Characteristics of Ventricular Activation and Recovery Patterns in the Rat

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ABSTRACT. Bipolar or unipolar epicardial and intraseptum electrograms were recorded from the *in situ* rat hearts with spontaneous sinus rhythm or myocardial electrical stimulation of 400 bpm in order to investigate the sequence and duration of cardiac activation in the rat. The ventricular epicardial isochronous maps in the rat showed the activation sequence comparable to those in humans and dogs, although the activation time (6 to 13 msec) of epicardium was approximately one-third of those of such species. The delay of activation between septum and ventricular surface in the rat was 5 to 8 msec, which was also shorter as compared with those in humans and dogs. However, the conduction velocity of the ventricular epicardium in the rat was approximately 40 cm/sec, mostly equal to that of humans. These findings might be accounted for by the possible presence of the bundle branch-purkinje system, at least functionally, similar to humans. The local QT interval in the septum were relatively longer (69 to 123 msec), compared with that of the ventricular surface (50 to 98 msec), and such durations of ventricular epicardium and septum were considerably less than those of dogs and humans. These results are consistent with the configuration of QRS-T on the limb lead ECG.—**KEY WORDS :** conduction sequence, ECG, electrogram, isochronous map, rat, ventricular activation.

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As compared with the other common domestic mammals, the rat heart manifests some noticeable characteristics such as shortness in QT interval and lack of ST segment on ECG [16, 21]. Moreover, concerning ventricular activation process, Hamlin and Smith [4] categorized the rat heart as “category A” according to the body surface electrocardiogram (ECG) (leads I, aV_F and V₁₀), in which ventricular excitation occurred initially in an apex site in the left ventricular endocardium, and then in both ventricular free walls, and finally in the bases of both ventricles. These characteristics are considered to be derived from the cellular electrophysiological property [10, 16, 21], and also from the propagation (activation sequence) and duration of electrical activity through the whole heart [2, 3, 11, 16]. Therefore, the investigation on the activation and recovery patterns in the ventricular conduction system and proper muscle, as well as in the single myocardial cell, could provide fundamental comprehension on the formation of limb lead electrocardiogram (ECG) in the rat.

Although the excitation property of cardiac cells in this species has been well established [10, 16, 21], the data on ventricular activation patterns have been limited to the conduction sequence to the ventricular surface [13, 16]. In this study, the ventricular activation and recovery patterns in the rat heart were investigated by analyzing the epicardial activa-

tion time, the delay of activation between the intraseptum and epicardium, and local QT interval.

In general, the investigations of ventricular activation and recovery patterns have been utilized as powerful diagnostic methods for several cardiac dysfunctions, clinically and experimentally [8, 9, 14, 17, 20]. Therefore, the establishments of the nature in the ventricular activation and recovery patterns in the rat may be also useful for the accurate evaluations of the drug effects on pharmacological and toxicological works, as this species has been most frequently used for various drug assessing tests.

MATERIALS AND METHODS

Animals and surgical procedure: Twenty nine male Wistar rats aging 12 to 16 weeks were used in this study. All rats were anesthetized with pentobarbital sodium (40 mg/kg, i.p.) and restrained in supine position on an operating table warmed 37°C during experiments. Artificial ventilation was maintained through an endotracheal tube by a positive pressure respirator at a frequency of 70 rpm. The chest was surgically opened at the ventral side so that the heart was disclosed.

Epicardial activation and isochronous epicardial mappings: In this study, the time from the earliest activity to the latest activity represented in each ventricle was termed as ‘activation time’. In order to

obtain the epicardial activation time and isochronous epicardial mapping, a reference electrode and a bipolar roving electrode were used, because the onset of local electrical activity can be easily detected by this method [8]. The reference electrode with a pair of enamel-coated copper wires (diameter, 0.06 mm; intraelectrode distance, 1 mm) was placed on the subepicardium at a central part of right ventricle. The roving electrode made of a pair of teflon-coated stainless steel wires (diameter, 0.2 mm; intraelectrode distance, 1 mm) was moved and lightly touched on the surface of epicardium at discrete fifteen points in each ventricle, according to the ordinal isochronous mapping methods as described previously [1, 8, 13, 16, 17]. The time of arrival (onset) of activation just under the position of the electrode attachment was defined at the peak of the largest monophasic complex or the first crossing point to baseline in the case of the largest biphasic complex [8, 16]. The delay from the onset in reference electrode activity or QRS complex on ECG to the onset in roving electrode activity was measured for determining the activation sequence through the isochronous epicardial mapping. These isochronous maps were made by referencing the delay of activation at various epicardial sites from the earliest activation at a certain site. In order to obtain the local 'QT' intervals (local activation duration) at various epicardial sites, an unipolar roving electrode was used, because the electrical activity on the unipolar electrogram was reported to reflect directly the local cardiac activity around the electrode [7]. These values were also referred to ECG. These methods of measurements used in the present study were essentially conformable to several previous reports by Iwa and Mukai [8], Josephson *et al.* [9], Spear [16], Whydham *et al.* [19], and Wiener *et al.* [20].

Furthermore, in order to investigate the overall conduction process in the heart, the electrical activity was recorded at given epicardial sites of right atrium and the intraseptum as well as right and left ventricular free wall.

Ventricular conduction velocity: Electrical stimulation with a frequency of 400 bpm to the surface of mid-portion between the apex and base in the right or left ventricular free wall was performed through bipolar stimulus electrodes connected to an electrical stimulator which delivered a square wave pulse (2 msec, 3.0 V). During electrical stimulation, bipolar epicardial electrograms at 5 mm distant from

the stimulus electrode were obtained together with the records of limb lead ECG. The conduction velocity (C.V.) was calculated by the following equation;

$$\text{C.V. (cm/sec)} = \frac{0.5 \text{ (cm)}}{\text{Time of arrival of activation from a stimulus site to a recording site (sec)}}$$

In all experiments electrical signals were amplified by biophysical amplifiers (San-ei Sokki: 1205C), using a time constant of either 0.01 sec for bipolar electrograms or 1.5 sec for unipolar electrograms and limb lead ECG. All these signals were stored on a micro-computer system (Epson: 286US) equipped with an analog-digital converter (Softron: SAR-8). The onset of local excitation and the local QT interval were accurately measured by the ECG-analysis program (Softron: SP-2) for micro-computer usage. These electrical signals were printed out on a laser printer (Ricoh: LP-1060-SP3), when required.

Statistical analyses were performed by Student's paired *t*-test, and a significant difference was considered if *P* value was less than 0.05.

RESULTS

Isochronous epicardial mappings: Representative recordings of the bipolar and unipolar electrograms from ventricular epicardium, as well as limb lead ECG and reference activity, are shown in Fig. 1. The appearance of these epicardial activations was well corresponded to the QRS duration or QT interval on the limb lead ECG.

The isochronous maps of ventricular epicardial activation are illustrated in Fig. 2. In most of rats, the earliest epicardial breakthrough (the earliest onset of activation in the ventricular surface) occurred on the center in the right ventricular free wall and on the apex site in the left ventricle, respectively. The onsets of activation on right ventricular surface tended to be earlier than those on the left ventricular surface. Mean times initiating electrical activity at different sites in the right ventricle of each rat were 3.1, 3.8, 5.9, 5.7, 2.8 and 3.4 msec (average 4.1 msec, *n*=6), while these values in the left one were 3.3, 5.0, 7.7, 8.9, 4.6 and 2.7 msec (average 5.4 msec, *n*=6). They were corresponded to the last half

Fig. 1. Bipolar and unipolar electrograms recorded from right ventricular surface. REF: Electrogram through a bipolar reference electrode. ROV Bipolar: Electrogram through a bipolar roving electrode. ROV Unipolar: Electrogram through an unipolar roving electrode.

of QRS duration on the limb lead ECG (Table 1). Epicardial activation times, i.e., times from the earliest to the latest activation, were somewhat shorter in the right ventricle than in the left one, indicating 5, 6, 6, 7, 8 and 8 msec (average 6.7 msec) in the right and 6, 6, 7, 8, 9 and 13 msec (average 8.2 msec) in the left.

Table 2 shows average local QT intervals in each ventricular epicardium. There were no significant differences in local QT intervals between the right and left sites on ventricular surface. These values were almost comparable with QT interval on the limb lead ECG recorded simultaneously, although the epicardial QT intervals tended to be longer than those of the limb lead ECG.

Ventricular conduction velocity: The representative bipolar electrograms during electrical stimulation at a frequency of 400 bpm as well as sinus rhythm are shown in Fig. 3. The average conduction

Fig. 2. Ventricular isochronous maps obtained from recordings at 30 points on each heart in 6 rats. The numbers in the heart indicate the time of arrival of activation (msec) when the earliest activation is 0 msec. RA: right atrium, LA: left atrium, RV: right ventricle, LV: left ventricle, PA: pulmonary artery, AO: aorta.

Table 1. Times of arrival of activation on ventricular surface compared with the limb lead ECG

Animals	Right ventricle	Left ventricle	QRS duration
1	11.1±1.8 ^{a)}	11.3±2.0	15
2	9.9±2.4	11.0±1.9	14
3	9.3±1.7	9.4±2.5	15
4	8.6±2.6	11.1±2.9	14
5	9.4±1.5	9.3±2.0	15
6	9.3±1.9	7.6±1.8	15
Average	9.6±2.2	10.0±2.6	14.7±0.47

In the right and left ventricles the values were expressed as delay (msec) from the beginning of QRS complex on the limb lead ECG.

a) Mean±SD (from 15 electrode positions in each ventricle).

velocity was 37.5 cm/sec in the right and 39.1 cm/sec in the left ventricles. There were no significant difference between these values.

Ventricular conduction sequence: Figure 4 shows an example of the bipolar and unipolar electrograms recorded from right atrial and bilateral ventricular

Table 2. Local QT intervals in the ventricular epicardium

Right Ventricle (n=90/6 ^{a)})	75.3±7.7 ^{b)}
Left Ventricle (n=90/6)	79.0±8.4
Both Ventricles (n=180/6)	77.4±8.2
QT interval on ECG	72.0±8.7

a) Number of experiments v.s. number of animals.

b) Mean±SD (msec).

Fig. 3. Bipolar electrograms under sinus rhythm and electrical stimulation of 400 bpm. The distance between each ROV and stimulation electrode was 5 mm. ROV (1), (2): Electrograms from two different electrodes. Stim.: Event marker (square wave-pulse) corresponded to the electrical stimulation.

surfaces, and intraseptum under the condition of spontaneous sinus rhythm. These bipolar and unipolar electrograms were consistent with the phase of QRS or QT on the limb lead ECG. In Table 3 electrical activation times at the different sites were compared with corresponded parameters of the limb lead ECG. The earliest onset of activation occurred in the intraseptum, and the activation times in all sites were within QRS duration on the limb lead ECG. The local QT interval in intraseptum tended

Fig. 4. Analog records of bipolar and unipolar electrograms from right atrial (RA), right and left ventricular (RV and LV) epicardia, and intraseptum (Sep).

Table 3. Activation sequences compared with the limb lead ECG, and local QT intervals

Times of activation	
Q ^{a)} to Sep ^{b)}	3.0±1.5 ^{d)}
Q to Right VE ^{c)}	8.2±2.6
Q to Left VE	9.2±3.5
Sep to Right VE	5.3±1.9
Sep to Left VE	7.4±3.1
(QRS duration on the ECG	15.2±1.8)
Local QT intervals	
Sep	95.8±15.9
Right VE	89.5±19.5
Left VE	87.6±12.3

Number of animals: 11 rats.

a) The beginning of QRS complex on ECG, b) Septum, c) Ventricular epicardium, d) Mean±SD (msec).

s: Significantly different ($p<0.05$, paired t -test).

to be longer than that in ventricular surfaces, indicating the statistical difference between the intraseptum and the left ventricular surface ($p<0.05$) but not between the intraseptum and the right ventricle.

DISCUSSION

Epicardial isochronous maps in the *in situ* rat heart showed that the activation sequence on both ventricular surface was essentially similar to those previously described in the rat heart [13, 16], and also the same as those of the human and dog hearts [2, 3, 5, 8, 16]. These times for activation in ventricular epicardium were well corresponded to the last half of QRS duration on the limb lead ECG simultaneously recorded. It was also clear that the septum was activated earlier than both ventricular epicardium. The major difference in the rat heart was that the activation times (6 to 13 msec) of the ventricular surface were approximately one-third of those in the human and dog hearts. Furthermore, the delay of onsets of activation between septum and ventricular surface in the rat was 5 to 8 msec, which was also one-third of those in the human and dog. Such a very short activation time in the rat ventricle was considered to reflect the short QRS duration on the rat ECG.

The conduction velocity of the *in situ* rat ventricular epicardium was approximately 40 cm/sec, comparable to that of the human heart [15]. The fact suggests that the nature of conduction factors (i.e. gap junction, dv/dt , membrane resistance and capacitance and cytoplasmic resistance) in the proper cardiac cells of rat ventricle might be similar to that of the human ventricle [2, 15, 18]. The short conduction time from septum to ventricular surface and the slower conduction velocity through ventricular muscle lead to an assumption that the functional development of bundle branch-purkinje system is present in the rat heart [3, 6, 15]. Moreover, the similarity in the mode of epicardial activation sequence between the rat and the human or the dog suggests that the distribution of purkinje fiber in the rat ventricle may be also similar in these animals. The precise histological evidences including the distribution of purkinje fiber network in the rat is required for supporting our findings because histological investigations on the impulse-conduction system in the rat heart were limited to only sinoatrial and atrioventricular nodes [12]. Furthermore, the activation process including endocardial sites is needed to elucidate entirely the ventricular activation process in the rat.

The local QT intervals in the intraseptum in the rat heart tended to be longer than those in the ventricular surface, as in the case of the human

heart. However, these values (50 to 98 msec) were considered to be very short, if compared with those of the human heart (200 to 250 msec) [18]. These evidences are assumed to contribute to the configuration of short QT interval and positive T wave on the rat limb lead ECG, while the shortness of action potential duration (APD) of the ventricular cell [7, 11, 21] might be also important.

In conclusion, it became clear that the characteristics of ventricular activation and recovery patterns in the rat heart was essentially similar to those of the human and dog hearts, and the results will provide a capability of utilizing this animal for several kinds of cardiac experiments.

REFERENCES

1. Burgess, M. J., Lux, R. L., Ershler, P. R., and Menlove, R. 1990. Determination of transmural location of onset of activation from cardiac surface electrogram. *Circulation* 82: 1335-1342.
2. Douglas, P. Z. 1988. Genesis of cardiac arrhythmias: electrophysiological considerations. pp. 581-595. In: *Heart Disease* (Eugene, B. ed.), W. B. Sanders Company, New York.
3. Durrer, D. R. T., Freud, G. E., Janse, M. J., Meijler, F. L., and Arzbaecher, R. C. 1970. Total excitation of the isolated human heart. *Circulation* 41: 899-912.
4. Hamlin R. L. and Smith C. R. 1965. Categorization of common domestic mammals based upon their ventricular activation process. *Ann. New York Acad. Sci.* 127: 195-203.
5. Houer R. N. W., Zwart M. T., Bakker J. M., Hitchcock J. F., Penn, O. C. K., Nijsen-Karelse, M., and Medina, E. O. 1986. Endocardial catheter mapping: wire skeleton technic for representation of computed arrhythmogenic sites compared with intraoperative mapping. *Circulation* 74: 1346-1354.
6. Houer, R. N. W., Heethaar, R. M., Zwart, M. T., Duk, R. N., Tweel, I., Borst C., and Medina E. O. R. 1986. Endocardial catheter mapping: validation of a cineradiographic method for accurate localization of left ventricular sites. *Circulation* 74: 862-868.
7. Huh, M. D., Hirose, H., and Sugano, S. 1987. Etiology of bifid T wave in electrocardiogram of rats. *Jpn. J. Vet. Sci.* 49: 965-972.
8. Iwa, T. and Mukai, K. 1983. Epicardial mapping. *Medicina Philosophica* 2: 257-262.
9. Josephson, M. E., Horowitz, L. N., Spielman, S. P., Waxman, H. L., and Grenspan, A. M. 1982. Role of catheter mapping in the preoperative evaluation of ventricular tachycardia. *Am. J. Cardiol.* 49: 207-220.
10. Kojima, M. and Sperelakis, N. 1983. Age-dependent changes in electrophysiologic characteristics of fast and slow action potentials in rat papillary muscle. *Can. J. Physiol. Pharmacol.* 91: 1509-1515.
11. Langer, G. A. 1978. Variation in myocardial physiology: the anomalous rats. *Environ. Health Perspec.* 26: 179-185.
12. Malax, H. and Lesson, T. S. 1970. Fine structure of the impulse-conducting system in rat heart. *Can. J. Zool.* 48:

- 837-839.
13. Nakata, T., Satou, S., Sekiya, S., Tsutsumi, K., and Nagata, H. 1988. The epicardial activation map of the isolated rat heart. *Adv. Anim. Cardiol.* 21: 27-35.
14. Nishimura, M., Watanabe, Y., and Toda, H. 1984. The genesis of bifid T waves: experimental demonstration in isolated perfused rabbit hearts. *Int. J. Cardiol.* 6: 1-14.
15. Shephend, J. T. and Vauhouutte, P. M. 1979. Components of the cardiovascular system: how structure is geared to function. pp. 13-61. *In: The Human Cardiovascular System* (Shephend, J. T. and Vauhouutte, P. M. eds.), Raven Press, New York.
16. Spear, J. F. 1980. Relationship between the scalar electrocardiogram and cellular electrophysiology of the rat heart. pp. 29-40. *In: The Rat Electrocardiogram in Pharmacology and Toxicology* (Budden, R., Detweller, D. K., and Zbinden, G., eds.), Pergamon Press, Oxford.
17. Spurell, R. A. J., Yates, A. K., Thorburn, C. W., Sowton, G. E., and Deuchar, D. C. 1985. Surgical treatment of ventricular tachycardia after epicardial mapping studies. *Br. Heart J.* 37: 115-126.
18. Toyama, J., Kodama, I., and Katoh, R. 1987. Sindenzu. pp. 103-132. *In: Mechanisms of Arrhythmias and Antiarrhythmic drugs* (Kannno, M., Nishi, K., and Hashimoto, K. eds.), Kanehara & Co., Ltd., Tokyo (in Japanese).
19. Whydham, C. R., Meeran, M. K., Smith, T., Saxena, A., Engelman, R. M., Levesky, S., and Rosen, K. M. 1979. Epicardial activation of the intact human heart without conduction defect. *Circulation* 59: 161-168.
20. Wiener, I., Mindich, B., and Pitchon, R. 1982. Epicardial activation of the human ventricle: effect of left ventricular hypertrophy. *Am. J. Cardiol.* 50: 1095-1098.
21. Yamamoto, T., Hirose, H., and Sawazaki, H. 1983. Alternation with age in cardiac activities of rat. *Jpn. J. Vet. Sci.* 45: 53-58.