Change of P-Q Intervals of the Electrocardiogram in the Rat Hearts Sensitized with the Killed Group A Streptococci

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SUMMARY

Prolongation of P-Q interval in the anesthetized rat was observed by repeated injection of killed group A streptococci. The prolongation was clearly recognized at about the 11th week after the first injection, but afterwards P-Q interval recovered to the normal level in spite of continuous injection of killed streptococci. His bundle electrogram recorded from isolated heart revealed the prolongation of A-H interval in the treated rat. Moreover, the transmembrane action potential in the atrioventricular nodal region of treated rat was slightly deteriorated, but the action potentials in the other cardiac muscles were not changed. It was deduced from the above results that P-Q prolongation was transiently brought about by the injection of killed group A streptococci and that deterioration of muscle in the atrioventricular nodal region might take a main part in the P-Q prolongation.

Additional Indexing Words:
Heart disease His bundle electrogram Microelectrode Action potential in A-V nodal region

It is well known that the prolongation of P-Q interval of the electrocardiogram (ECG) is a sign of the rheumatic heart disease induced by the infection of group A streptococci. There have been many experiments intended to produce experimentally the heart disease by the application of group A streptococci or of the cell-constituents of this organism to the experimental animals such as rabbit.1-4) Terawaki produced the change of wave form of ECG in crabeating monkey by the repeated injections of M protein or lipopolysaccharide of group A streptococci.5) Present investigation was undertaken in order to ascertain the pattern of P-Q interval prolongation by the

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918
application of killed streptococci to the intact rats and to decide the portion initially involved by using the His bundle electrogram and the intracellular microelectrode technique.

**Materials and Methods**

*Experimental animals and anesthesia:*
Wister male rats weighing around 300 Gm were used in this experiment. Since the P-Q interval of the rat's ECG is relatively long in spite of the fairly frequent heart-rate, the P-Q interval could be easily measured. All of the experimental procedure were performed by using the rats anesthetized with the intraperitoneal injection of Na-pentobarbital, 25 mg/Kg.

*Preparation of the formalin-killed organisms:*
Group A streptococci D58 strain, that had been subcultured in our laboratory, were cultured in 5 L of Todd-Hewitt broth at 37°C, and the bacteria were harvested by centrifugation at 8000 r.p.m. for 15 min from the 48-hr-old cultures. The cells were washed 3 times with sterile phosphate-buffered saline (pH 7.2), resuspended to a concentration of 8 mg (wet weight)/ml in sterile physiological saline. This bacterial suspension, to which formalin was added to give a 1% (v/v) final concentration, was preserved for 2 weeks at 4°C, and thereafter performed to sterility test.

*Sensitization of the rats:*
Three series of experiments were performed. In the first experiment, the animals were divided into 2 groups each of which consisted of 20 rats, respectively. Twenty rats belonging to one group were injected with 1 ml of streptococcal antigen (8 mg) with 1 ml of Freund's complete adjuvant into the muscle of thigh twice a month. The other group was a non-treated control group.

The second experiment was carried out in 4 groups of animals consisted of 10 rats each. The same amount of the antigen and adjuvant as the first experiment were injected to the animals of first group. The killed streptococci alone was injected to the second group and the adjuvant alone to the third group. The fourth group was a non-treated control group.

The procedure of the third experiment was the same as the first one and moreover the His bundle electrogram and the transmembrane action potentials were recorded from the isolated heart quickly removed from the rat with prolonged P-Q interval.

*Recording of ECG:*
Standard limb lead II and III ECG were recorded on the ink-writing oscillogram (Nihon Kohden, Type 3047-52, Tokyo) through an ECG preamplifier (Hitachi, low noise ECG preamplifier, Tokyo), once a week during whole experimental periods.

*Recording of His bundle electrogram and transmembrane action potential:*
The free walls in the atria and the ventricles were cut out from the quickly removed heart and then the septum preparation isolated from the right heart was
immediately pinned on a paraffin block in a plastic chamber continuously perfused with oxygenated Tyrode solution at 37°C. The Tyrode solution used in the present study had the following composition (millimolar): NaCl, 140; KCl, 2.7; CaCl₂, 1.8; NaHCO₃, 11.9; NaH₂PO₄, 0.42; dextrose 16.5; and was aerated with a mixture of 95% O₂ and 5% CO₂.

Bipolar Ag-AgCl electrodes with 0.5 mm of tip diameters were used to record extracellularly the His bundle electrogram, and electrical activities were displayed on the dual-beam cathode-ray oscilloscope (Nihon Kohden, Type VC-9A, Tokyo) through a biophysio-preamplifier (Nihon Kohden, Type AVB-9, Tokyo).

The transmembrane action potentials were obtained by the routine microelectrode technique and displayed on the same oscilloscope as the His bundle electrogram through a high input impedance preamplifier (Dia Medical System, DPZ-11, Tokyo). The records of both electrical activities were photographed with a oscilloscope camera (Nihon Kohden, RLG-6101, Tokyo).

**Measurement of serum ASO and ASK:**
The blood samples obtained by cutting of rat's tails were separated into serum within 1 hr. Serum ASO and ASK values were measured by means of the 2-fold series dilution method in microplate that was generally used.

**Results**

**Change of P-Q interval in the sensitized rats:**

Fig. 1 shows a typical pattern of P-Q prolongation in the first experiment. A definite P-Q prolongation was recognized in the ECG of rats at the 11th week after the first sensitization. The results of first experiment were summarized in Fig. 2. The P-Q interval showed a trend of gradual prolongation from the 7th week after the first injection of the antigen and reached a maximum value at the 11th week. However, the interval shortened gradually after the 11th week and almost recovered to the control level at the 13th week in spite of the continuous injection of the antigen. So far as the

Fig. 1. Typical wave form of ECG recorded from the same rat in the first week (upper trace) and in the 11th week (bottom trace) after the first injection of killed group A streptococci. Prolongation of P-Q interval is recognized in the record of the the 11th week.
Fig. 2. Total illustration of the results of first experiment. Bottom curves show the change of P-Q intervals in the control rats (filled circles) and in the treated rats (open circles), respectively. Middle bars indicate the titers of ASK (dotted bar) and ASLO (solid bar) respectively. Uppermost arrows indicate the injection of killed group A streptococci with Freund's complete adjuvant.

Fig. 3. The time courses of change of P-Q interval in the second experiment are illustrated by the separate graphs of 4 groups. The first group; treated with killed group A streptococci with Freund's complete adjuvant, second; treated with killed group A streptococci alone, third; treated with Freund's complete adjuvant alone, and fourth; untreated control. Ordinate mean P-Q interval of ECG in msec, abscissa week after the start of experiment.
first experiment, the P-Q prolongation in the 11th week was statistically significant (p<0.05).

The second experiment was undertaken in order to discriminate whether the P-Q prolongation in the first experiment was induced by the injection of antigen or could be induced by the application of adjuvant alone. Fig. 3 shows the results of second experiment. P-Q prolongation was recognized in both the streptococci-adjuvant group and the streptococci alone group. However, the statistical significance of P-Q prolongation in the second experiment was rather smaller than in the first experiment (0.1>P>0.05). On the other hand, neither control group nor adjuvant alone group exhibited any significant prolongation of the P-Q interval.

The results of the third experiment showed a similar pattern of P-Q prolongation as the first experiment. And the most of animals in the third experiment were used to measure the His bundle electrogram and transmembrane action potentials.

In the measurement of P-Q interval during whole experiment, the ECG which showed an extreme bradycardia was excluded from routine measurement.

Effect of sensitization on the His bundle ECG:

A typical pattern of His bundle electrogram recorded from the isolated heart of a control rat is shown in Fig. 4-A. A spike of H wave was very small but clearly distinguishable. Mean value of A-H interval and H-V interval in the control rats were 73±8.4 msec (M±S.E.) and 18±3.4 msec (n=12), respectively. Therefore, the mean value of A-V interval was calculated as about 91 msec and this value was longer than the P-Q interval in the anesthetized whole animal. This discrepancy may be probably due to the difference of experimental procedure between whole animal and the isolated heart. On the other hand, in the sensitized rats, the mean value of H-V interval was not largely increased by the sensitization (21±5.8 msec, n=9), but A-H interval was relatively prolonged by the injection of streptococcal antigen (102±17.2 msec, n=9), as shown in Fig. 4-B.

Effect of sensitization on the transmembrane action potential:

A series of typical action potential are shown in Fig. 5. Most of the action potentials except those of the A-V nodal region were not affected by the sensitization with streptococcal antigen. However, the configuration of A-V nodal action potential was slightly deteriorated. Since stable insertion of the microelectrode into the A-V nodal cell was very difficult, satisfactory picture of the A-V nodal action potential could not easily photographed.
Fig. 4. His bundle electrogram recorded from isolated rat heart. A; an example of control rat, B; a typical example of prolonged A-H interval in the treated rat. Bottom schema indicates the meaning of each spike, A; atrium, H; His bundle, and V; ventricle.

Fig. 5. Action potentials and extracellular potentials recorded from various fibers in the rat heart. Uppermost panel (Ext) is an extracellular potential, and the meaning of each abbreviation is the same as the scheme in Fig. 4. In the following 4 panels, top curve is an extracellular potential and bottom is an action potential respectively. Abbreviation A; atrium, AV; atrioventricular nodal region, His; His bundle, V; ventricle. The time of onset of action potential is compared with the extracellular potential, and localization of each intracellular action potential is ascertained.

Therefore, the action potentials recorded from the A-V nodal region could not be statistically treated.

Change of serum ASO and ASK:

Values of serum ASO and ASK were slightly elevated in the 8th week of the first experiment. However, such elevations were never observed in the other experiments. Therefore, it was concluded that the elevation of seurm
ASO and SAK was not brought about by the procedure of present experiment.

**DISCUSSION**

Recently Terawaki reported that the heart murmur and the change in the wave form of ECG were brought about in the crabeating monkeys treated with M protein or lipo-polysaccharide of group A streptococci,\(^5\) and that these monkeys showed clinical patterns similar to the rheumatic heart disease. Whether the prolongation of P-Q interval is an initial symptom in the rheumatic heart disease or not, is not yet clear, but the P-Q prolongation is actually one of the minor symptoms in the rheumatic heart disease. In the present experiment, a trend of transient P-Q prolongation was clearly demonstrated in the rats treated with the killed group A streptococci. Moreover, it was in proved by one of the authors that the P-Q prolongation was produced in early by the application of killed group A streptococci in the germ-free mice, and that such disturbance of the excitation conduction system in the heart might be due to direct cytolysis or antibody-dependent cell-mediated cytotoxicity of activated lymphocytes or macrophages.\(^6\) Therefore, the P-Q prolongation in the present experiment seems to reflect an immunological response to the heart muscle produced by the treatment with the group A streptococci. However, this response may be only transient and does not mean any direct development to the rheumatic heart disease.

A-H intervals in the His-electrogram exhibited relatively longer value in the P-Q prolonged rats than that in the control rats. These large differences were not expected from the degree of P-Q prolongation in the treated rats. The reasons of these remarkable prolongations might be due to the experimental procedure in which hearts were excised and perfused extracorporeally by Tyrode's solution. Since the safety factor for the impulse conduction through the atrioventricular node is really lower than in the other cardiac muscle\(^7\) and A-V interval in the heart excised from control animal is also longer than the P-Q interval in the whole animal, the A-V interval in the treated rat might be sensitively prolonged by the slight and probably functional disturbance in the atrioventricular conduction system. Any way, A-H interval was relatively prolonged in the rat treated with killed group A streptococci.

Since the insertion of microelectrode to the A-V node was rather difficult than the insertion to the other cardiac muscle, measurement of the action potentials of the A-V node could not be statistically performed. However, a slight deterioration by the sensitization of group A streptococci was
observed from the configuration of action potentials obtained successfully from A-V nodal region. Since the action potentials in the other cardiac muscles were not changed by the application of group A streptococci, it was suggested that streptococci may damage the A-V nodal region. However, such microelectrode studies are necessary to continue extensively.

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