiments.

**CALCIUM CONCENTRATION**

The positive chronotropic response of the sinus node to sympathetic stimulation is decreased by decreasing the Ca concentration (4). If the resistance of the sinus node to high K concentrations is in part due to the sympathetic release, removal of Ca from the perfusing solution should make the sinus node more sensitive to K. This point was tested in seven dogs. Six of these dogs had had previous atriotomy. At 2.7 mM K, the sinus node pacemaker maintained dominance in the presence and the absence of Ca. However, even at 5.4 mM K, in three dogs in the absence of Ca the pacemaker shifted toward the base of the atrium. At 10.8 mM K, loss of sinus dominance occurred in two dogs in the presence and three dogs in the absence of Ca; at 21.6 mM K, the corresponding figures were three and six. It is interesting that in five dogs with chronic sympathectomy the presence or the absence of Ca made no difference in the loss of sinus node dominance. This finding would be expected if catecholamines are liberated by K primarily from sympathetic nerve endings. In this connection it must be pointed out that the acceleratory action of catecholamines is greater at high external Ca concentrations (15). Also, it has been shown that in the absence of Ca no catecholamine release occurs in response to sympathetic nerve stimulation (16) or high K concentrations in the adrenal medulla (13).

**Discussion**

The results obtained in this study suggest that catecholamines play a role in the resistance of the sinus node to high K concentrations. The inhibitory effect of high K concentrations on sinus node automaticity increases progressively as the influence of tonic sympathetic discharge and neural, extra-neural, and circulating catecholamines is progressively eliminated.

The reason for the different sensitivity of different cardiac pacemakers to high K concentrations is not understood. That such a difference should exist is not surprising, for it is clear that pacemaker tissues of the heart differ in several aspects. For example, lowering the standard Na concentration causes a decrease in spontaneous activity in Purkinje fibers (17) but little change in the sinus node rate (3, 18, 19). Tetrodotoxin, which blocks fast Na channels (20), suppresses Purkinje fibers (15, 21) but not sinus node cells (15, 22, 23). High Ca decreases the rate of spontaneous discharge of Purkinje fibers (24) but increases that of the sinus node (19, 25-29), even after abolition of autonomic influences (25, 28, 29). Although the slowing in Purkinje fibers is due to a shift in threshold (24), the acceleration in the sinus node is due to a steepening of diastolic depolarization (25, 27). Manganese (Mn) suppresses the activity of the sinus node but not that of Purkinje fibers (15). Increasing the external Ca concentration partially counteracts the Mn-induced suppression (15). The present experiments illustrate yet another difference. After complete elimination of sympathetic and vagal influences, the sinus node continues to discharge at a K concentration of 10.8 mM, but Purkinje fibers become quiescent at half this concentration (1). In fact, the sinus node was shown in this study to remain active even at higher K concentrations but only if catecholamines were available. In this regard, it is significant that the innervation of the sinus node is more abundant than that of Purkinje fibers (30). Chemical and fluorescence histochemistry determinations of catecholamine content have shown that the sinus node receives a far more extensive sympathetic innervation than does the ventricular Purkinje fiber network and has a higher content of catecholamines (norepinephrine and dopamine) than any other region of the heart (31, 32).

It seems convenient to discuss now the mechanism by which K may depress the automaticity of different pacemaker tissues. The mechanism underlying the diastolic depolarization has been extensively studied in Purkinje fibers and found to be a time-dependent fall of a slow K current (33, 34). No detailed analysis of diastolic depolarization in the sinus node is available. However, both the Purkinje fibers and the sinus node tissues respond to an increase in membrane conductance to K (gK) with a decrease in automaticity. In Purkinje fibers, gK is increased by increasing K (35): when K is doubled to 5.4 mM, the membrane resistance is halved and fibers become quiescent (1). In the sinus node, gK is increased by acetylcholine, and this effect also results in suppression of spontaneous
activity. There are, however, differences between the response of the sinus node and that of Purkinje fibers to different K concentrations. Thus, when K in the perfusion solution is reduced from a high to a normal level, the maximum diastolic potential of the sinus node increases (3, Fig. 5) but less than that of Purkinje fibers (36, Fig. 17). The smaller increase in the maximum diastolic potential in the sinus node may be due to a significantly higher membrane conductance for Na at normal K levels (18), implying that, when K is increased, gK of sinus node cells increases and partially compensates for the decrease in K equilibrium potential. This possibility is supported by the finding that, in the sinus node, vagal stimulation hyperpolarizes more at low than at high K concentrations (3). A contributory factor in accounting for the persistence of sinus node discharge at high K concentrations may be the fact that sinus node excitability is less dependent on the resting potential than is Purkinje fiber excitability (37).

The above factors may be involved in the ability of the sinus node to discharge in a solution containing 10.8 mM K. However, the present experiments show that catecholamines are required if sinus node activity is to be maintained at the higher concentration of 21.6 mM K. That such a requirement exists is not too surprising since the reciprocal finding has been described; namely, a high K concentration has been shown to depress the positive chronotropic action of both exogenous catecholamines (23) and sympathetic stimulation (4). In other words, K antagonizes the positive chronotropic effect of catecholamines and catecholamines antagonize the negative chronotropic effect of K. The mechanism by which catecholamines counteract the depressant effect of K is not known. Among several possible explanations, it is conceivable that the inward current flowing during diastolic depolarization of sinus node cells could include a Ca component (15) and this component could be increased by norepinephrine. If, at the same time, the threshold for the slow Na and Ca currents flowing during the action potential is shifted to more negative values by norepinephrine (38), less depolarization would be required during diastole to maintain the spontaneous discharge of the sinus node. Another possibility is that norepinephrine shifts to more positive values the voltage dependence of activation of a slow K current, as has been shown in Purkinje fibers (39). The effect of such a shift would be to force the slow K current to suitably decrease during diastole to a threshold value. Such a mechanism would imply that a time-dependent fall in the slow K current underlies diastolic depolarization in the sinus node cells as it does in Purkinje fibers.

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References

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