Action of Manganese Ions and Tetrodotoxin on Atrioventricular Nodal Transmembrane Potentials in Isolated Rabbit Hearts

By Douglas P. Zipes and Carlos Mendez

ABSTRACT

In the isolated rabbit heart, it was shown that (1) manganese ions (Mn²⁺) in concentrations of 5 × 10⁻⁶ g/ml Tyrode’s solution rendered atrial and His bundle fibers inexcitable without preventing AV nodal potentials of automatic origin and without affecting the rate of rise of cells located in the N region, and (3) in the presence of TTX (5 × 10⁻⁶ g/ml of Tyrode’s solution) the addition of epinephrine (2 µg/ml solution) restored atrial activity. It was concluded that the different effects of Mn²⁺ and TTX on AV nodal action potentials is consistent with the postulate, made by Rougier et al. (8) and Vassort et al. (9) for frog atrial fibers treated with TTX, that AV nodal action potentials probably result from the operation of a slow ionic channel.

KEY WORDS

propagation in AV node
slow ionic channel
AV nodal automaticity
His bundle fibers
atrial fibers
epinephrine

AV conduction block

The main characteristic of atrioventricular (AV) nodal action potentials is their slow-rising depolarization phase (1–5). This slow rate of voltage change (dV/dt) and the selective effect of acetylcholine on AV nodal action potentials led Paes de Carvalho et al. (6, 7) to postulate that the ionic mechanisms responsible for regenerative responses in nodal tissue differ from those responsible for regenerative responses in the rest of the myocardium. According to this hypothesis, typical AV nodal action potentials result from a small positive inward current and a concomitant decrease in resting potassium (K⁺) conductance.

With the voltage-clamp technique, Rougier et al. (8) and Vassort et al. (9) showed the existence of two ionic channels responsible for regenerative responses in isolated frog atrial strands. They demonstrated that the first channel provides a rapid inward current carried by sodium ions (Na⁺). The current is voltage and time dependent, and the channel is blocked specifically by tetrodotoxin (TTX). The second channel permits a slow inward current, also voltage and time dependent, carried by Na⁺ and calcium ions (Ca²⁺). This second channel is not blocked by TTX but is inhibited by manganese ions (Mn²⁺). These authors proposed that the slow channel might play an important role in the genesis of AV nodal action potentials.

In the present study this hypothesis was tested, although no attempts were made to elucidate the ionic mechanisms of AV nodal activity. The results obtained agreed with the concept that an ionic channel blocked by Mn²⁺ but not affected by TTX is responsible for the generation of AV nodal active responses.

Methods

Experiments were performed in isolated rabbit hearts according to the technique described by Hoffman et al. (2). The preparation, consisting of the AV nodal region and attached portions of the atrium and the His bundle, was perfused with continuously flowing Tyrode’s solution; unless otherwise indicated in the Results, the millimolar composition of the Tyrode’s solution was: NaCl 136.8, KCl 2.7, NaH₂PO₄ 0.4, CaCl₂ 1.8, MgCl₂ 1.1, NaHCO₃ 11.9, and dextrose 5.5. The temperature of the tissue bath was maintained at 36 or 37 ± 0.4°C.
In all experiments the sinoatrial (SA) node was excised, and the preparation was driven by stimuli conducted through bipolar silver electrodes applied to the right atrial roof or to the His bundle. Stimuli were rectangular pulses 1 msec in duration obtained from a Tektronix pulse generator and passed through an isolation transformer.

In all experiments one or two simultaneous records of transmembrane potentials were obtained by conventional techniques. The electrical signals were displayed on a 565 Tektronix oscilloscope and photographed with a Grass camera. In some experiments atrial electrograms were recorded from bipolar silver electrodes located in the crista terminals close to the ostium of the coronary sinus.

Electronic differentiation of the transmembrane action potential was accomplished with an operational amplifier having a variable time constant appropriate to the rates of change encountered in the several tissues. The system was linear. A ramp of known slope was fed to the microelectrode to initiate. With either TTX or MnCl₂ repeated impalements encountered cells with membrane potentials in excess of this arbitrary limit (Table 1).

The subdivision of the AV node into three main regions (AN, N, and NH) proposed by Paes de Carvalho and de Almeida was retained in this study; classification of cell types was based on action potential configuration and temporal relationships during AV propagation. An ocular micrometer attached to a dissecting microscope was used to obtain control and corresponding test records from essentially the same location within each subdivision of the node.

Results

The depressant effects of MnCl₂ on transmission of the cardiac impulse were primarily confined to the AV node; block occurred within the nodal area while propagated impulses could still be initiated within atrial tissue or the His bundle. The effects of TTX were exerted primarily on cells in the atrium and the His bundle, in which propagated activity was suppressed at a time when spontaneous impulses were recorded from cells within the AV node.

Because it was usually impossible to maintain impalements of the same AV nodal cell throughout control, experimental, and recovery periods, we limited the analysis of results to cells in which the resting membrane potential exceeded 50 mv regardless of whether propagated responses could be initiated. With either TTX or MnCl₂, repeated impalements encountered cells with membrane potentials in excess of this arbitrary limit (Table 1).

During the control period, the action potential of all nodal cells tested had a clear-cut overshoot (Table 1).

<table>
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<tr>
<th>Tissue</th>
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Many impalements during drug effects yielded resting membrane potentials within the normal range. Only those cells that were studied completely are included in the table.

*No significant alterations in resting membrane potential were observed in any of the cells studied with the possible exception of atrial cells exposed to TTX.
‡Most of the observations of atrial and His bundle activity during exposure to Mn²⁺ were made from extracellular recordings; in no case did propagation fail within these tissues.
§Measurements of overshoot and dV/dt are listed only for cells studied prior to the development of complete block.

TABLE 1

Effects of Mn²⁺ and TTX on Membrane Potentials, Overshoot, and dV/dt

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EFFECT OF MANGANESE IONS

The observations described in this section were obtained with Tyrode's solution lacking NaH$_2$PO$_4$ to avoid precipitation when MnCl$_2$ was added.

In concentrations ranging from 2 to 4 mM, MnCl$_2$ caused complete conduction block in the rabbit's AV node. These concentrations of Mn$^{2+}$ did not suppress atrial or His bundle activity when those tissues were directly stimulated. Figure 1 illustrates characteristic results. Records in A and B were obtained from the same cells under control conditions. Between B and C, MnCl$_2$ was added to the perfusion solution to a final concentration of 2 mM, and 30 minutes was allowed for equilibration. C shows two superimposed records obtained during antegrade propagation. The newly impaled AN cell had an action potential without overshoot. Its peak reached only $-9$ mv, although the resting potential was well within normal values. Only a subthreshold depolarization was obtained from the impaled His bundle fiber in one sweep; in the next sweep activity reached and discharged the His bundle, but only after a conspicuous delay in conduction. D, obtained from the same cells immediately after C but with stimuli applied to the His bundle, shows that retrograde propagation was blocked within the node; the AN cell yielded only a subthreshold depolarization, and no activity reached the atrium. In all eight experiments performed with MnCl$_2$, retrograde propagation failed before propagation in the normal direction. Between D and E, the Mn$^{2+}$ concentration was increased to 4 mM. The AN response shown in E had an extremely long rise time and its peak reached only $-22$ mv, although the resting potential of the impaled cell, 62 mv, was close to that in C. The His bundle fiber did not show even a subthreshold depolarization, because antegrade conduction through the node was abolished. The records in F, obtained from the same cells immediately after E, show that the His bundle was still capable of firing actively when it was directly stimulated, although the recorded action potential was distorted—the last part of its upstroke was rather slow. Retrograde propagation was absent, and the AN cell did not show any change in its resting transmembrane potential. Although it was often difficult to obtain reliable records from the AN region, the tracings in Figure 1 show that in this region of the AV node Mn$^{2+}$ depressed the action potential magnitude without altering the resting membrane potential.

A comparison of the effects of Mn$^{2+}$ on the action potentials of cells from the atrium, the N region, and the His bundle obtained from the same representative experiment is illustrated in Figure 2. A and B correspond to transmembrane potentials from atrial fibers. The repolarization phase of the action potential evoked by direct stimulation of the atrium was altered by Mn$^{2+}$: the early phase of repolarization was accelerated and the last phase was retarded. In this experiment, $dV/dt$ after Mn$^{2+}$ perfusion was slightly greater than it was in the control cell. However, since the recordings were not made from the same cell before and after MnCl$_2$ application, we do not know the control $dV/dt$ for...
FIGURE 2

Effects of MnCl₂ on atrial, N, NH, and His bundle transmembrane potentials. Left: Control observations. Right: After administration of MnCl₂ (3 mM). In each section, the top trace shows transmembrane potentials, and the bottom trace corresponds to dV/dt. A and B: Atrial responses. C and D: N cells. E and F: NH cells. G and H: His bundle cells. In each section the horizontal lines preceding or above action potentials indicate zero potential for each impaled cell. Calibrations: 100 msec and 50 mV for all panels. Vertical calibration: 40 mV/sec in A, B, E, G, and H and 20 mV/sec in C, D, and F. See text for discussion.

the cell in B and, conceivably, it could have exceeded the value recorded after Mn²⁺ perfusion. C and D correspond to transmembrane potentials of N cells. After Mn²⁺ was added, only a local subthreshold potential was obtained when the His bundle was stimulated. However, the resting potential remained within normal values. With the same impalement, atrial activation caused an even smaller depolarization (not shown in Fig. 2). E and F, obtained with stimulation of the His bundle, correspond to tracings from cells in the NH region. A response which was probably electrotonic rather than active was recorded in the presence of Mn²⁺ (F); its dV/dt was approximately a third of that obtained in the control situation (see calibrations in the figure legend). No response was recorded from this cell following atrial stimulation. G and H correspond to records obtained from the His bundle when this tissue was directly stimulated. The response in the His bundle cell was regenerative with a considerable overshoot, i.e., propagation was still possible. The slower rise time and the lower resting membrane potential at this time could have been due to a poor impalement.

Results qualitatively similar to those shown in Figure 2 were obtained in five additional experiments. Since it was not possible in each experiment to obtain good impalements in all the regions explored, the poor resting potential shown in Figure 2F was probably the result of faulty technique. However, in three cases in this series of experiments, the resting potential of NH cells obtained in the presence of Mn²⁺ was within the normal range, but the peak of the responses initiated by direct stimulation of the His bundle often failed to cross the zero-potential line (Table 1).

**EFFECT OF TETRODOTOXIN**

TTX³ at concentrations higher than those needed to block the first rapid channel in the frog atrium (8) completely suppresses excitability of atrial fibers in isolated rabbit hearts (10). In the present study, TTX also caused inexcitability of the His bundle, yet the drug did not abolish the activity of AV nodal cells. Figure 3 illustrates this point. A, recorded during the control period, shows the transmembrane action potential of a cell located in the crista terminals. Between A and B, TTX was added to the perfusion solution to a final concentration of 5 × 10⁻⁶ g/ml Tyrode's solution. B shows the

³Tetrodotoxin, lot no. 001839, was purchased from Calbiochem.
absence of atrial activity (bottom) and the zero-potential level (top). Multiple impalements in atrial tissue showed a complete absence of regenerative activity even when large currents were passed through the external stimulating electrodes. It was also impossible to initiate activity in the His bundle by direct stimulation, but in the same experiment and under the same conditions active responses of automatic origin were recorded in regions of the AV node (C). The action potentials were recorded on moving film; the top trace in C corresponds to an N cell and the bottom trace to an NH cell. It is clear that the N cell fired earlier than the NH cell. Therefore, the spontaneous activity originated in tissue which was unaffected by TTX and located proximal to the site where the NH cell was impaled. The automatic activity could not have traveled over atrial fibers, since those fibers were inexcitable. This phenomenon will be briefly analyzed in the Discussion.

Effect of Epinephrine in the Presence of Tetrodotoxin

It has been shown that epinephrine can restore activity in frog atrial muscle rendered inexcitable by TTX (9, 11), and the same phenomenon is
Figure 5
Effect of TTX on NH action potentials (top) and the corresponding dV/dt (bottom). A: Control. B: After administration of TTX. Horizontal lines preceding action potentials indicate zero potential. Calibrations: 100 msec, 50 mv, and 40 v/sec for A and 100 msec, 50 mv, and 20 v/sec for B. See text for discussion.

Effect of TTX on NH action potentials (top) and the corresponding dV/dt (bottom). A: Control. B: After administration of TTX. Horizontal lines preceding action potentials indicate zero potential. Calibrations: 100 msec, 50 mv, and 40 v/sec for A and 100 msec, 50 mv, and 20 v/sec for B. See text for discussion.

Present in atrial tissue of the rabbit. In this species action potentials in the presence of TTX can again be recorded after the administration of epinephrine, but they have a considerably lower dV/dt than they do under control conditions. Figure 6 illustrates this point. A represents an atrial action potential recorded during the control period. Its dV/dt was 116 v/sec. After this record was obtained and after the addition of TTX, the atrial tissue was inexcitable, but resting potentials were within the normal range (not shown in Fig. 6). In the presence of TTX, epinephrine was added to the tissue bath to a final concentration of about 2 μg/ml; atrial activity was restored as shown in B. The active response was automatic in origin. The sweep was half that used in A, and the dV/dt of the response was only 13 v/sec (see calibrations in the figure legend). These results are very similar to those reported by Vassort et al. (9) for frog atrium.

Discussion
Nature of AV Nodal Potentials
Rougier et al. (8) and Vassort et al. (9) proposed that the depolarization phase of active responses in the frog atrium results from the successive operation of two ionic channels. According to these authors, the first part of the ascending phase of the action potential results from a rapid inward current carried by Na+ that can be blocked with TTX. The second part of the ascending phase corresponds to a slowly developing inward current carried by Na+ and Ca2+. This slow channel is not influenced by TTX but is inhibited by Mn2+.

Lenfant et al. (10) provided suggestive evidence that a slow channel is involved in the generation of SA nodal potentials in the rabbit heart, and Vassort et al. (9) proposed that AV nodal action potentials might also result primarily from the operation of a slow channel. The results obtained in the present study agreed with this suggestion. Thus, Mn2+ did not significantly alter the resting potentials of nodal cells but suppressed the regenerative activity of AN, N, and NH cells. Under the influence of Mn2+ the atrial and the His bundle action potentials elicited by direct stimulation of the corresponding tissue had a marked overshoot; the magnitude of nodal responses, however, decreased considerably and depended on the location of the impaled cell relative to the input provided. With atrial inputs, the larger responses were found in the AN region, and yet their peaks often fell well below the zero-potential reference (Table 1). Under the same conditions, the magnitude of the responses decayed with distance: small depolarizations were obtained from the N region, and only faint changes were detected in the NH region. The reverse was true when the His bundle was stimulated directly, i.e., relatively large depolarizations were found in the NH region, smaller depolarizations were recorded from the N region, and practically nothing was detected in the AN region. Obviously, complete antegrade and retrograde block was established under these conditions.

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In the presence of Mn$^{2+}$ the small depolarizations observed in the N region and their spatial decay were very probably electrotonic in nature. However, the relatively large depolarizations obtained, when the proper input was used, from the AN and NH regions might have contained a small active component. But even if that was the case, these responses were not capable of propagation.

It can be argued that, with the procedure used, it was not possible to determine the effects of Mn$^{2+}$ on the N region because the antegrade or the retrograde inputs, AN and NH, respectively, were too small to fire the N cells. However, just before complete block was established, the overshoot of the recorded N potentials was grossly depressed or totally suppressed. Therefore, it seems safe to conclude that Mn$^{2+}$ had the same qualitative effect on the N region as it did on the AN and NH regions.

TTX prevented the initiation of regenerative responses in the atrium and the His bundle, but the spontaneous action potentials recorded from N cells remained within normal limits for resting potential, overshoot, and dV/dt of the action potential.

It is therefore clear that the effects of TTX and Mn$^{2+}$ are quite different in AV nodal tissue and in the surrounding tissues (atrial and His bundle fibers). The discrepancy would be readily explained if, as proposed by Rougier et al. (8) and Vassort et al. (9), the slow channel was mainly responsible for active responses in the AV node. Mn$^{2+}$ suppressed nodal activity, but TTX did not, at least in some regions of the node, affect the magnitude or the dV/dt of action potentials. However, in the atrium and the fibers of the His bundle, the first rapid channel plays a predominant role during the fast depolarization. Accordingly, TTX rendered these tissues inexcitable, but Mn$^{2+}$ did not prevent the initiation and the propagation of regenerative responses.

More study is needed to account for the changes caused by Mn$^{2+}$ on the configuration of atrial and His bundle action potentials. At present only tentative explanations can be given. Thus, the initial rapid repolarization of the atrial response shown in Figure 2B might be due to inhibition of the slow channel. However, the later prolongation of repolarization does not fit the same explanation. Rougier et al. (8) found that Mn$^{2+}$ at a concentration of 1 or 2 mmoles/litre shortens the action potentials of the frog atrium. However, they also indicated that lower concentrations of Mn$^{2+}$ can increase the total duration of atrial action potentials, and they attributed the phenomenon to a lowering of the membrane K$^+$ permeability. It is conceivable that in the rabbit the concentrations of Mn$^{2+}$ used also decreased the K$^+$ conductance. This phenomenon would explain the prolongation of the action potential duration, but we do not have evidence to prove that it actually was the case.

The decreased dV/dt of His bundle action potentials caused by Mn$^{2+}$, especially during the later part of the upstrokes, is puzzling. The possibility exists that the second channel contributed to the ascending phase of His bundle responses and that Mn$^{2+}$ suppressed that component. Other alternatives are of course possible; for instance, Mn$^{2+}$ could affect the behavior of the first rapid channel in His bundle fibers, although this possibility seemed not to be the case in atrial tissue. It is also possible that the interaction between the His bundle and the adjacent nodal fibers could retard depolarization in the His bundle cells. If this hypothesis is correct, a zone of transition should exist in the proximal part of the AN region and in the distal part of the NH region, in agreement with the observations of Paes de Carvalho (6). In the transitional zones, the relative contributions of the first rapid channel and the second slow channel would change rapidly with distance until in typical nodal tissue only the slow channel would operate. This notion, well illustrated in the experiments of Paes de Carvalho (6), could explain the action of TTX on distal NH fibers (Fig. 5B). In these transitional cells, the first rapid channel might contribute to the upstroke of the action potential, and the observed decrease in dV/dt would correspond to the blocking action of TTX.

Vassort et al. (9) showed that epinephrine does not affect the behavior of the first rapid channel in frog atrial fibers but considerably increases the inward current flowing through the slow channel. In their records, it is clear that, once the first channel is blocked by TTX, the rate of rise of the regenerative responses provided by the slow channel increases under the influence of epinephrine. In the present study, epinephrine was also shown to restore activity to atrial cells in the presence of TTX.

The present study did not provide information on the ionic nature of AV nodal action potentials. It seems tempting to extrapolate that the same ionic species postulated by Rougier et al. (8) for the slow channel of atrial fibers carry the inward current in the AV node. However, definitive evidence can only
be obtained using the voltage-clamp technique, a procedure that, at present, cannot be employed in AV nodal tissue. Furthermore, the possible pitfalls of this technique when applied to bundles of cardiac fibers have been analyzed recently by Johnson and Lieberman (12). Accordingly, only future developments will decide the true nature of AV nodal action potentials.

It seems important to emphasize that our results do not contradict those reported by Paes de Carvalho (6) and Paes de Carvalho et al. (7). If the AV nodal action potentials result from inward current flowing through the slow channel, the action of acetylcholine can be explained with the simple assumption that this agent increases the permeability of the K+ channel. In other words, the AV nodal action potentials might well be due to an inward current carried perhaps by Na+ and Ca2+, but this current could be effectively counteracted by an outward K+ current resulting from the increased K+ permeability induced by acetylcholine.

ORIGIN OF AV NODAL RHYTHMS

It has been our experience and that of other investigators that the only region within the AV node of the rabbit that clearly exhibits phase-4 depolarization is the distal part of the NH region. The AN and N regions seem to be devoid of this property. Yet in Figure 3C, in the presence of TTX, it is clear that the automatic activity originated proximal to the impaled NH cell. It is doubtful that the spontaneous activity arose from specialized atrial fibers. Multiple impalements on the specialized posterior tract never showed regenerative activity in the presence of TTX. Furthermore, the SA node was eliminated in our preparations. A possible alternative is that the automatic activity originated in the AV ring (5). Under this condition, AV nodal rhythms could start in this region and in the electrocardiogram the P wave could shortly precede the QRS complex. In other cases, if the automatic activity predominated in the NH region, the QRS complex could precede the P wave. We have not explored this point in detail, but it seems that TTX can be employed as a useful tool in the elucidation of AV nodal rhythms.

Acknowledgment

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References

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