Effects of Cyclopiazonic Acid on Triggered Activities in Ventricular Muscle and Cardiomyocytes Isolated from Hamster Hearts

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Abstract

The present experiments were performed to study the actions of cyclopiazonic acid on triggered activities generated *in vitro* in ventricular papillary muscle and cardiomyocytes isolated from the hearts of healthy male Syrian hamsters (Biobreeders F1B). Action potentials (APs) of ventricular muscle with a diameter around 1.5 mm were recorded using a microelectrode technique and force was recorded using a transducer. Ventricular preparations were driven at 2 Hz in high $[Ca]_o$ (9 mM)-low $[K]_o$ (1 mM) solution to induce delayed after depolarizations (DADs). Triggered activities were induced on resumption of electrical stimulation after a rest period of 20 sec. Effects of cyclopiazonic acid (3~10 μM) on steady-state rhythms and post-rest triggered activities were determined. Results revealed that cyclopiazonic acid initially enhanced the amplitude of DADs and induced post-rest triggered rhythms. However, after several minutes of cyclopiazonic acid exposure, AP duration (APD) was prolonged and DADs were significantly depressed. The effects on APD and DADs were reversible after washout of cyclopiazonic acid, but the diastolic potential during rest period oscillated and was able to generate high-frequency spontaneous APs at a reduced potential level. In ventricular myocytes isolated enzymatically, ionic currents were measured using of whole-cell patch-clamp techniques. In a high $[Ca]_o$-low $[K]_o$ solution, a series of oscillatory transient inward currents (I_{ti}) were obtained on repolarization to the holding potential (-45 mV) after a depolarizing pulse to the test potential of +20 mV for 1.2 sec. Cyclopiazonic acid (10 μM) reduced significantly the magnitude of I_{ti}. The present results in hamster ventricular cells suggested that cyclopiazonic acid by inhibiting the sarcoplasmic reticulum (SR)-Ca^{2+} pump would gradually deplete the amount of Ca^{2+} within the SR. The consequent reduction in the amount of Ca^{2+} released into the cytoplasm by cyclopiazonic acid might inhibit triggered arrhythmia through a reduction of DADs and I_{ti}.

Key Words: action potential, hamster ventricular muscle, oscillatory transient inward currents, post-rest potentiation, SR Ca^{2+}-pump inhibitor, triggered arrhythmias

Introduction

The contribution of various Ca^{2+} transport systems located on the sarcolemma and the sarcoplasmic reticulum (SR) in the regulation of cytosolic $[Ca^{2+}]$ and contractility of heart muscle depending on types of tissue, species, age and health condition of the individual (5). One way to establish the functional significance of the SR membrane system is to obliterate the SR functions by using potent inhibitors of SR Ca^{2+}-pumping ATPase such as thapsigargin and cyclopiazonic acid (5).

In our earlier study on ventricular muscle of 17-25 week-old Syrian hamsters driven at 2 Hz in high

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Received: February 17, 2004; Revised: April 30, 2004; Accepted:May 3, 2004.

Ca-low K superfusate (to induce intracellular [Ca^{2+}] overload, see 19), we found that thapsigargin (3 μM) reduced the amplitude of DADs and abolished the triggered APs developed after resting interval of 20 sec (16). In a subsequent study on dog Purkinje fibers and ventricular muscle fibers, we found that both ventricular tissues underwent post-rest potentiation of contraction (see ref 1) after rest periods ranging from 5 to 60 sec (2). The speed of post-rest potentiation of contraction may be indicative of the activity of the SR Ca^{2+}-pumping ATPase, since over-expression of this Ca^{2+}-pumping ATPase accelerated post-rest potentiation (8). We found that in dog ventricular trabecular muscle, cyclopiazonic acid (3-10 μM) decreased the post-rest potentiation of contraction but not the steady-state contraction. In dog Purkinje fibers, however, cyclopiazonic acid depressed AP plateau, induced oscillations of diastolic potential during rest interval and facilitated the onset of spontaneous activity (2). Thus cyclopiazonic acid induced differential effects in 2 types of cardiac tissues from the same species of animal. In a most recent article we reported our experiments on the role of SR in altered AP and contraction of myopathic ventricular muscles from explanted human hearts versus young (17-27 week-old) and old (39-43 week-old) hamster hearts (23). We found an impaired function of the SR contributed to the age-related progressive deterioration of ventricular function in dilated cardiomyopathy. The impairment was increased by cyclopiazonic acid, and there were similarities and differences between human and hamster ventricles.

The aims of the present study was to establish the Syrian hamster as an experimental model for investigation in the role of SR in altered AP and triggered arrhythmias under conditions of Ca^{2+} overload. Syrian hamsters were used because the strain Bio 14.6 of this species has an in-born defect in the SR Ca^{2+} reuptake process and is prone to develop intracellular Ca^{2+} overload (9, 14). Cyclopiazonic acid as an inhibitor of the SR Ca^{2+}-pumping ATPase was used to exaggerate the malfunction of the SR (5), as revealed by means of the measurement of post-rest potentiation of contraction against various rest intervals (2, 8, 23).

Materials and Methods

The present experiments conformed to the guidelines of National Defense Medical Center for the care and use of laboratory animals. Male Syrian hamsters (strain F1B, age 17 to 25 weeks) were obtained from Bio Breeder Inc. (Fitchburg, MA, USA). Animals were injected subcutaneously with heparin (100 μg/kg) 30 min prior to the intraperitoneal injection of sodium pentobarbital (50 mg/kg). Then, when the animals were under full anesthesia, thoracotomy was performed to rapidly retrieve the hearts. Six healthy hamsters were used for ventricular tissue studies. Nine healthy hamsters were used for studies on isolated single ventricular myocytes.

Ventricular Tissue Preparations

The hearts were placed in Tyrode solution saturated with a gas mixture of 97 % O_2 and 3 % CO_2 at room temperature. The atria were removed and ventricles cut open. Strips of papillary muscle with a diameter around 1.5 mm and a length of 5 mm were excised from ventricles and placed in a tissue bath containing (in mM) NaCl 137, KCl 4, $NaHCO_3$ 15, $CaCl_2$ 2.7, $MgCl_2$ 0.5, NaH_2PO_4 0.5 and dextrose 5.5.

One end of the specimen was fixed to the bottom of the tissue bath and the other end was connected through a silk thread to a force-displacement transducer (FT 03C, Grass Instrument CO, Quincy, MA, USA). The action potentials (APs) were recorded by means of glass microelectrode filled with 3 M KCl and connected to a WPI Duo 773 Electrometer (World Precision Instruments, New Haven, CT, USA). Electrical and mechanical events were displayed simultaneously on an oscilloscope (model 4072, Gould Instruments, Cleveland, OH, USA) and a recorder (model TA11, Gould). Electrophysiological parameters were measured as described previously in detail (17, 23).

Ventricular Myocyte Preparations

In nine hamsters, ventricular myocytes were isolated for ionic current measurements by means of the whole-cell patch-clamp techniques as described previously (18) with minor modifications (4). In brief, the heart was perfused in retrograde *via* a polyethylene tube through the aorta with oxygenated normal Tyrode solution at 37 °C containing (in mM) NaCl 137, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 10 and glucose 11 (the pH was adjusted to 7.4 by NaOH). The perfusion lasted for about 15 min until effluent fluid was without blood. The perfusate was then replaced with oxygenated Ca^{2+}-free Tyrode solution containing 150 units/ml collagenase (Sigma, Type I) and 0.1 units/ml protease (Sigma, Type XIV) for 8~15 min and thereafter the heart was washed with oxygenated Ca^{2+}-free Tyrode solution for 10 min. After that, the left ventricle was removed from the heart and cut into fine pieces with gentle shaking until single cardiomyocytes were obtained. The cells were stored in oxygenated Tyrode solution at lease 30 min before use. Only ventricular myocytes with clear cross striations were used for electrophysiological studies.

An Axopatch 1D amplifier was used in voltage-

clamp mode for the measurement of ionic currents. The pipette solution contained (in mM) KCl 120, $MgCl_2$ 1, Na_2ATP 5, HEPES 10, EGTA 0.5 and $CaCl_2$ 0.01. Depolarization-induced currents were elicited at clamped potentials from -45 to +20 mV for 1.2 s at a frequency of 0.1 Hz. The myocytes were initially perfused in a control solution containing 1.8 mM $[Ca]_o$ and 5.4 mM $[K]_o$, followed by a test solution containing high $[Ca]_o$ (9 mM) and low $[K]_o$ (1 mM) solution, and then CPA (3 and/or 10 μM) were added. Maximum outward K current (I_K) on depolarization from the holding potential (-45 mV) to a test potential of +20 mV and the first transient inward oscillatory current (I_{ti}) on repolarization from +20 mV to -45 mV were measured as described previously (3).

Chemicals

Cyclopiazonic acid and electrolytes were purchased from Sigma Chemicals (St. Louis, MO, USA). CPA was dissolved in dimetylsulfoxide (DMSO, Fluda Chemie AG, Switzerland) in stock concentrations of 0.1~0.03 M and was diluted 10,000 times in Tyrode solution for experiment. For the control, DMSO (0.01 %) was added to drug-free Tyrode solution.

Statistics

All quantitative data are expressed as mean ± SEM. Effects of cyclopiazonic acid (Sigma Chemicals) on electrophysiological characteristics were evaluated by paired *t*-test before and after treatment. A value of $P < 0.05$ was considered to be statistically significant.

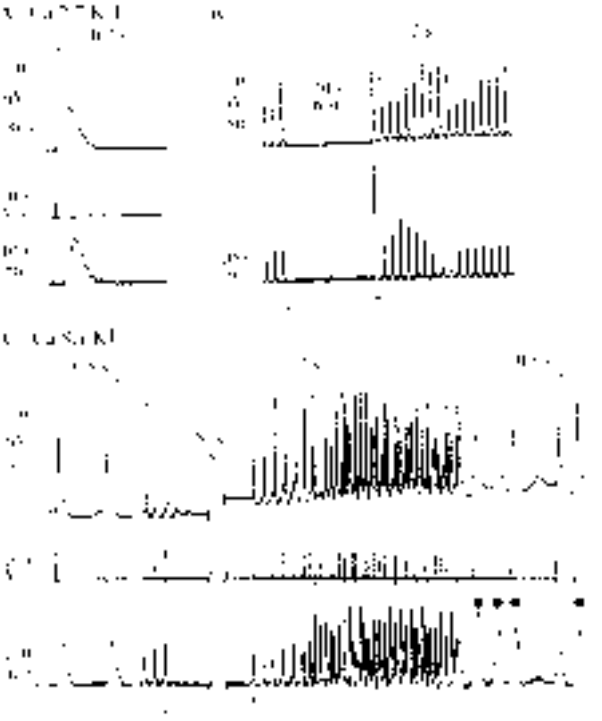

Fig. 1. Panel A shows traces of action potential (AP), its first derivative (dV/dt) and twitch curve of a hamster ventricular muscle preparation driven at 2 Hz in normal Tyrode solution (Ca 2.7 K 4). Panel B shows slow-speed recordings of APs and contractions of the same preparation. Downward and upward arrows indicated the time when electrical stimuli were interrupted for 20 sec (rest interval). Note significant post-rest potentiation of contractions lasted several beats after rest. In panel C, the $[Ca]_o$ of the perfusate was increased to 8.1 mM and $[K]_o$ was reduced to 1 mM. Note that after 20 sec rest, tachyarrhythmia occurred and the automatic rhythms (indicated by dots) were intermingled with driven APs.

Results

Steady-State and Post-Rest Electromechanical Activity of Hamster Ventricular Muscle

Figure 1A illustrated steady-state action potential and twitch force of a healthy ventricular muscle preparation driven at 2 Hz in normal Tyrode (panel A, containing Ca 2.7 mM and K 4 mM) and in high Ca-low K solution (containing Ca 8.1 mM, K 1 mM). A rest interval of 20 sec was used to induce post-rest changes in electro-mechanical activity as shown in Fig. 1B. Evidently, the first beat after rest had a smaller AP spike but an increased contractile force. Exposure to high Ca-low K solution significantly prolonged AP duration near final repolarization (APD_{90}), shifted the maximum diastolic potential to a more negative level and induced delayed afterdepolarizations (DADs) during diastole as was illustrated in Figure 1C. On resumption of electrical stimulation the amplitude of DADs increased progressively and eventually triggered AP occurred which intermingled with the driven AP.

Similar experimental protocols were performed in ventricular muscles of 6 hamsters. Values of the key electrophysiological parameters of these six healthy preparations are summarized in Table 1. Evidently, as compared to perfusion with normal Tyrode solution, high Ca-low K perfusion induced significant arrhythmogenic DAD and post-rest triggered activity.

Electromechanical Actions of Cyclopiazonic Acid

Effects of cyclopiazonic acid on the APs and contractile force were then tested before and after a 20 sec rest period. Figure 2A shows the control APs and twitch force of a ventricular muscle recorded in high Ca-low K solution. Interruption of electrical stimulation (at 2 Hz) revealed the usual presence of DAD in high Ca-low K solution (albeit smaller than that shown in Fig. 1C). Figure 2B~C illustrated ac-

Table 1. Effects of cyclopiazonic acid on hamster ventricular muscle driven at 2 Hz in high $[Ca]_o$ (8.1 mM) and low $[K]_o$ (1 mM) Tyrode solution.

Parameters	APA (mV)	APD_{90} (ms)	DAD (mV)
DMSO	99±5	86 ± 10	9.0 ± 1.5
CPA 10 μM	92±10	128 ± 16	1.8 ± 1.2
difference	7±6	+42.5 ± 12.5*	-7.2 ± 1.7*

Values are mean ± SEM. Number of preparations was 6. APA, action potential amplitude. APD_{90}, action potential duration at 90% repolarization level. DAD, delayed afterdepolarization. *$P < 0.05$, significant difference by paired Student's *t* test between control (8.1 mM $[Ca]_o$ and 1 mM $[K]_o$ Tyrode solution containing vehicle 0.01% DMSO) and 10 μM cyclopiazonic acid (CPA).

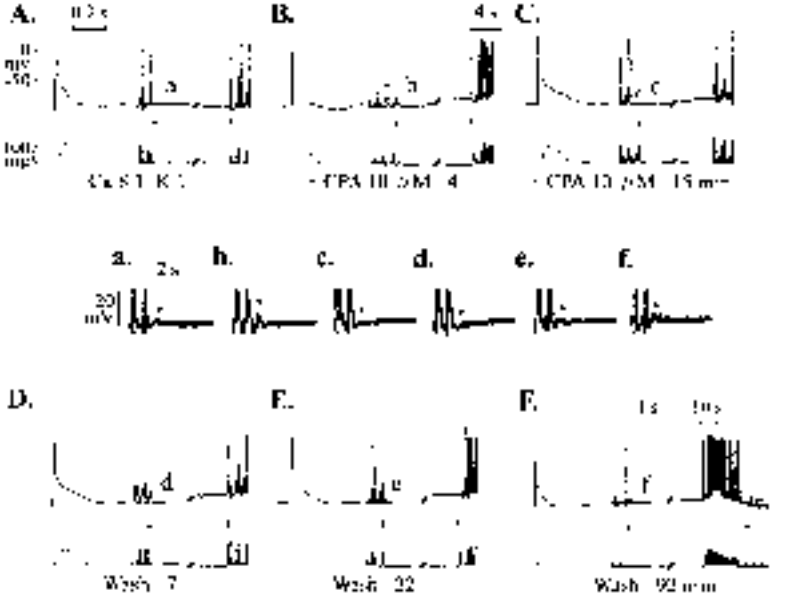

Fig. 2. Effects of 10 μM cyclopiazonic acid on action potentials and contractions of ventricular muscle. Panel A shows the control recordings. Panels B and C were recorded during 4th and 15th min of cyclopiazonic acid perfusion. Panels D~F were recorded during washout of cyclopiazonic acid for 7, 22 and 92 min, respectively. The lower part of last two driven action potentials were magnified 2.5 times and listed in the middle panels. DADs immediately after the last driven APs were identified with solid triangles. The small letters a~f indicated the times when DADs were magnified.

tions of 10 μM cyclopiazonic acid on the same preparation. Cyclopiazonic acid initially enhanced the amplitude of DADs and induced post-rest triggered rhythms (Fig. 2B). However, after a longer period of cyclopiazonic acid exposure, AP duration was prolonged and DADs were significantly suppressed (Fig. 2C). The effects on APD and DADs were reversible after washout of cyclopiazonic acid, but the diastolic potential during late stage of rest period oscillated (Fig. 2D,E) and was able to generate high-frequency spontaneous APs at a reduced potential level (Fig. 2F).

Effects of 10 μM cyclopiazonic acid on electrical parameters (AP amplitude, APD_{90} and DAD) of 6 ventricular muscles are summarized in Table 1. Evidently, cyclopiazonic acid induced a significant prolongation of APD_{90} and inhibition of DAD.

Effects of Cyclopiazonic Acid on Ionic Currents

To explore the ionic mechanisms responsible for the electrophysiological actions of cyclopiazonic acid, outward currents on depolarization and inward currents on repolarization were measured in isolated ventricular myocytes. Fig. 3A shows the clamp protocol. Panels B and C illustrate current traces recorded in a ventricular myocyte. In the control solution (containing 1.8 mM $[Ca]_o$ and 5.4 mM $[K]_o$), depolarizing step from -45 to +20 mV induced an inward current which inactivated quickly and returned to a

Table 2. Effects of cyclopiazonic acid on peak outward K currents (I_K) and oscillatory transient inward currents (I_{ti}) in hamster ventricular myocytes.

Ca9 K1 Control		CPA 3μM		CPA 10μM	
I_K	I_{ti}	I_K	I_{ti}	I_K	I_{ti}
144±31	63±10 pA				
(n=9)					
142±53	65±17 pA	285±118	49±26 pA		
(n=5)		+109±43%	-39±17%		
168±35	69±10 pA			247±64	0 pA*
(n=7)				+37±16 %	-100%*

Values are given in mean ± SEM. The total number (n) of ventricular myocytes was 9. Both low (3 μM) and high concentrations of cyclopiazonic acid (10 μM CPA) were tested in three of the nine myocytes. All high [Ca]-low [K] solutions contained 0.01% DMSO (vehicle). I_K and I_{ti}, peak outward K current on depolarization (from -45 to +20 mV) and peak transient inward current on repolarization, respectively. Percentage changes in values are given underneath the currents data. *$P < 0.05$, significant difference between control and 10 μM cyclopiazonic acid by paired Student's *t* test.

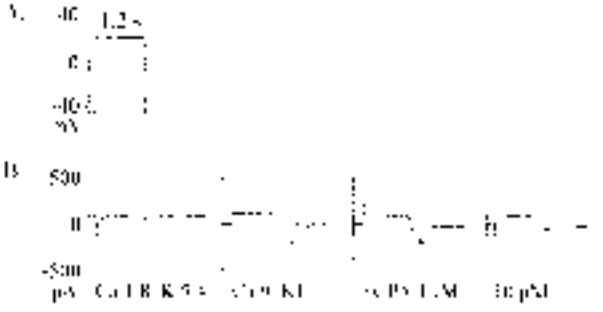

Fig. 3. Ionic current traces induced by depolarizing pulses from a holding potential of -45 to +20 mV for 1.2 sec. Panel A shows the clamp protocol. The left panel B shows the currents recorded in normal Tyrode solution. The 2nd, 3rd and 4th panels B show currents (open triangles, outward K currents; upward arrows, transient inward currents) recorded in high-$[Ca]_o$ low-$[K]_o$ solution in the absence and presence of 3 and10 μM cyclopiazonic acid in sequence.

level closed to the holding current before the depolarizing step. When $[Ca]_o$ was increased to 9 mM and $[K]_o$ was reduced to 1 mM (to provoke intracellular Ca overload), the depolarizing step induced an immediate outward K current (without prior inward current) which was rather well sustained. On returning to the holding potential (-45 mV), a series of oscillatory transient inward currents (I_{ti}) were induced. Adding 3 μM cyclopiazonic acid in the high-Ca low-K solution changed little the outward current but reduced the I_{ti} generated on repolarization. A higher concentration of cyclopiazonic acid (10 μM) abolished the I_{ti} (Fig. 3B).

Results of five experiments with 3 μM cyclopiazonic acid and seven experiments with 10 μM cyclopiazonic acid are summarized in Table 2. Cyclopiazonic acid depressed the I_{ti} in a concentration-dependent manner in all ventricular myocytes tested. In contrast, 10 μM cyclopiazonic acid did not significantly change the outward K currents (increased in 5 but slightly reduced in 2 out of 7 myocytes).

Discussion

The present findings suggest that cyclopiazonic acid might enhance the release of Ca^{2+} from the SR of cardiac cells and thus induce the initial increase in the amplitude of DADs. As a result of inhibition of the SR Ca^{2+} pump by cyclopiazonic acid, however, the amount of Ca^{2+} within the SR would be gradually depleted with a consequent reduction in the amount of Ca^{2+}-induced Ca^{2+}-release into the cytoplasm (thus caused a smaller DADs). After washout of cyclopiazonic acid and a gradual relief of its inhibition on SR Ca^{2+} pump, the elevated level of cytosolic Ca^{2+} would increase the sarcolemmal ion conductance with subsequent membrane depolarization and generation of fast rhythms.

The novel finding that in cardiac muscle cyclopiazonic acid induced an initial potentiation of DAD and post-rest triggered APs (Fig. 2, see ref. 22) is in agreement with the observation that cyclopiazonic acid could induce a release of Ca^{2+} from the SR Ca^{2+} stores in aortic smooth muscle (11). Cyclopiazonic acid also induced an enhancement of post-rest potentiation of contraction in dog Purkinje fibers (2). The initial action of cyclopiazonic acid on hamster ventricular muscle is comparable to that induced by 1 mM caffeine in cardiac Purkinje fiber (21). Caffeine increases and rapidly decreases force in Purkinje fiber. This methylxanthine is known to induce an initial enhanced release of Ca^{2+} from the SR and thus increase the oscillatory potential and arrhythmia (6, 10) before it eventually inhibits Ca^{2+} reuptake into the SR and then depletes the intracellular Ca^{2+} store (7).

Similar biphasic electromechanical actions of cyclopiazonic acid on hamster ventricular muscle could be explained by the same mechanisms.

During washout of cyclopiazonic acid in high Ca-low K solution, DAD after the last driven AP gradually returned (Fig. 2E and 2F) presumably as a result of the relief of inhibition of the SR Ca^{2+}-pump and the replenishment of the Ca^{2+} stores. However, before DADs were fully regenerated, there were oscillations in diastolic potential during late stage of 20 sec rest period (Fig. 2D). The magnitude of these oscillations (ThV_{os}, see 13) could reach threshold and eventually very high-frequency triggered APs developed at a depolarized level (Fig. 2F). Electrogenic extrusion of Ca^{2+} by the Na^{+}-Ca^{2+} exchanger (I_{ex}) (15, 19, 20) could be one of the important ionic mechanisms responsible for the depolarization and the triggered APs induced under this condition.

Results of present experiments have provided important control data for comparison with the preparations obtained from the dilated myopathic Syrian hamster (strain Bio 14.6) to be performed in our future studies on the arrhythmogenesis in the myopathic hearts.

Acknowledgments

The present study was supported by grants from National Science Council (NSC92-2320-B016-037) and National Defense Medical Center (DOD92-124), Taipei, Taiwan, R.O.C.

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