The Influence of Mersalyl and some Sulphydryl Group Reagents on the Pacemaker Action Potential and the Contraction in Isolated Guinea Pig Atria.

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In order to analyze the cause of a cardiotoxic action of mersalyl, its direct effects of transmembrane action potential in cardiac pacemaker cells and on contractility of the atrial muscle fibers were investigated in isolated guinea pig hearts. The effect was compared with those of sod. arsenite, iodoacetamide, N-ethylmaleimide and iodosobenzene acetate. All of these SH reagents caused a shortening of the duration and a reduction of the propagated action potential. The former change is considered as a cause of the rhythm accelerating effect of these reagents, and the latter as a cause of arrhythmia. The reagents act on the contractility different ways, while iodoacetamide and sod. arsenite having a marked depolarizing character cause the contracture.

It has long observed that the mercurial preparation has the strong cardiotoxic effects i. e., its high concentration causes cardiac arrhythmia. That results acute death in animals and also in human beings following the intravenous infusion of mercurial preparations (1, 2, 3). And the toxic action has been attributed to a blocking action of sulphydryl radicals in the tissue (4). Recently, E. Muscholl and others reported the effect of mercurial preparations on transmembrane action potential in diaphragm muscle fibers and cardiac proper fibers (5, 6). However, nothing is known about the direct action of the agents and other types of sulphydryl reagents upon cardiac pacemaker cells.

In the present study attempts were made to observe influences of mercurial preparations on the membrane activity of the pacemaker cell in isolated guinea pig atria with an aid of intracellular microelectrode technique and on the contractility of atrial muscle fibers, because it is thought as the most basic problem to consider the mode of the cardiotoxic action. And the effects of the mercurial preparation is compared with those of other types of sulphydryl reagents subjected to analyze pharmacologically.
METHODE and MATERIALS

The hearts taken from exsanguinated guinea pigs of either sex weighing 180g to 350g were used throughout the experiment. The atrium was quickly separated from the ventricle and placed in an oxygen saturated nutrient fluid bath at about 30°C. Isometric record of contractions of the naturally beating atria was carried out by using a strain gage transducer (Shinkoh UL-10-120) and an automatic balancing strainrecorder (Shinkoh AS-2) with an initial tension of 600mg.

Transmembrane action potentials of the pacemaker cell in the right atrium were led from its pacemaker region via intracellular microelectrode of suspended type (with 80μ copper wire) to a low input high impedance amplifier (Nihonkohden MZ-3B) and displayed in two cathode ray oscilloscopes, utilizing one for record, the other for monitor. The location of the pacemaker area and the procedure of its exposure will be described in the next chapter. In the current study, stable records of a pacemaker action potential having a distinct prepotential were at least 10mV conveniently.

The solution composed of NaCl 154mM, KCl 5.6mM, CaCl₂ 2.2mM, Glucose 5mM, NaHCO₃ 5.95mM per liter was used as a nutrient medium. The chemicals used in this experiment were mersalyl sodium (Sigma), iodoacetamide (Hani Co.), iodosobenzene acetate (Wako Co.), sodium arsenite (Hani Co.) and N-ethylmaleimide (Sigma).

RESULTS

A. Location of pacemaker cells in the guinea pig.

A. P. de Carvalho and et al. have studied extensively about the location of specialized fibers in the rabbit atrium and have drawn its distribution map (7). However it was confirmed in the current investigation that the map could not be adapted for the guinea pig atrium. Although a report concerning activity of pacemaker cells of the guinea pig atrium was already published from our laboratory, yet precise location of the area has not been presented. So the approximate site and procedure of the exposure are for the first time displayed in this paper.

A right atrium was isolated from the heart together with interatrial septum and the tricuspid valve. In the atrium, an incision from atrioventricular orifice to superior vena cava was taken, then the cut was extended along with free anterior edge of the atrium from atrioventricular orifice to the triangular cusp of the right atrium. The dissected atrium was fixed with stainless steel needles on a cork block in a horizontal bath. The appearance is likely to Fig. 1. In this drawing, a shaded trapezoidal portion was found as the most probable site which gives a typical pattern of the pacemaker action potential having a diastolic slow depolarization. In the practice, impalement of a microelec-
Fig. 1. Schematic drawing the site of the pacemaker area in endocardial side of the right guinea pig atrium. A triangle is postulated between one point at the ostium of inferior vena cava and two points on the crista terminalis intersecting with large endocardial foldings. And the trapezoidal portion was found as the most probable site of the pacemaker area in guinea pig atria.

trode was made at arbitrary point in this portion and some places had to be changed to have the sufficient prepotentials. Actual records of the transmembrane action potentials taken from different points in this portion in one experiment were shown in Fig. 2.

B. Effects of sulfhydryl group reagents on the isolated guinea pig atrium.

1. Mersalyl (mersalyl sodium).

A gradual decrease in the contractile tension and a slight transient increase in the atrial beat followed by a decrease were produced by application of mersalyl to the bath in a final concentration of 0.8mM. Five to fifteen minutes after the application, the contraction has abruptly stopped in most instances. The contractions could be obtained by a repetitive electrical stimulation even in this state of atrium, but these contractions decreased again in process of time. And a slight elevation of resting tension was observed after the contractile response had been inhibited markedly (Fig. 3A).

The pacemaker propagated action potential reduced gradually its
Fig. 2. Transmembrane action potentials recorded from the pacemaker area and its vicinity in an experiment. Tracing 1 to 5 correspondingly represents a record from the point of the same number in Fig. 1.
Fig. 3. A. Effect of mersalyl 0.8mM (0.4mg/ml) on the contractile tension of guinea pig atria. Upper numbers indicated time in minutes after application, and the lower indicated atrial beats every 20 sec..

STIM.: electrical driven stimuli (120 c/min).

R: restraining the stimulation for 10 sec..

B. Effect of mersalyl 0.8mM on the transmembrane action potential of the pacemaker area in the guinea pig atrium. Each photograph after the application were superimposed upon the original record. The last photograph was superimposed upon original recrod and upon that 10 minutes later.
Fig. 4. A. Effect of sodium arsenite 15.5mM (2mg/ml) on the contractile tension of guinea pig atria. Upper numbers indicated time in minutes after application, and the lower indicated atrial beats every 20 sec. STIM.: electrical driven stimuli.

B. Effect of sodium arsenite 15.5mM on the transmembrane action potential of the pacemaker area in the guinea pig atrium. Each photograph after the application were superimposed upon the original record. The last photograph was superimposed upon original record and upon that 10 minutes later.

height and duration after application of mersalyl, but the prepotential remained in no change for first few minutes. Consequently rate of slow depolarization of the prepotential became so slow, that a marked reduction of pacemaker rhythm was resulted. Height of the prepotential was not influenced so much until the pacemaker rhythm being so slow, but it has also diminished finally (Fig. 3B).

2. Sodium arsenite.

Marked increase of contractile tension and irregurality of the beat were observed transiently within few minutes after applying sodium arsenite(15.5mM), and then increase of the resting tension became so marked and the beat was stopped completely about 5 minutes later. But electric stimulation could activate the atrium to contract even though the resting tension increased considerably (Fig. 4A). Change in the pacemaker action potential appeared first as a shortening of the propagated action potential with slight acceleration of the rhythm, which was followed by marked elevation of the resting potential. Consequently height of the action potential reduced markedly, and it was no longer to activate the contractile atrial fibers (Fig. 4B).

3. Iodoacetamide.

Almostly similar change in contractile tension by applying of sodium arsenite was also produced by iodoacetamide (6.5mM), that is, marked and transient increase of the contractile tension and elevation of the resting tension was observed (Fig. 5A). Height of the prepotential increased slightly during first few minutes was followed by a marked reduction. Speed rate of slow depolarization of the prepotential was slightly accelerated, and consequently frequency of the pacemaker rhythm was accelerated moderately. However, only a slight shortening of the duration in the propagated action potential was observed. And reduction both in the resting potential and in the action potential was observed finally (Fig. 5B).


Contractile tension of the guinea pig atrium was increased moderately with a slight acceleration of the beat by applying of NEM in a final concentration of $1.2 \cdot 10^{-1}$mM. The resting tension was elevated gradually and the effect lasted over a period of ten to twenty minutes. More marked increase in contractile tension was produced by higher conce-
Fig. 5. A. Effect of iodoacetamide 6.5mM (1.2mg/ml) on the contractile tension of guinea pig atria. Upper numbers indicated time in minutes after application, and the lower indicated atrial beats every 20 sec.

STIM.: electrical driven stimuli.

B. Effect of iodoacetamide 6.5mM on the transmembrane action potential of the pacemaker area in the guinea pig atrium. Each photograph after the application were superimposed upon the original record. The last photograph was superimposed upon original record and upon that 10 minutes later.
Fig. 6. A. Effect of NEM on the contractile tension of guinea pig atria.
1. NEM $1.2 \cdot 10^{-1}$mM ($1.5 \cdot 10^{-2}$mg/ml)
2. NEM $6 \cdot 10^{-1}$mM ($7.5 \cdot 10^{-2}$mg/ml)
3. NEM $8 \cdot 10^{-1}$mM (0.1mg/ml)
Upper numbers indicated time in minutes after application, and the lower indicated atrial beats every 20 sec.
STIM.: electrical driven stimuli

B. Effect of NEM $1.2 \cdot 10^{-1}$mM on transmembrane action potentials of the pacemaker area in the guinea pig atrium. Each photograph after the application were superimposed upon the original record.
ntration such as $8 \cdot 10^{-1}$ mM, and the effect lasted shorter, causing with irregularity of the beat and elevation of the resting tension (Fig. 6A). The pacemaker action potential, its height of the prepotential was lowered temporarily after NEM application, then elevated moderately, and duration of the propagated action potential was shortened slightly, but speed rate of the slow depolarization was not influenced. Finally the resting potential reduced moderately (Fig. 6B). It is considered as a concomitant change with elevation of the resting tension in the atrium.

Iodosobenzene acetate (4mM) showed the similar effect with those of NEM (1.2.10$^{-1}$ mM) on the contractile tension and on the configuration of action potential.

**DISCUSSION**

The study on transmembrane action potential of cardiac pacemaker cells of the guinea pig heart has already been published from our laboratory (8). But an exact location of the pacemaker area of a guinea pig atrium has never been pointed out, because sizes of both the tip of a microelectrode and the area in the guinea pig atrium are very small. So the first attempt was made to determine the exact location of the pacemaker site in the guinea pig atrium. Though impalement of the microelectrode into the pacemaker area is suggested by A. P. de Carvalho (7) and T. C. West (9) in a rabbit atrium, it failed to get any evidence of the pacemaker action potential. The present mapping of the pacemaker in the guinea pig atrium clearly indicated that there were a considerable species difference between the rabbit and the guinea pig in site of the pacemaker.

All SH group reagents used in the current experiment give changes in configuration of the pacemaker action potential. The changes found in effects of the reagents throughout are a shortening on duration of the propagated action potential and a reduction on height of the action potential. The transient rhythm accerelating effect of the reagents seen in soon after its administration could adequately be explainable by the former change. The latter change, the reduction of the action potential, may participate in producing of arrhythmia, since it is very probable that the inhibited and propagated action potential in the pacemaker cell could not activate cardiac proper fibers to contraction, so an ectopic excitation replaces the normal rhythm. On the height of the prepotential and speed rate in slow depolarization, these SH reagents do not show any uniform effect, so reducing in height, and shortening in duration of the propagated action potential and diminution of the resting potential are of the general effects attributable to the SH group blocking action.

The reagents showing marked reduction of the resting potential
such as iodoacetamide and sod. arsenite produce also a marked elevation of the mechanical resting tension of the atrium. This suggests that the elevation of the resting tension is due to depolarizing character of these agents.

A positive inotropic action found in these reagents with the exception of mersalyl calls an attention through its mechanism of the pharmacological action. Although the reason is not explainable, it seems to result from any inhibitor action of them. Because it has been already known that some drugs having inhibitory qualities such as quinidine (10), fluoride (11–15), cardiotonic glycoside (16) etc. also exert a positive inotropic action. And another possibility has appeared recently (17), that some of these agents could interact directly with adrenergic receptor, because NEM was suggested as an antiadrenergics through its blocking action of the receptor in a guinea pig heart.

Differences in the mode of action of each SH blocking agent on the atrium could be attributable to degree of penetration into the tissue (18, 19), mode of binding to tissue SH (20, 21), specificity of the SH blocking action and other characters of each reagent.

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