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Extracellular detection of delayed afterdepolarization of cardiac fibers using signal averaging technique

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KEY WORDS arrhythmia; electrophysiology; Purkinje fibers; strophanthidin; bufanolides; computer-assisted; signal processing;

ABSTRACT

AIM: To detect delayed afterdepolarizations (DAD) in extracellular electrograms using signal averaging technique. **METHODS**: DAD were induced by acetylstrophanthidin ($0.25\ \mu mol \cdot L^{-1}$, $n = 9$) and resibufogenin ($0.52\ \mu mol \cdot L^{-1}$, $n = 5$) in sheep cardiac Purkinje fibers. Intracellular voltage was recorded with a conventional microelectrode, and simultaneous extracellular electrograms were recorded differentially from widely spaced electrodes placed in the tissue bath. Noise of electrograms was reduced using signal averaging technique. **RESULTS**: Acetylstrophanthidin and resibufogenin both induced DAD in the intracellular recording and extracellular DAD (DAD-E) in the extracellular electrogram in sheep heart Purkinje fibers. Acetylstrophanthidin and resibufogenin induced typical changes in the action potential including decrease in action potential amplitude, resting potential, maximum diastolic potential, and action potential duration. Similar shortening occurred in the "Q-T interval" recorded by the extracellular electrogram. With either acetylstrophanthidin or resibufogenin, shortening of stimulation cycle length from 990 ms to 690 ms reduced the coupling interval between action potential upstroke and peak voltage of the DAD ($P < 0.01$), and the coupling interval between the "QRS" and DAD-E recorded extracellularly ($P < 0.01$). **CONCLUSION**: DAD can be detected using the extracellular electrograms combined with the high resolution, signal averaging technique.

INTRODUCTION

The mechanisms thought to cause cardiac arrhythmias are often grouped as reentrant, abnormal automaticity, and triggered activity. Triggered activity is caused by afterdepolarizations[1]. These are oscillations of membrane voltage that are dependent on a preceding action potential for their initiation. When one or more afterdepolarizations attain voltage threshold, they can trigger action potentials in isolated tissue, or trigger arrhythmias in the intact heart. Afterdepolarizations can arise before repolarization of an action potential is completed (*early afterdepolarizations* or EAD) or following its complete repolarization (delayed afterdepolarizations or DAD)[2-5].

The detection of mechanisms of arrhythmias in intact hearts remains difficult, and most existing electrophysiological techniques are not primarily oriented towards the detection of mechanisms (eg, programmed stimulation) or they are intended to detect reentry (eg, body surface signal averaging).

We tested the idea that since afterdepolarizations are oscillations in membrane voltage and they occur 'late' during the cardiac cycle, they should be detectable in extracellular electrograms combined with signal averaging technique[6]. We studied DAD as the prototypical experimental model and used isolated cardiac Purkinje fibers which permitted the membrane voltage to be directly monitored. DAD were induced using mildly toxic concentrations of acetylstrophanthidin[7-9] and resibufogenin[10-13]. Thus, the purpose of the present study was to detect DAD induced by acetylstrophanthidin or resibufogenin in extracellular recordings with high resolution, signal averaging technique.

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METHODS

Preparation Sheep hearts were obtained at a local slaughterhouse in the Chicago area and transported to the laboratory in cooled oxygenated Tyrode's solution and were rapidly dissected. Single, free-running Purkinje fibers from the left ventricle, having a length of (15.8 ± 1.2) mm and width of (1.2 ± 0.1) mm ($x \pm s$) were used. Single, free-running Purkinje fibers were placed in a 2-mL tissue bath and superfused with Tyrode's solution, 1 – 2 mL/min .

Solution Acetylstrophanthidin (Sigma, Chemical Company) was dissolved in distilled water to form a stock solution that was diluted with Tyrode's solution to a final concentration of 0.25 μmol·L^{-1} when used. Resibufogenin ($C_{24}H_{32}O_4$), whose chemical structure is similar to that of digitoxigenin, was extracted and separated from a traditional Chinese medicine, Chan Su, which is obtained from the skin gland venom of toads[10-13]. Resibufogenin was diluted for each experiment with Tyrode's solution to a concentration of 0.52 μmol·L^{-1} when used. The procedure for making Tyrode's solution was described in our previous report[13].

Electrical recording The transmembrane action potential was measured with conventional glass microelectrodes (WPI, 1B150F-4 glass) filled with KCl 3 mol/L, and having resistances of 10 – 20 MΩ and small tip potentials. To record the extracellular electrogram, two glass patch pipettes (100 precision, Capillary Tubes, Drummond Scientific Co) with tip openings of about 5 – 10 μm were filled KCl 3 mol/L – 2 % agar, and were placed in the tissue bath at opposite ends of the fiber. They were connected via Ag/AgCl half-cells to the input of a low-noise, low-drift, differential intrumentation amplifier (AD 521, Analogue Devices, Norwood, Mass). The signal was then amplified (typical gain 20 000) and filtered (2-pole butterworth, – Db@225 Hz). The signals were displayed on a storage oscilloscope (Tektronix 5111), and digitized in 12-bit resolution (minimum of 3.6 ms/sample) and stored on a laboratory computer (IBM compatible) using commercial software (P-Clamp version 5.5, Axon Instrument). Single bit conversions permitted us to resolve extracellular voltage changes to 0.2 μV and intracellular voltage changes to 0.1 mV. The preparation was stimulated using bipolar extracellular silver electrodes placed near one end of the fiber. Stimulating current pulses (World Precision Instruments) usually were 2 ms in duration with amplitudes of two times threshold. Data acquisition was synchronized with the stimulus pulse.

In most experiments, data were signal averaged online to reduce noise using the P-Clamp software routine. In some experiments, we also used fast-fourier transforms and area calculations of data records that were performed off-line using programs written in Asyst (Kiethly/Asyst).

Experimental protocol In each experiment, the Purkinje fiber was equilibrated at a stimulation cycle length of 990 ms for a minimum of one hour before study. Because DAD can occur in freshly isolated fibers, our experimental protocols required that every fiber for study to have normal resting and action potentials, be free of automaticity and afterdepolarizations in the transmembrane action potentials, and have a large T-wave amplitude in the extracellular electrogram.

Data were acquired during a control period, a drug exposure period, and a washout period. In addition, since electrophysiological properties of DAD (coupling interval to the previous action potential, DAD amplitude, etc) depend on the rate of stimulation, each fiber was stimulated two cycle lengths. At the end of the equilibration period, control measurements were made first at a 990-ms cycle length and then at a 690-ms cycle length. The preparation was then superfused with Tyrode's solution containing drug, usually for 60 min. This drug exposure period was usually adequate to produce DAD that did not reach voltage threshold for triggered activity. Fibers were again stimulated at cycle lengths of 990 and 690 ms during which data were collected. Fibers were then returned to normal Tyrode's solution for a 60-min washout period, at the end of which data were again collected at stimulation cycle lengths of 990 and 690 ms.

Three groups of fibers were studied. In Group Ⅰ, Purkinje fibers were studied initially to assess the number of sweeps needed for optimal signal averaging ($n = 6$). Fibers in Group Ⅱ were exposed to acetylstrophanthidin ($n = 9$) during the drug exposure period. Fibers in Group Ⅲ were exposed to resibufogenin ($n = 5$) during the drug exposure period.

Electrophysiological measurements The following electrophysiological parameters were measured from the transmembrane action potential records, the resting potential (RP), action potential amplitude (AP_{AMP}), maximum diastolic potential (MDP), and action potential duration at 50 % of repolarization (APD_{50}). When DAD were induced during the drug exposure period, DAD amplitude (DAD_{AMP}) was measured as the difference between the maximum diastolic voltage and the most

positive voltage of the DAD. The coupling interval of the DAD (DAD_{CI}) was measured as the time difference between the onset of action potential to the most positive voltage of the DAD. In the extracellular electrograms, the Q-T interval (Q-T) was measured as the difference between the onset of the QRS/stimulation artifact complex and the return of the T-wave to its baseline. T-wave amplitude (T_{AMP}) was measured as the difference between the peak voltage of the T-wave and its baseline voltage. When DAD were recorded during drug exposure, an additional, low frequency, deflection occurred in the extracellular electrogram following the T-wave, which we termed the extracellular delayed afterdepolarization (DAD-E). DAD-E amplitude ($DAD\text{-}E_{AMP}$) was defined as the difference between the peak voltage of the DAD-E and the baseline voltage after the termination of the T-wave. The DAD-E coupling interval ($DAD\text{-}E_{CI}$) was measured as the time difference between the QRS/stimulus artifact and the peak deflection of the DAD-E[13].

Data analysis Data were analyzed using a paired t test or unpaired t test and are presented as $\bar{x} \pm s$. P value < 0.05 was considered statistically significant.

RESULTS

Effects of signal averaging on the electrograms Fig 1 shows recordings of the extracellular electrograms (left-hand panels) and their power density spectra (right-hand panels) from Group Ⅰ. In each extracellular electrogram, three stimulated complexes are present, with the stimulus applied at the arrows. In the upper traces, a single electrogram shows the non-signal averaged noise level. The power density spectrum shows broad bands of noise with the greatest density at very low frequencies and near 60 Hz. The middle traces show the effect of averaging 25 sequential electrograms while the lower traces show the effect of averaging 50 sequential electrograms. These data show that signal averaging of 25 sequential electrograms greatly reduced the amplitude of random noise. These data also show that incremental averaging of up to 500 sequential electrograms (data not shown) resulted in only small additional decreases in broadband noise. The power density spectra show that low frequency content of the extracellular electrograms was altered little by incremental averaging. For these reasons, we averaged 25 sequential electrogams for all data reported.

Signal averaged detection of DAD in the extracellular electrogram Fig 2 shows signal averaged data of action potentials (panels A, B) and extracellular electrograms (panels C, D) recorded during control conditions and following 45 min of exposure to acetylstrophanthidin ($0.25\ \mu mol \cdot L^{-1}$) at the pacing cycle length of 990 ms from Group Ⅱ. For control conditions (panel A), the RP was -89.6 mV, APAMP was 112.5 mV, and APD_{50} was 224.0 ms. In the presence of acetylstrophanthidin (panel B), the RP was -81.3 mV, AP_{AMP} was 92.2 mV, APD_{50} was 128.0 ms. Following the repolarization of action potential, a DAD 4.0 mV in amplitude was present (arrow) that had a DAD_{CI} of 768.0 ms. The control extracellular electrogram (panel C) shows the QRS/stimulus artifact complex followed by a T-wave. In the presence of acetylstrophanthidin (panel D), an additional low frequency, voltage deflection (DAD-E, arrow) was present following the T-wave. This DAD-E had a $DAD\text{-}E_{CI}$ of 774.4 ms which corresponds closely in time to that of the DAD recorded intracellularly.

DAD amplitude and coupling interval depend on the stimulation cycle length[13-15]. The effect of two stimulation cycle lengths (990 and 690 ms) on electrophysiological properties of DAD recorded intra- and extracellularly was observed in this study. Fig 3 shows a typical example of the effects of stimulation cycle lengths of 990 ms (left hand) and 690 ms (right hand) on acetylstrophanthidin-induced DAD and DAD-E on transmembrane action potentials and extracellular electrograms. For control conditions (Panel A), no DAD and DAD-E were present in intra- and extracellular recordings. At both stimulation cycle lengths, the RP, AP_{AMP}, and MDP were similar, whereas APD_{50} decreased from 224.0 ms to 217.6 ms. After exposure to acetylstrophanthidin ($0.25\ \mu mol \cdot L^{-1}$) for 60 min (Panel B), DAD and DAD-E were present in the intra- and extracellular recordings. As expected, the intracellular recordings show that at the shorter stimulated cycle length DAD amplitude was greater (6.3 mV *vs* 4.7 mV) and the coupling interval decreased (608 ms *vs* 806 ms). The extracellular recordings show that at the shorter stimulated cycle length DAD-E amplitude was slightly greater (0.014 mV *vs* 0.013 mV) and the coupling interval decreased (640 ms *vs* 800 ms). Following 60 min of washout with Tyrode's solution (Panel C), DAD had disappeared and both the action potential and electrogram recordings had returned nearly to control conditions. Similar results were obtained using resibufogenin to induce DAD and DAD-E in Group Ⅲ.

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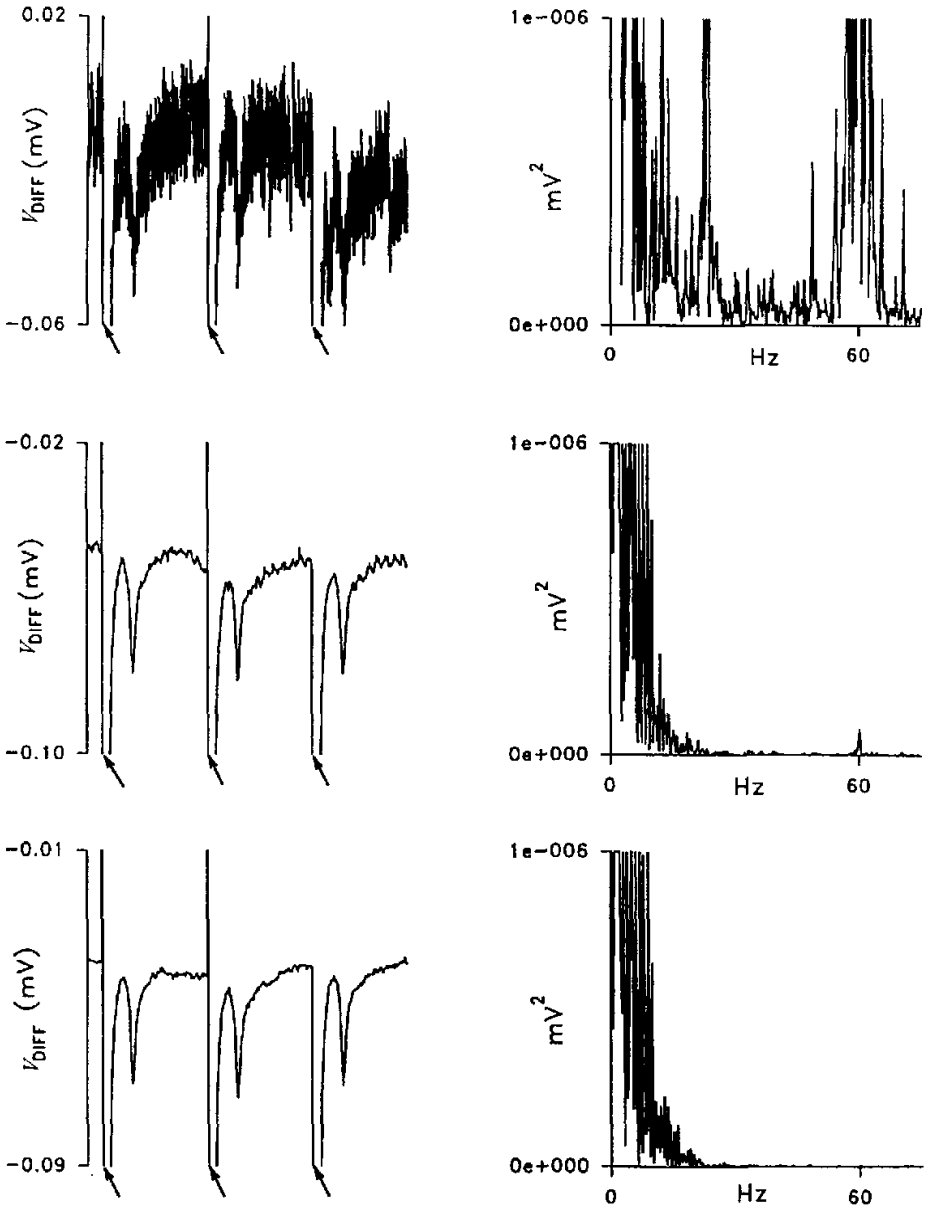

Fig 1. Recordings of the extracellular electrogram and their power density spectra. In each extracellular electrogram, three stimulated complexes were present with stimulus applied at the arrows. Left hand panels: The extracellular electrograms. Right hand panels: The power density spectra of the electrograms. Upper traces: A single electrogram shows the non-signal averaged noise level. The power density spectrum shows broad bands of noise with the greatest density at very low frequencies near 60 Hz. Middle traces: Effect of 25 averaging sequential electrograms. Lower traces: Effect of 50 averaging sequential electrograms.

Tab 1 summarizes the data showing the effects of acetylstrophanthidin and resibufogenin on DAD and DAD-E recorded intra- and extracellularly. DAD_{AMPS} at the shorter cycle length of 690 ms were higher than at 990 ms in Group Ⅱ [from (6.3 ± 0.7) to (4.2 ± 0.4) mV, $P < 0.05$] and in Group Ⅲ [from (7.3 ± 1.9) to $(5.1 \pm$

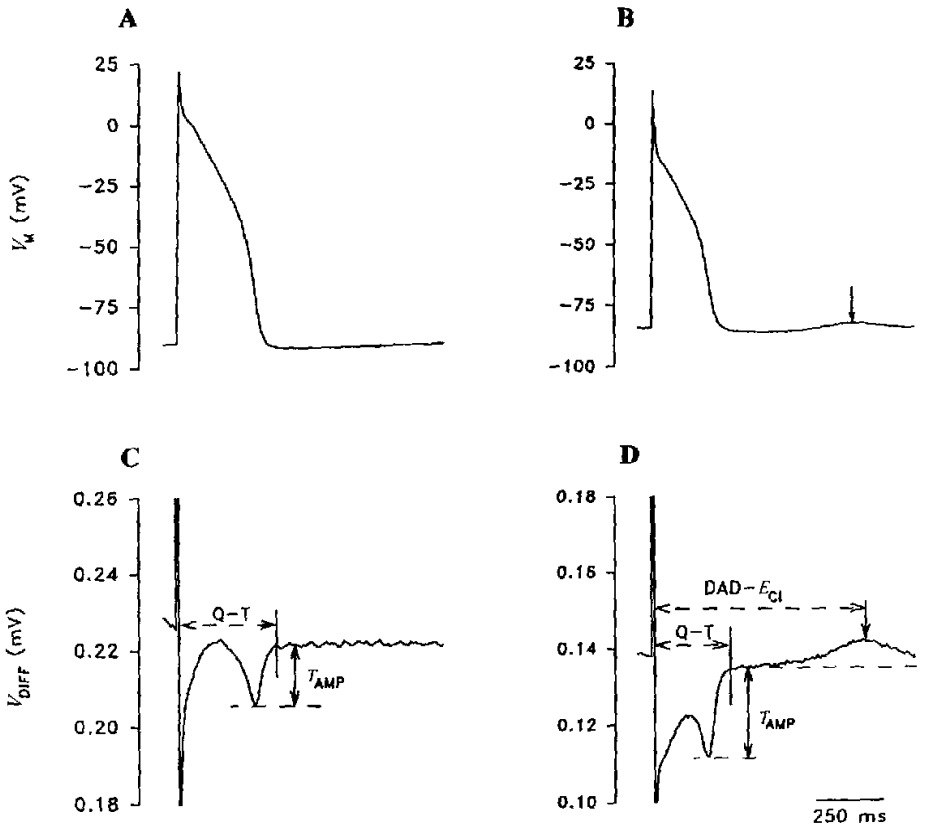

Fig 2. Twenty-five signal averaging recordings of transmembrane action potentials and extracellular electrograms and effects of acetylstrophanthidin (0.25 μmol·L^{-1}) at a basic cycle length of 990 ms. A and B are 25 signal averaging recordings of transmembrane action potential. (A) is at the control condition. (B) is 45 min following exposure to acetylstrophanthidin. An arrow shows a DAD. C and D are 25 signal averaging recordings of extracellular electrograms. (C) is at the control condition. (D) is 45 min following exposure to acetylstrophanthidin. An arrow shows a DAD-E.

0.9) mV, $P > 0.05$]. Both DAD and DAD-E coupling intervals at 990 ms were significantly longer than at 690 ms in the two groups above ($P < 0.01$). The results were similar to those of previous reports[7-9,13-15]. In general, DAD and DAD-E were induced by acetylstrophanthidin at the same time, which were (40.0 ± 4.3) min at 990 ms and (38.3 ± 4.4) min at 690 ms ($P > 0.05$). The DAD coupling intervals [(848 ± 22) ms at 990 ms and (580 ± 19) ms at 690 ms] were similar to those of DAD-E coupling intervals [(852 ± 26) ms, and (576 ± 22) ms, respectively, $P > 0.05$] at 60 min after drug was added in the group Ⅱ. Similar results were obtained from the group Ⅲ (Tab 1). Therefore, DAD in the transmembrane action potentials and DAD-E in the extracellular electrograms could be induced by acetylstrophanthidin and resibufogenin at the same time and similar position without any significant difference. Tab 1 also shows that resibufogenin has the effects similar to acetylstrophanthidin on electrophysiological property and toxicity.

Effects of acetylstrophanthidin and resibufogenin on electrophysiological characteristics Tab 2 displays the effects of acetylstrophanthidin and resibufogenin on the transmembrane action potentials and the extracellular electrograms in sheep cardiac Purkinje fibers. Following exposure to acetylstrophanthidin for 60 min, AP_{AMP}, absolute values of RP and MDP decreased progressively both at the cycle lengths of 990 and 690 ms (at 990 ms, AP_{AMP}: from (114.1 ± 2.2) to (82.4 ± 3.5)

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Tab 1. Comparison of acetylstrophanthidin (0.25 μmol/L) and resibufogenin (0.52 μmol/L) for induced DAD and DAD-E in sheep cardiac Purkinje fibers. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* 990 ms group. $^dP > 0.05$ *vs* acetylstrophanthidin.

	Acetylstrophanthidin Group ($n = 9$)		Resibufogenin Group ($n = 5$)	
	990 ms	690 ms	990 ms	690 ms
DAD_{AMP} (mV)	4.2 ± 0.4	6.3 ± 0.7[b]	5.1 ± 0.9[d]	7.3 ± 1.9[bd]
DAD_{CI} (ms)	848 ± 22	580 ± 19[c]	844 ± 15[d]	618 ± 9[cd]
DAD-E_{AMP} (mV)	0.012 ± 0.003	0.015 ± 0.002[b]	0.013 ± 0.001[d]	0.018 ± 0.003[bd]
DAD-E_{CI} (ms)	852 ± 26	576 ± 22[c]	822 ± 36[d]	626 ± 19[c]

DAD_{AMP}: Delayed afterdepolarization amplitude.
DAD_{CI}: Delayed afterdeopolarization coupling interval.
DAD-E_{AMP}: Extracellular delayed afterdepolarization amplitude.
DAD-E_{CI}: Extracellular delayed afterdepolarization coupling interval.

Tab 2. Effects of acetylstrophanthidin (0.25 μmol/L) and resibufogenin (0.52 μmol/L) on the intracellular action potentials and the extracellular electrograms of sheep cardiac Purkinje fibers. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* control.

Stimulus	Acetylstrophanthidin Group ($n = 9$)			Resibufogenin Group ($n = 5$)		
BCL (ms)	Control	AS	Washout	Control	RBG	Washout
			Action potential amplitude (mV)			
990	114.1 ± 2.2	82.4 ± 3.5[b]	113.8 ± 2.0[a]	119.0 ± 3.2	98.9 ± 2.7[b]	116.0 ± 2.5[a]
690	116.1 ± 2.0	77.7 ± 2.0[b]	116.5 ± 1.7[a]	119.8 ± 3.2	94.7 ± 2.7[b]	118.2 ± 2.7[a]
			Resting potential (mV)			
990	−92.0 ± 1.6	−76.5 ± 2.1[b]	−90.2 ± 1.3[a]	−92.5 ± 0.7	−81.3 ± 1.7[b]	−94.8 ± 1.5[a]
690	−92.5 ± 1.5	−74.8 ± 3.0[b]	−91.0 ± 1.2[a]	−93.7 ± 0.8	−80.0 ± 1.9[b]	−94.1 ± 0.9[a]
			Maximum diastolic potential (mV)			
990	−93.3 ± 1.5	−79.5 ± 2.1[b]	−91.5 ± 1.2[a]	−93.1 ± 0.8	−84.4 ± 1.5[b]	−95.2 ± 1.8[a]
690	−93.1 ± 1.4	−77.6 ± 3.2[b]	−91.4 ± 1.1[a]	−93.9 ± 0.8	−84.6 ± 1.6[b]	−94.3 ± 0.9[a]
			APD_{50} (ms)			
990	216.2 ± 9.5	93.2 ± 13.0[b]	204.8 ± 8.3[a]	271.8 ± 19.5	129.3 ± 13.5[b]	248.3 ± 19.5[a]
690	203.4 ± 9.5	82.7 ± 12.8[b]	192.3 ± 9.1[a]	240.0 ± 15.4	97.9 ± 8.2[b]	233.0 ± 10.2[a]
			T-Wave amplitude (mV)			
990	0.03 ± 0.01	0.02 ± 0.003[a]	0.03 ± 0.016[a]	0.04 ± 0.01	0.03 ± 0.004[a]	0.03 ± 0.01[a]
690	0.03 ± 0.01	0.02 ± 0.004[a]	0.03 ± 0.01[a]	0.03 ± 0.01	0.03 ± 0.009[a]	0.02 ± 0.01[a]
			Q-T interval (ms)			
990	404.6 ± 29.0	229.7 ± 15.8[b]	379.7 ± 17.3[a]	442.9 ± 33.7	304.6 ± 30.1[b]	407.0 ± 28.9[a]
690	386.4 ± 22.5	204.8 ± 18.7[b]	355.6 ± 16.3[a]	410.9 ± 28.6	259.8 ± 26.9[b]	380.2 ± 26.2[a]

BCL: Basic cycle length of stimulation.
AS: Acetylstrophanthidin.
RBG: Resibufogenin.
APD_{50}: Action potential duration at 50 % of repolarization.

mV, RP: from (−92.0 ± 1.6) to (−76.5 ± 2.1) mV and MDP: from (−93.3 ± 1.5) to (−79.5 ± 2.1) mV, $P < 0.01$). APD_{50} and Q-T interval were shortened significantly ($P < 0.01$). All of the electrophysiological parameters recovered basically after wash out for 60 min ($P > 0.05$). The similar effects in acetylstrophanthidin group on the electrophysiological characteristics occurred in resibufogenin group without any signifi-

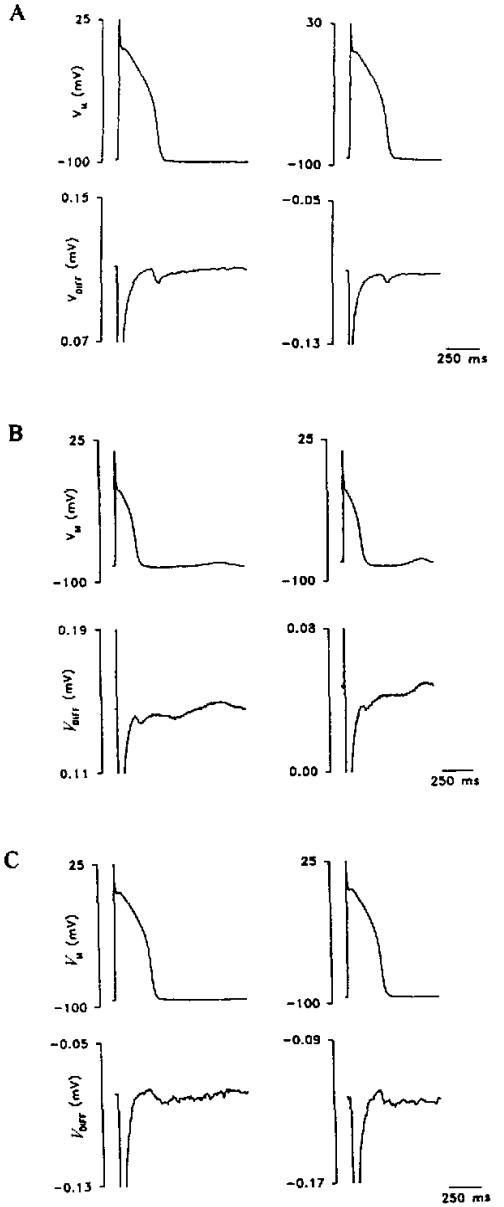

Fig 3. A typical example of the effects of stimulation cycle lengths of 990 ms and 690 ms on acetylstrophanthidin-induced DAD and DAD-E on intracellular action potentials and extracellular electrograms in isolated sheep cardiac Purkinje fibers. Panel A: control conditions. Panel B: after exposure to acetylstrophanthidin (0.25 μmol·L^{-1}) for 60 min. Panel C: following 60 min of washout with Tyrode's solution. Left hand: stimulation cycle lengths of 990 ms. Right hand: stimulation cycle lengths of 690 ms. Upper traces in each panel: the transmembrane action potentials. Lower traces in each panel: the extracellular electrograms.

cant difference (Tab 2).

DISCUSSION

The findings of the present study are as follows. 1) We have demonstrated firstly that it is possible to detect DAD induced by toxic concentration of acetylstrophanthidin and resibufogenin using the extracellular electrogram with signal averaging technique. 2) Both acetylstrophanthidin and resibufogenin can induce DAD in the transmembrane action potential and DAD-E in the extracellular electrogram in isolated sheep cardiac Purkinje fibers at cycle lengths of 990 ms and 690 ms. They have similar electrophysiological and toxic characteristics between them, without any significant differences.

Can DAD be detected in the extracellular electrogram using signal averaging technique? Our experiments indicated that signal averaging of 25 sequential electrograms greatly reduced the noise amplitude and analysis of the power density spectra, shown by the low frequency content of the extracellular electrograms (60 Hz). Thus, we used the signal averaging of 25 sequential electrograms to detect DAD of intracellular action potentials.

In the present study, following exposure to toxic concentrations of drugs, DAD in the intracellular action potentials and DAD-E in the extracellular electrograms can be induced simultaneouly at different cycle lengths of stimulation (990 and 690 ms). As cycle length decreases, DAD and DAD-E tend to increase in amplitude, and coupling intervals of DAD and DAD-E become shorter (Tab 1). As a result, at faster drive rates or heart rates, DAD demonstrate progressive increases in amplitude until they attain threshold potential and induce ectopic or triggered arrhythmias. These electrophysiological results were similar to previous reports[7-9, 13-15]. On the other hand, DAD in the intracellular recordings were induced at the same time and at the similar positions with DAD-E in the extracellular electrograms at both cycle lengths of 990 and 690 ms. These results show that DAD and DAD-E were not only induced simultaneously, but also had a similar position in the intracellular and extracellular recordings. It is possible, therefore, that DAD-E in the extracellular electrogram could be applied to detect DAD in the intracellular action potential using the signal averaging technique. This study suggests that DAD may be detected by an EKG, a routine clinical extracellular electrogram with the signal averaging technique.

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Origin of the extracellular delayed afterdepolarization (DAD-E) The precise electrophysiological mechanism of normal and abnormal U-wave in the surface electrocardiograms and in the electrograms is still unclear[16,17]. The previous reports suggested that normal U-wave could correspond to terminal repolarization or prolonged repolarization of cardiac Purkinje fibers[17,18]. In the present experiments, when DAD were present during drug exposure, a low frequency and extracellular deflection of voltage occurred following the T-wave, which we termed as "the extracellular delayed afterdepolarization" (DAD-E). The results suggest that a significant DAD in the intracellular action potential can give rise to a distinct and prominent DAD-E in the extracellular electrogram. According to our observation and analysis of DAD and DAD-E coupling intervals, we suggest that the genesis of DAD-E should be divided into two kinds. First, some DAD-E coupling intervals correspond closely in time to DAD_{CI}, and DAD-E may be generated in the same cell as DAD. Secondly, other intervals are not identical or close with DAD_{CI}, and DAD-E may develope in different cells of cardiac Purkinje fibers or other mechanisms.

In summary, our data indicate that the characteristics of DAD-E in the extracellular electrogram using signal averaging technique can reflect the DAD in the intracellular electrical activity. DAD-E in electrogram, thus, has potential to be applied in the detection of DAD in the intracellular recording. The electrogram with signal averaging technique has a direct implication to EKG in clinical setting to detect DAD and triggered arrhythmia.

REFERENCES

1 Hiraoka M, Kawano S, Hirano Y, Furukawa T. Role of cardiac chloride currents in changes in action potential characteristics and arrhythmias. Cardiovasc Res 1998; 40: 23 – 33.
2 Cranefield PF, Aronson RS. Cardiac arrhythmias: the role of triggered activity and other mechanisms. Mount Kisco (NY): Futura Publishing Company; 1988.
3 Terek RM, January CT. Excitability and ocsillatary afterpotentials in isolated sheep cardiac Purkinje fibers. Am J Physiol 1987; 252: H645 – H52.
4 Zipes DP. Cardiac electrophysiology: Promises and contributions. J Am Coll Cardiol 1989; 13: 1329 – 52.
5 Rosen MR. The concept of afterdepolarizations. In: Rosen MR, Jamse MJ, Wit AL, editors. Cardiac electrophysiology: a textbook. Mount Kisco (NY): Futura Publishing Company; 1990. p 267 – 81.
6 Kim EM, Katoh T, Hayakawa H. Analysis of initial potential of signal-averaged QRS complex in patients with acute myocardial infarction. Nippon Ika Daigaku Zasshi 1996; 63: 31 – 8.
7 Ferrier GR, Moe GK. Effect of calcium on acetylstrophanthidin-induced transient depolarizations in canine Purkinje tissue. Circ Res 1973; 32: 508 – 15.
8 Ferrier GR, Saunders JH, Mendez C. A cellular mechanism for the generation of ventricular arrhythmias by acetylstrophanthidin. Circ Res 1973; 32: 600 – 09.
9 Sounders JH, Ferrier GR, Moe GK. Conduction block associated with transient depolarizations induced by acetylstrophanthidin in isolated canine Purkinje fibers. Circ Res 1973; 32: 610 – 24.
10 Tsukada K. Biochemical studies on the cadiotonic action of senso. Metabolism 1973; 10: 775 – 77.
11 Xie JT, Belic N, McCullough J, Singer DH. The electrophysiological action of resibufogenin on mammalian cardiac fibers. Chin J Physiol Sci 1988; 4: 145 – 54.
12 Xie JT. Bufogenin. Drugs Future 1987; 12: 837 – 9.
13 Xie JT, Cunningham PM, Adriana M, Cadman CS, Shorofsky S, January CT. Induction of delayed afterdepolarizations and triggered arrhythmias in isolated cardiac Purkinje fibers: Comparison of resibufogenin and acetylstrophanthidin. Acta Pharmacol Sin 1994; 15: 97 – 102.
14 Davis LD. Effect of changes in cycle length on diastolic depolarization produced by ouabain in canine Purkinje fiber. Circ Res 1973; 32: 206 – 14.
15 Karagueuzian HS, Katzung BG. Voltage clamp studies of transiant inward current and mechanical oscillations induced by ouabain in ferret papillary muscle. J Physiol (Lond) 1982; 327; 255 – 71.
16 Surawicz B. U wave: facts hypotheses, misconceptions, and misnomers. J Cardiovasc Electrophysiol 1998; 9: 1117 – 28.
17 El-Sherif N, Craelius W, Boutjdir M, Gough WB. Early afterdepolarizations and arrhythmiogenesis. J Cardiovasc Electrophysiol 1990; 2: 145 – 60.
18 January CT, Fozzard HA. Delayed afterdepolarizations in heart muscle: Mechanisms and elevance. Pharmacol Rev 1988; 40: 219 – 27.

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用细胞外信号叠加技术探测心肌纤维的延迟后除极

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关键词 心律失常; 电生理学; 浦肯野氏纤维; 毒毛旋花子甙元; 蟾酥甾类; 计算机辅助信号处理

目的: 用信号叠加技术探测心肌细胞延迟后除极(DAD). **方法**: 用乙酰毒毛花甙元(AS 0.25 μmol/L, $n=9$)和脂布福吉宁(RBG 0.52 μmol/L, $n=5$)诱发羊心肌浦氏纤维的 DAD. 胞内记录用常规微电极技术, 胞外电图用一般电极技术. 用信号叠加技术减少电图的噪声干扰. **结果**: AS 和 RBG 均可诱发羊心肌浦氏纤维 DAD, 并可在胞内、外同时记录到. AS 和 RBG 引发动作电位的典型改变包括降低振幅, 减小静息电位和最大舒张期电位, 缩短动作电位间期及 Q-T 间期. 缩短刺激周长可缩短细胞内外记录的 DAD 的偶联间期($P<0.01$). **结论**: 用信号叠加技术可在细胞外探测心肌细胞 DAD.

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