Effects of ectopic pacing on repolarization of the chicken left ventricle

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Summary

Effects of ectopic pacing on left ventricular repolarization were studied in six anaesthetized open-chest chickens. In each animal, unipolar electrograms were acquired from as many as 98 sites with 14 plunge needles (seven transmural locations between epicardium and endocardium in each needle). Activation-recovery intervals (ARIs), corrected to the cycle length, were used for estimating repolarization. At baseline, the nonuniform ARI distribution in the left ventricle resulted in the apicobasal differences being greater than the transmural gradient. Nonuniform ARI prolongation caused by ectopic pacing resulted in decreasing the transmural repolarization gradient and increasing the differences in the apex-to-base direction. The basal, but not apical transmural differences contributed to the total left ventricular transmural gradient. The total left ventricular apicobasal gradient was contributed by the apicobasal differences in mid-myocardial and subendocardial layers more than in subepicardial ones. Thus, in *in vivo* chicken hearts, the transmural and apicobasal ARI gradients exist within the left ventricle with the shortest ARIs in the basal subepicardium and the longest ARIs in the subendocardium of the apical and middle parts of the left ventricle. Apicobasal compared to transmural heterogeneity of local repolarization properties more contributes to the total left ventricular repolarization gradient.

Key words

Repolarization, Activation-recovery interval, Heart ventricles, Pacing, Avian heart

Introduction

Three repolarization gradients at least exist in the heart ventricles of mammalian species: transmural (Higuchi and Nakaya, 1984; Yan and Martin 2003, Hlaing *et al.* 2005), apicobasal (Nishimura *et al.* 1984, Cowan *et al.* 1988, Janse *et al.* 2005, Ramanathan *et al.* 2006, Azarov *et al.* 2007, 2008), and interventricular (Nishimura *et al.* 1984). However, several studies have demonstrated the absence of the former in *in vivo* mammalian heart ventricles (Anyukhovsky *et al.* 1996, Taggart *et al.* 2001, Wang *et al.* 2002, Janse *et al.* 2005, Coronel *et al.* 2007, Zhang *et al.* 2007). Most likely, the ventricular repolarization gradients are differently expressed in the heart and are differently affected by various factors (e.g. electrolyte balance, temperature, ectopic excitation etc.).

The use of different model animals contributes to understanding the evolution of a heart function and mechanisms of electrogenesis in the heart. In our investigations, birds have been chosen as model animals. Vast majority of avian research of cardiac repolarization has centered only on the morphology of the T wave. Little is known about repolarization gradients in the heart ventricles in birds. Our recent studies have demonstrated the apicobasal repolarization differences on the epicardial surface of the heart ventricles (Kharin 2004) and the transmural repolarization gradient in the left ventricular free wall (Kharin *et al.* 2007) at sinus rhythm in chickens. Which of the ventricular repolarization gradients (apicobasal or transmural) is more expressed in avian hearts is unknown. Effects of various factors on ventricular repolarization in birds have not been addressed .

The objective of the present study is to evaluate the effects of ectopic pacing of the heart on left ventricular repolarization across and along the left ventricular free wall and to determine a contribution of apicobasal and transmural repolarization heterogeneity to the total left ventricular repolarization gradient in chickens.

Methods

Animals and surgical procedure

The study conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No 85-23, revised 1996). Six Brown laying hens *Gallus gallus* (weight 1.3-1.8 kg, 9-12 months old) were purchased from a poultry farm. Birds were anaesthetized with an intramuscular injection of sodium thiopental (120-150 mg/kg) and placed in dorsal recumbency. After installation of artificial ventilation, the heart was exposed via bilateral thoracotomy. Throughout the experiment, the temperature of the avian body was in the range of 41-42°C. The heart was prevented from cooling and drying by warm saline (0.85% NaCl). For this purpose, the heart was moistened by regular irrigation.

Electrodes

Unipolar electrograms were acquired from as many as 98 myocardial sites of the left ventricular free wall with plunge needles at sinus rhythm and under ectopic pacing. Fourteen plunge needles (diameter, 0.35–0.40 mm; length, 5.0–7.5 mm), each consisting of seven unipolar electrodes, were used. The diameter of each lead point was 0.07 mm. A distance from centre to centre between electrode points was equal for each plunge needle (0.65, 0.80 or 1 mm). Thus, the distance from centre to centre between the first and seventh electrode points was 3.9, 4.8 or 6 mm. Electrodes were made of copper insulated wires (diameter, 0.07 mm) fixed on a steel needle with an epoxy resin. Plunge needles were inserted into the myocardial wall according to one of schemes (Fig. 1).

Figure 2 shows the original tracings of intramural electrograms.

Needle electrodes were placed through the skin of the medial part of each shoulder (the red electrode – the right wing, the yellow electrode – the left wing) and through the skin of each thigh close to the knee joint (the green electrode – the left foot, the indifferent electrode – the right foot) to obtain ECG recordings with the standard bipolar limb leads.

Signal acquisition

All unipolar electrograms and standard bipolar limb lead electrocardiograms were recorded simultaneously. The signals were isolated, amplified, multiplexed and recorded by a custom-designed 128-channel data acquisition system with the bandwidth of 0.05 to 1000 Hz at a sampling rate of 4000 Hz and an accuracy of 16 bits.

Stimulation protocol

Unipolar stimulation was used to generate ectopic beats of the heart ventricles. To obtain a stabile state of ectopic excitation, the heart was driven for 30 ms. Hearts were paced with right-angled impulses of 3 ms duration at a frequency of 3 Hz (approximately 15-30% higher than sinus rhythm). Amplitude of impulses was 3–7 V. The base and apex of the left ventricle were paced from epicardial and endocardial points.

Data analysis

To estimate local repolarization properties of myocardium, activation-recovery intervals (ARIs) were measured from unipolar electrograms as the interval between local depolarization and local repolarization times (Burgess *et al.* 1972). Local depolarization and repolarization times were defined as the minimum first derivative and the maximum first derivative of the unipolar electrogram in the period corresponding to the QRS complex and T wave in ECG, respectively. The computer-chosen (automatically) depolarization and repolarization times were reviewed and corrected if required. 322 electrograms of 588 ones (6 chickens × 14 plunge needles × 7 electrodes) were analysed; rest electrograms were unsatisfactory to analyse the T wave. All ARIs were corrected to the cardiac cycle length according to the Bazett's equation. Electrodes 1 and 2 of each plunge needle were considered to be in the subendocardial position, electrodes 3, 4 and 5 in the mid-myocardial position and electrodes 6 and 7 in the subepicardial position (i.e. what is stated in Table 1 and 4 and in Figure 3).

Data are presented as means ± SD. Statistical comparisons were carried out by paired and unpaired Student's *t*-test.

Results

ARI distribution within the chicken left ventricular free wall at sinus rhythm

The pooled ARI in the whole of the left ventricular free wall was 257±31 ms at sinus rhythm, which was 150±14 beats per minute (Table 1, "Average" line).

Apicobasal differences in ARIs. ARIs in the basal third of the left ventricular free wall were the shortest , whereas ARIs in the middle third were the longest (Table 2). ARIs in the apical third of the left ventricular free wall were intermediate but closer to the latter.

The apicobasal ARI distribution in each of three layers (subendocardial, mid-myocardial, subepicardial) of the left ventricular wall coincided with the total apicobasal distribution in the left ventricular wall (Fig. 3, Table 2). The difference between ARIs in the apical and middle thirds of the left ventricular free wall was negligible in the subepicardial layer (252±24 vs. 257±29 ms, respectively), whereas it was slightly more in the mid-myocardial (259±24 vs. 269±23 ms, respectively) and subendocardial (265±23 vs. 277±19 ms, respectively) layers. The difference between ARIs in the middle and basal thirds of the left ventricular free wall was the greatest in the mid-myocardial layer (269±23 vs. 224±48 ms, respectively), whereas it was less in the subepicardial (257±29 vs. 219±48 ms, respectively) and subendocardial (277±19 vs. 240±43 ms, respectively) layers. The latter was similar to the difference between the apical and basal ARIs in the mid-myocardial (35 ms) and subepicardial (33 ms) layers, which was more than that in the subendocardial layer (25 ms).

Transmural differences in ARIs. ARIs in the subepicardial layers were shorter than in the subendocardial ones (Table 1). The lengthening of ARIs from the subepicardium to the subendocardium was smooth and even in some regions of the ventricular wall, whereas sharp in

others. However, in some regions of the left ventricular free wall, ARIs were shorter in the subendocardial than in subepicardial layers. As a result, the transmural gradient of ARIs (the difference between ARIs recorded from the subepicardial and subendocardial layers of myocardium) had a large variation and was in the average 16±14 ms (Table 3).

ARI prolongation across the left ventricular free wall was statistically more significant in the middle and apical thirds than in the basal third (Table 4). In all thirds of the ventricular wall, the subepicardial ARIs were, as a rule, shorter than the subendocardial ones. As a result, the direction of the transmural gradient of ARIs in each third of the ventricular wall coincided with the direction of the total transmural gradient in the left ventricular wall (Table 3).

Effect of ectopic pacing on the pooled ARI

The pooled ARI was significantly prolonged under ectopic pacing (Table 1, "Average" line). Across the ventricular wall, this prolongation resulted mainly from the lengthening of the subepicardial and mid-myocardial ARIs, but not the subendocardial ones (Table 1). Prolongation of the pooled ARI along the ventricular wall was mostly due to the lengthening of ARIs in the basal and middle thirds of the ventricular wall under basal pacing and in the middle and apical thirds under apical pacing (Table 2). ARI prolongation was greater along than across the left ventricular wall. Epicardial pacing of the left ventricular apex resulted in ARI prolongation to a lesser degree compared with other pacing sites (Tables 1 and 2).

Effect of ectopic pacing on transmural differences in ARIs

Under ectopic pacing, excluding epicardial pacing of the left ventricular apex, ARIs were significantly prolonged in all myocardial layers (Table 1). This prolongation was nonuniform across the ventricular wall. As a rule, the subepicardial ARIs were more prolonged than the subendocardial ones that resulted in decreasing the total transmural gradient of ARIs in the left ventricular wall (Table 3).

Compared with baseline, ARI prolongation across the left ventricular free wall was statistically significant mainly in the middle and apical thirds under basal and apical pacing, respectively (Table 4). Although there was nonuniform prolongation of the subepicardial and subendocardial ARIs under ectopic pacing, the subepicardial ARIs were, as a rule, shorter than the subendocardial ones in all thirds of the ventricular wall, excluding the basal third under basal pacing (Table 4). As a result, the transmural gradient was decreased in the basal and middle thirds, but not in the apical third of the left ventricular free wall (Table 3). The decrease of the transmural gradient was more expressed in the basal third of the ventricular wall than in the middle one. Besides, the transmural gradient in the basal third was inverted under basal pacing of the left ventricle.

Effect of ectopic pacing on apicobasal differences in ARIs

Under ectopic pacing, ARI in all three layers of the left ventricular wall were prolonged most of all in the middle third of the ventricular wall (Fig. 3). In comparison with each other, a degree of prolongation of the basal and apical ARIs depended on a pacing site. In all three layers, ectopic pacing from the left ventricular base lengthened the basal ARIs with the apical ARIs being not or insignificant prolonged. The contrary situation was observed for ARIs in all three layers under endocardial pacing of the left ventricular apex. ARI prolongation under epicardial pacing of the left ventricular apex was more complex and insignificant.

The differences in ARIs between the middle and basal thirds of the left ventricular wall were changed insignificantly except under endocardial pacing of the left ventricular apex Table 2). In contrast, the ARI differences between the middle and apical thirds of the left ventricular wall were increased more than twofold, excluding under apical epicardial pacing. At the same time, the ARI differences between the apical and basal thirds of the left ventricular wall changed insignificantly.

Under endocardial ectopic pacing of both the apex and base of the left ventricle, the apicobasal gradient in each layer of the left ventricular wall (Fig. 3) coincided with the total apicobasal

gradient in the left ventricular wall (Table 2). In contrast, under subepicardial pacing of the left ventricular apex, the apicobasal gradient in the subendocardial and mid-myocardial layers of the left ventricular wall coincided with the total apicobasal gradient in the left ventricular wall. Only the apicobasal gradient in the mid-myocardial layer of the left ventricular wall corresponded to the total apicobasal gradient in the left ventricular wall under subepicardial pacing of the left ventricular base.

Discussion

The major findings of the present study are as follows. 1) At baseline, the nonuniform ARI distribution in the left ventricle resulted in the apicobasal gradient being greater than the transmural gradient. 2) Nonuniform ARI prolongation caused by ectopic pacing resulted in a decreasing of the transmural repolarization gradient and increasing of the differences in the apex-to-base direction. 3) The basal, but not apical transmural differences contributed to the total left ventricular transmural gradient. 4) The total left ventricular apicobasal gradient was contributed by the apicobasal differences in mid-myocardial and subendocardial layers more than in subepicardial ones.

Investigations of repolarization describe either only transmural or apicobasal differences in local repolarization durations, action potential durations or monophasic action potential durations. Majority of the above investigations were made on ventricular preparations or isolated myocytes, but not *in vivo* hearts. Our study describes both the transmural and apicobasal gradients of ARIs in the *in vivo* chicken left ventricle.

No differences in repolarization among myocardial layers were shown in *in vivo* intact normal hearts of dogs (Anyukhovsky *et al.* 1996, Janse *et al.* 2005, Coronel *et al.* 2007), humans (Taggart *et al.* 2001), sheep (Wang *et al.* 2002, Zhang *et al.* 2007). The transmural repolarization gradient in the *in vivo* chicken left ventricle was such that ARIs in the subepicardial layer were shorter than in the subendocardial one. These results are consistent with observations of action potential durations (Main *et al.* 1998, Xu *et al.* 2001, Aiba *et al.* 2003, Wan *et al.* 2003, Idriss and Wolf

2004) and monophasic action potential durations (Liu *et al.* 2003) in mammals and with the ARI distribution in chicken hearts (Kharin *et al.* 2007). In chickens, the basal transmural differences contributed to the total left ventricular transmural gradient more than the apical ones.

M cells contributing to transmural heterogeneity of action potential durations, have been detected by microelectrode techniques in canine (Sicouri and Antzelevitch 1991), human (Drouin *et al.* 1995), guinea pig (Sicouri *et al.* 1996), porcine (Stankovicova *et al.* 2000), rabbit (Li *et al.* 2002), murine (Liu *et al.* 2003), feline (Aiba *et al.* 2003) left ventricular myocardium. The above descriptions of M cells have been made on ventricular preparations, myocardial strips or enzymatically isolated myocytes. At the same time, a distinct layer of M cells may not be observed in the left ventricle of smaller animal species (Hlaing *et al.* 2005). *In vivo* results have shown no differences in repolarization among myocardial layers in intact normal hearts of dogs (Anyukhovsky *et al.* 1996, Janse *et al.* 2005, Coronel *et al.* 2007), humans (Taggart *et al.* 2001), sheep (Wang *et al.* 2002, Zhang *et al.* 2007). At present, there are no evidences of an existence of M cells in the avian left ventricle. This question remains to be answered. M cells are absent in the chicken left ventricle or are masked by intercellular coupling (Conrath *et al.* 2004). Our previous study (Kharin *et al.* 2007) and the present results do not demonstrate the long ARIs in the mid-myocardial region of *in vivo* chicken hearts at baseline and during ectopic pacing. The transmural ARI distribution in the normal chicken left ventricle is a smooth continuum.

In mammals, action potential durations or ARIs were demonstrated to be shorter in the apex compared with the base (Nishimura *et al.* 1984, Laurita *et al.* 1996, Baker *et al.* 2000, Choi and Salama 2000, Szentadrassy *et al.* 2005, Mantravadi *et al.* 2007, Ramanathan *et al.* 2006, Azarov *et al.* 2007). In other investigations, the longer repolarization was found in the apex compared with the base (Toyoshima *et al.* 1981, Cowan *et al.* 1988, Cheng *et al.* 1999, Janse *et al.* 2005). The present results are consistent with both above observations. The differences in the apex-to-base direction in the chicken left ventricle were such that the apical ARIs were longer than the basal ones and shorter than ARIs in the middle part of the left ventricle, and the latter were the

longest. Besides, the apicobasal differences in the subendocardial and mid-myocardial layers contributed to the total left ventricular apicobasal gradient more than in the subepicardial layer. There is some discrepancy with our recent investigation, concerning the ARI distribution on the chicken ventricular epicardium with ARIs increasing progressively from the apex to the base (Kharin 2004). This discrepancy can be related to regional heterogeneity in expression of ionic currents throughout ventricular myocardium (Bryant *et al.* 1998, Main *et al.* 1998, Cheng *et al.* 1999, Xu *et al.* 2001, Szabó *et al.* 2005, Szentadrassy *et al.* 2005) and in their sensitivity to different factors, such as ionic concentrations (Wan *et al.* 2000), hormones (Daleau and Turgeon 1994, Wang *et al.* 1999), adrenoceptor stimulation (Bosch *et al.* 2002, Volders *et al.* 2003, Rocchetti *et al.* 2006), the autonomic nervous system tone (Tatewaki *et al.* 2003, Conrath and Opthof 2006) and spatial heterogeneity of effects of the autonomic nervous system on ventricular repolarization (Mantravadi *et al.* 2007). Besides, the difference in the ARI distributions might be related to a dissimilar dependence of ARIs in different ventricular parts on heart rate. In the present study, heart rate was 150±14 beats per minute, whereas it was 225±20 beats per minute in the previous investigation (Kharin 2004).

Unexpectedly, the ARIs at sinus rhythm and under epicardial pacing of the left ventricular apex were rather close (Fig. 3). An additional analysis is needed to explain this observation. However, we suppose that it might be related to dependence of the ARI distribution and repolarization sequence on the activation sequence. On the whole, ARI prolongation in the chicken left ventricle under ectopic pacing is in agreement with action potential prolongation in chronically paced mammalian hearts (Kääb *et al.* 1996, Tsuji *et al.* 2000), and with action potential prolongation following a period of the altered activation sequence in wedge preparations of canine ventricles (Libbus and Rosenbaum 2003). Our results indicate that the apicobasal repolarization differences are greater than the transmural ones at both baseline and ectopic pacing. Under ectopic pacing, ARI prolongation is greater in the apex-to-base compared to transmural direction. Both our observations support the suggestion that apicobasal heterogeneity in local repolarization properties

contributes to the total left ventricular repolarization gradient more than transmural heterogeneity.

In conclusion, in the *in vivo* chicken heart at sinus rhythm, the transmural and apicobasal gradients of ARIs exist within the left ventricular free wall with the shortest ARIs in the basal subepicardium and the longest ARIs in the subendocardium of the apical and middle parts of the left ventricle. This study provides data in support of predominance of apicobasal compared to transmural heterogeneity of local repolarization properties, and of a greater contribution of the apicobasal gradient to the total left ventricular repolarization gradient compared with the transmural gradient. A correlation between the changes in the repolarization gradients and changes in the electrocardiographic T wave during ectopic pacing of the avian heart ventricles is of certain interest for the genesis of the T wave. It was no the object of the present study. Effects of the changes in the repolarization gradients on the T wave in birds remains to be elucidated.

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Table 1. Activation-recovery intervals in seven myocardial layers of the left ventricular free wall in all animals under sinus rhythm and ectopic pacing of the left ventricle.

Locations of electrodes	**Sinus rhythm**	**Pacing of the base**		**Pacing of the apex**	
		endocardial	**epicardial**	**endocardial**	**epicardial**
1 (endocardium)	270±28 **	291±47 $^{\#\#}$	295±54 $^{\#}$	292±49 $^{***, \#}$	273±29 ***
2	261±28 **	283±49 $^{\#\#}$	286±53 $^{***, \#}$	291±55 $^{\#}$	265±28
3	260±30 **	284±48 $^{\#}$	283±55 $^{\#}$	287±56 $^{\#}$	263±29
4	256±32 **	284±57 $^{\#}$	287±62 $^{\#}$	291±57 $^{*, \#}$	263±26
5	254±32 *	277±53 $^{***, \#}$	284±57 $^{\#}$	284±57 $^{\#}$	260±37
6	250±32 **	274±54 $^{**, \#\#}$	287±59 $^{\#}$	283±57 $^{\#}$	253±31 *
7 (epicardium)	247±30 †	268±53 $^{\#\#}$	274±57 $^{\#}$	281±58 $^{\dagger, \#}$	255±32 †
Average	257±31	280±51 $^{\#}$	285±56 $^{\#}$	287±55 $^{\#}$	262±31 $^{\#}$

Data are mean ± S.D. * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$ vs. the below value; † $P < 0.001$ epicardium vs. endocardium; $^{\#}$ $P < 0.001$, $^{\#\#}$ $P < 0.01$ vs. sinus rhythm.

Table 2. Apico-basal differences in activation-recovery intervals in the left ventricular free wall in all animals under sinus rhythm and ectopic pacing of the left ventricle.

The part of the left ventricular wall	**Sinus rhythm**	**Pacing**			
		of the base		**of the apex**	
		endocardial	**epicardial**	**endocardial**	**epicardial**
The basal third	228±47	286±45 $^{\#}$	290±60 $^{\#}$	244±15	231±20
The middle third	267±24 $^{*, \dagger\dagger}$	319±22 $^{*, \dagger, \#}$	331±22 $^{*, \dagger, \#}$	307±52 $^{*, \dagger\dagger, \#}$	272±24 *
The apical third	258±24 §	260±51 §§§	262±53 §§§	284±56 $^{\#, \S\S}$	264±31 §

Data are mean ± S.D. * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$ the middle part vs. the basal one; † $P < 0.001$, †† $P < 0.01$, ††† $P < 0.05$ the middle part vs. the apical one; $^{\#}$ $P < 0.001$, $^{\#\#}$ $P < 0.01$, $^{\#\#\#}$ $P < 0.05$ vs. sinus rhythm; § $P < 0.001$, §§ $P < 0.01$, §§§ $P < 0.05$ the apical third vs. the basal one.

Table 3. Differences between activation-recovery intervals recorded from the subepicardial and subendocardial layers of the myocardium in the left ventricular free wall in all animals under sinus rhythm and ectopic pacing of the left ventricle.

The part of the left ventricular wall	**Sinus rhythm**	**Pacing of the base**		**Pacing of the apex**	
		endocardial	**epicardial**	**endocardial**	**epicardial**
The total wall	16±14	11±28	3±24 *	9±16 **	13±19
The basal third	22±24	-1±70	-45±42 **	3±9	6±35
The middle third	23±12	15±22	10±15 **	9±25 **	20±11
The apical third	11±10	11±18	9±15	10±12	13±17

Data are mean ± S.D. * $P < 0.01$, ** $P < 0.05$ vs. sinus rhythm.

Table 4. Activation-recovery intervals in seven myocardial layers in each of the apical, middle and basal thirds of the left ventricular free wall in all animals under sinus rhythm and ectopic pacing of the left ventricle.

Locations of electrodes	**Sinus rhythm**	**Pacing of the base**		**Pacing of the apex**	
		endocardial	**epicardial**	**endocardial**	**epicardial**
		The basal third of the left ventricular free wall			
1 (endocardium)	246±41	283±29	276±58	259±20	236±35
2	233±50	288±40	240±10	242±10	234±26
3	227±55	291±41	244±40	243±19	234±25
4	223±49	294±44	285±86	249±7	237±12
5	222±50 **	289±66 ***	272±67	232±4	234±10
6	217±51	286±65	350±39 $^{\#\#\#}$	235±10	225±10 $^{\#\#\#}$
7 (epicardium)	221±50	285±68	350±39 $^{\#\#\#}$	238±6	220±33 $^{\#\#\#}$
		The middle third of the left ventricular free wall			
1 (endocardium)	270±22 ***	289±16 $^{\#}$	294±23 $^{\#}$	292±47 $^{\#\#\#}$	276±21
2	261±16 ***	279±5 $^{***, \#\#}$	283±13 $^{\#}$	286±50 $^{\#\#\#}$	272±17
3	262±21	281±14 $^{\#}$	280±20 $^{\#}$	280±52	268±19
4	259±24 ***	280±28 $^{\#\#}$	280±19 $^{\#}$	283±50 $^{\#\#\#}$	263±30
5	256±25 ***	272±20 $^{\#\#}$	280±26 $^{\#}$	279±55 $^{***, \#\#\#}$	261±36 ***
6	253±27	271±22 $^{\#\#}$	279±27 $^{\#}$	276±57 $^{\#\#\#}$	256±10 ***
7 (epicardium)	247±30 †	266±35 $^{\#\#\#}$	271±29 $^{\dagger\dagger\dagger, \#\#}$	276±51	254±10 ††
		The apical third of the left ventricular free wall			
1 (endocardium)	268±24 ***	279±54 ***	279±56	284±52	276±27
2	262±22	271±52	276±55	290±56 $^{**, \#\#\#}$	271±26 $^{***, \#\#\#}$
3	261±22 **	265±49	262±53	284±58 $^{\#\#\#}$	266±29 $^{\#\#\#}$
4	258±24	257±54	258±56	285±59 $^{**, \#\#\#}$	259±22 $^{\#\#\#}$
5	257±26 ***	255±53	262±56	281±57 $^{\#\#\#}$	259±39
6	254±25 *	249±52	250±50	281±58 $^{***, \#\#\#}$	260±34
7 (epicardium)	249±24 †	249±51 †††	243±49	282±59 $^{\dagger\dagger, \#\#}$	260±34 $^{\dagger\dagger, \#\#\#}$

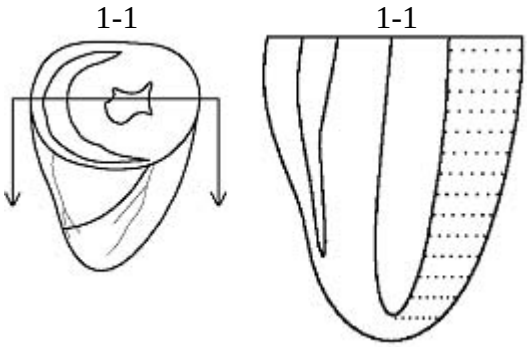
Data are mean ± S.D. * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$ vs. the below value; † $P < 0.001$, †† $P < 0.01$, ††† $P < 0.05$ epicardium vs. endocardium; $^{\#}$ $P < 0.001$, $^{\#\#}$ $P < 0.01$, $^{\#\#\#}$ $P < 0.05$ vs. sinus rhythm.

Figure 1 The schemes (a, b) of insertion of plunge needles into the myocardial wall of the left ventricle. Sections of the heart ventricles: 1-1, longitudinal section; 2-2, 3-3, 4-4, transversal sections.

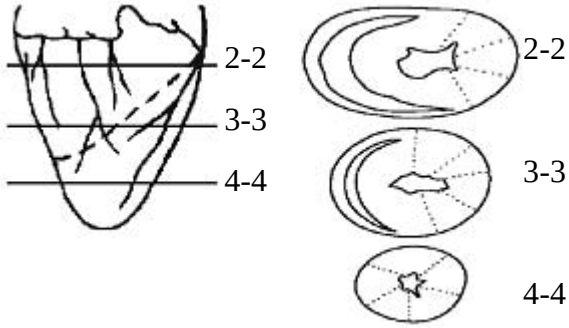
Figure 2 Examples of electrograms recorded with three plunge needles within the chicken left ventricular wall, and ECG in the standard bipolar limb leads under sinus rhythm (a) and endocardial pacing of the left ventricular base (b). I, II and III, ECG in the standard bipolar limb leads; endo, endocardium; epi, epicardium.

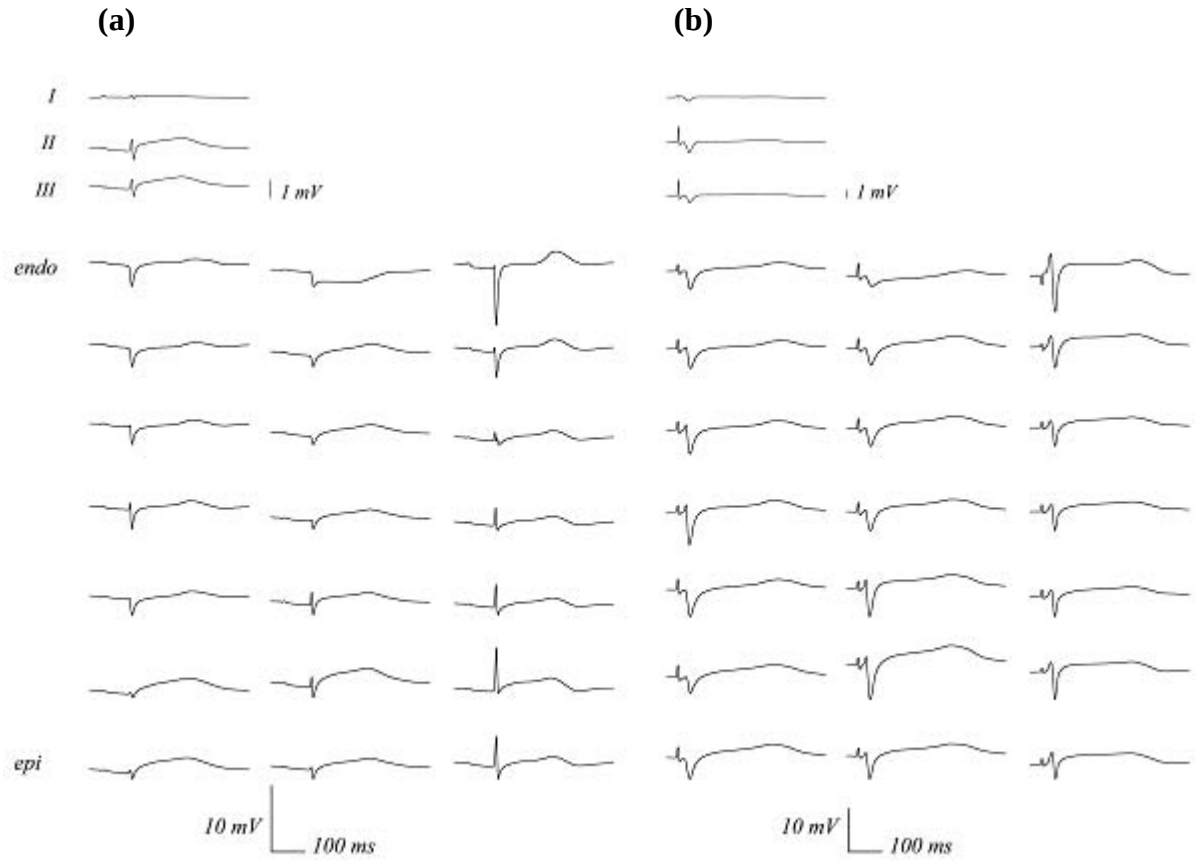
Figure 3 Apicobasal differences in activation-recovery intervals in the subendocardial (a), mid-myocardial (b) and subepicardial (c) layers of the left ventricular free wall in all animals under sinus rhythm and ectopic pacing of the left ventricle. Two, three and two electrodes were considered to be at subepicardial, mid-myocardial and subendocardial positions, respectively, in each seven-electrode plunge needle. SR, sinus rhythm; Bendo, endocardial pacing of the left ventricular base; Bepi, epicardial pacing of the left ventricular base; Aendo, endocardial pacing of the left ventricular apex; Aepi, epicardial pacing of the left ventricular apex. * $P < 0.001$, ** $P < 0.01$, *** $P < 0.05$ the middle / basal third vs. the apical one; † $P < 0.001$, †† $P < 0.01$, ††† $P < 0.05$ the middle third vs. the basal one; $^{\#}$ $P < 0.001$, $^{\#\#}$ $P < 0.01$, $^{\#\#\#}$ $P < 0.05$ vs. sinus rhythm.

(a)

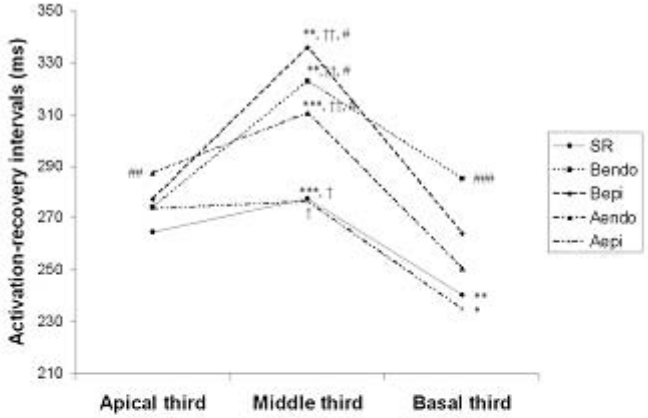


(b)

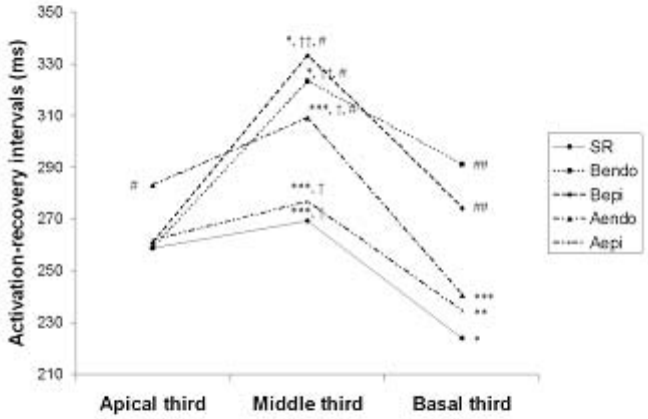



(a)
(b)
I
II
III
1 mV
endo
epi
10 mV
100 ms
1 mV
10 mV
100 ms

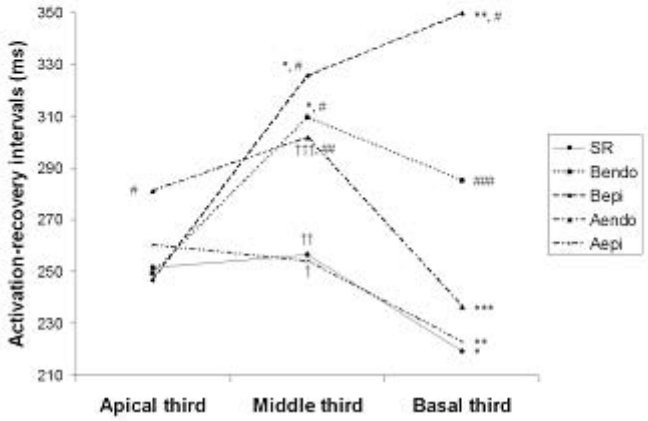
(a)


Activation-recovery intervals (ms)
350
330
310
290
270
250
230
210
Apical third
Middle third
Basal third
SR
Bendo
Bepi
Aendo
Aepi

(b)


Activation-recovery intervals (ms)
350
330
310
290
270
250
230
210
Apical third
Middle third
Basal third
SR
Bendo
Bepi
Aendo
Aepi

(c)


Activation-recovery intervals (ms)
350
330
310
290
270
250
230
210
Apical third
Middle third
Basal third
SR
Bendo
Bepi
Aendo
Aepi