EFFECTS OF VERAPAMIL ON SA AND AV NODAL ACTION POTENTIALS IN THE ISOLATED RABBIT HEART

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In the isolated rabbit heart, it was shown that verapamil suppressed the electrical activities of the SA and AV nodes without preventing regenerative responses in atrial muscle fibers. It was concluded that the different effects of verapamil on SA and AV nodal action potentials resulted from the operation of a different ionic channel (slow channel) from that of ordinary atrial muscle.

RECENT advances in the electrophysiological technique, particularly the voltage clamp method, have revealed that the rising phase of the action potential of the working myocardium and the Purkinje fiber have a similar origin as in the case of the squid fiber action potential,¹⁻³ although there is some controversy concerning the plateau and the repolarization of the action potential. However, the ionic mechanisms of the SA and AV nodes are less known and less investigated than those of the Purkinje fiber and the working myocardium due to the experimental difficulties. In 1969 Rougier *et al.*⁴ showed the existence of two distinctly separable ionic channels allowing the flow of two different inward currents during the action potential: one which is carried by sodium ions and specifically blocked by tetrodotoxin (TTX) contributes to the fast depolarizing phase; the other which is carried by both calcium and sodium ions and specifically blocked by manganese ions (Mn^{++}) contributes to the plateau. They proposed that the second channel (slow channel) might be the sole factor responsible for the action potential of the SA node of the rabbit. In the present study this

hypothesis was tested, although no attempts were made to elucidate the ionic mechanisms of the SA and AV nodal activities.

METHOD

Rabbits randomly selected were anesthetized with intravenous sodium pentobarbital (30 mg/kg). Under artificial respiration, the chest was opened and the heart was quickly removed. In order to obtain an isolated preparation, the cut was made between the SA and AV nodes so that these two tissues could be separated from each other. The tissues were mounted in a Clarex block and continuously perfused with Tyrode solution equilibrated with 95%O₂ + 5%CO₂. The millimolar composition of Tyrode solution was as follows: NaCl, 137 mM; KCl, 2.7 mM; CaCl₂, 1.8 mM; MgCl₂, 0.5 mM; NaH₂PO₄, 3.3 mM; NaHCO₃, 11.9 mM; dextrose, 5.5 mM. The temperature was maintained at 33.6 – 35.2°C. In some experiments, the SA node was stimulated with rectangular pulses of 1 msec at a frequency of 120 Hz applied through a pair of fine platinum wires insulated except for the tips (Nihon-Koden MSE-3). An isolated atrial muscle preparation was stimulated at the same frequency. Transmembrane potentials were recorded from the endocardial surface between an intracellular microelectrode and a distant indifferent electrode. The 3M-KCl filled microelectrode was connected through an Ag-AgCl₂ electrode holder to a high input impedance preamplifier with input capacity

Key Words:

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Fig. 1. Structure of verapamil.

Fig. 2. Action of verapamil (5×10^{-4} g/L) on SA node. Vertical bar represents 50mv and its top shows zero line. Horizontal bar represents 0.5sec. Fig. 3. Action of verapamil (2×10^{-3} g/L) on SA node. Description as for Fig. 2. Note artifacts during electrical stimulation.

neutralization (Nihon-Koden MZ-3B) and displayed on an oscilloscope (Nihon-Koden VC-7) and photographed on moving films at speeds of 2.0 and 20.0 cm/sec. In atrial muscle preparations, the records were photographed on films by triggering the horizontal axis of the oscilloscope instead of moving the films. The chemical formula of verapamil (α -isopropyl- α -(N-methyl-N-homoveratryl)- γ -aminopropyl]-3,4-dimethoxyphenylacetone nitrile hydrochloride* is presented in Fig. 1. In order to obtain the desired concentration, an appropriate amount of concentrated verapamil solution was injected through the syringe into the bath. The first time derivative of the action potential was obtained with the aid of an appropriate operational amplifier and was calibrated as follows: Triangular pulses of known amplitude and frequency were applied between the ground and the indifferent electrode. This signal was recorded by the microelectrode and amplified by the high input impedance preamplifier and delivered to the differentiator. The slope of the triangular pulses was determined and the corresponding output voltage of the differentiator was measured. It was linearly related to the slope of the triangular pulses up to 500 v/sec.

RESULTS

Figs. 2 and 3 showed the effects of verapamil on SA nodal action potential at a concentration of 5×10^{-4} g/L, 2×10^{-3} g/L respectively. At a concentration of 5×10^{-4} g/L, verapamil suppressed the maximum diastolic potential (MDP) and the membrane potential at the peak of depolarization and produced a decrease in the slope of diastolic depolarization and the rate of rise. However, the automaticity was not lost. At a concentration of 2×10^{-3} g/L, the SA node lost its automaticity 20 minutes after verapamil was injected into the bath and only local

* supplied by the Eisai Pharmaceutical Company.

Fig.4. Action of verapamil (5×10^{-4} g/L) on AV node.
Description as for Fig.2.

Fig.5. Action of verapamil (2×10^{-3} g/L) on AV node.
Description as for Fig.2.

responses were obtained by direct electrical stimulation. Figs. 4 and 5 showed the effects of verapamil on AV nodal action potential at the same concentration as in the SA preparation. At a concentration of 5×10^{-4} g/L, verapamil markedly suppressed the spontaneous firing rate of the AV node. However, it hardly affected MDP and overshoot of AV nodal action potential. The lower resting membrane potential in the bottom tracing of Fig. 4 could be due to a poor

impalement. Fig. 5 showed the effects of verapamil on AV nodal action potential at a concentration of 2×10^{-3} g/L. After 3 minutes of injection, overshoot was reduced without changing the MDP significantly. After 15 minutes, the AV node yielded only subthreshold oscillations. The effects of verapamil on ordinary atrial muscle was presented in Fig. 6 in comparison with that of verapamil on the SA and AV nodes. After 15 minutes, Max dv/dt

Fig.6. Action of verapamil (2×10^{-3} g/L) on atrial intracellular action potentials. In each section the records are as follows: Top tracing corresponds to dv/dt , middle two tracings to intracellular potentials at slow and fast ($\times 10$) speeds, bottom tracing to rectangular pulses (100Hz). Left and right vertical bars 50v/sec and 50mv, respectively.

of the atrial muscle was slightly lower than it was in the control cell, while the resting potential (RP) and the overshoot remained almost the same value. The early phase of repolarization (Phase 1) was accelerated and the late phase of repolarization (phase 3) was retarded. The configuration of the action potential became triangular in shape.

DISCUSSION

In 1969, *Rougier et al.*⁴ applied the sucrose gap method to frog atria and showed the existence of two distinctly separable ionic channels responsible for the cardiac electrogenesis, one carried by sodium ions and specifically blocked by TTX, the other carried by sodium and calcium ions. They called the latter ionic current the "slow channel"

and demonstrated that this ionic current was not blocked by TTX but was specifically blocked by manganese ions (Mn^{++}). In 1972, *Kohlhardt et al.*⁵ showed that this current was blocked by organic calcium antagonists such as verapamil and D 600 in a highly selective manner. In the present study, it was shown that verapamil suppressed the electrical activities of the SA and AV nodes, while it did not prevent the regenerative responses of atrial muscle. This difference of the actions of verapamil can be explained if, as *Rougier et al.* proposed, the slow channel is involved in the generation of SA and AV nodal action potentials. The fact that TTX hardly affected the electrical activities of the SA and AV nodes, while it resulted in the loss of the excitability of the ordinary atrial and ventricular

muscles of the rabbit (unpublished observation), further confirms the hypothesis of *Rougier et al.* and the fact that this suppressive effect of verapamil was compensated for by the elevation of extracellular calcium ions suggests that calcium ions play an important role in the generation of SA nodal action potential (unpublished observation). Although the subdivision of the AV node into three main regions (AN, N, NH region) was not performed, definite differences among verapamil induced-AP changes in the various portions of the AV node were not observed. Since it was not possible to make good impalements all the time during the experiments, statistical evaluation could not be made in the present study. However, we think that the actions of verapamil differed between the SA node and the AV node. As Figs. 2 and 4 demonstrated, in a concentration of 5×10^{-4} g/L verapamil reduced the MDP and the overshoot of the SA node, while it did not seem to change the MDP and the overshoot of the AV node. Even in a high concentration (2×10^{-3} g/L) verapamil seemed to maintain the MDP of the AV node at almost the same level as that in the control cell, though it reduced the overshoot markedly. At present we do not know the reason for this difference. The change of configuration of the atrial muscle induced by verapamil could be tentatively explained by the hypothesis that the acceleration of phase 1 was due to inhibition of the slow channel. However, the retardation of phase 3 could not be explained by the inhibition of the slow channel but could be explained by supposing that verapamil reduced the membrane potassium conductance and this would explain the reduction of the MDP of SA nodal action potential. Recently *Zipes et al.*⁶ observed the effects of manganese ions on the AV node in the isolated rabbit hearts and reached the same con-

clusion. Thus it is conceivable that the electrical activities of the cells with low resting membrane potential and small dv/dt , such as the SA and AV nodes, are generated by a common ionic channel (slow channel) different from that of ordinary atrial muscle.

CONCLUSION

It was concluded that the different effects of verapamil on SA and AV nodal action potentials resulted from the operation of a different ionic channel (slow channel) from that of ordinary atrial muscle.

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