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Comparison of early afterdepolarization induced by cesium chloride in mouse atrial and ventricular fibers

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AIM: To study the similarities and the differences in the induction of early afterdepolarization (EAD) by CsCl, a blocker of potassium channels, between mouse atrial and ventricular fibers. **METHODS**: Papillary muscle from left ventricle or a small piece of right atrium of Swiss Albino mouse was isolated. Transmembrane action potential (AP) and EAD were recorded using the conventional microelectrode method. **RESULTS**: CsCl induced EAD directly in the mouse atrial and ventricular fibers on the basis of elongation of action potential duration (APD). In both preparations EAD appeared after 5 — 15 min exposure to CsCl and the take-off potentials (TOP) were in the same range (−30 to −60 mV). But the inducing concentration in ventricular fibers (3 mmol·L^{-1}) was lower than that in atrial fibers (5 mmol·L^{-1}), and most of the EAD induced in atrium belonged to the triggered burst type (6/9) which demonstrated the property of cycle length (CL)-dependence while the EAD occurred in ventricle mainly belonged to the second plateau type (14/17) which showed no definite relation with the CL. **CONCLUSION**: Low concentration of CsCl induced EAD more easily in mouse ventricular fibers than in atrial fibers, and the types of EAD were also different from each other. These suggested that there might be some differences in potassium channels between mouse atrial and ventricular fibers.

KEY WORDS cesium; action potentials; myocardium

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Early afterdepolarization (EAD), as one of the important bases of triggered arrhythmias, may play an important role in the generation of long QT syndrome, the arrhythmias due to hypoxia, acidosis, ischemia and reperfusion, myocardial infarction, and even some anti-arrhythmic drugs[1-4]. Multiple hypotheses for EAD have been proposed, but the mechanism of EAD still remains unclear because of its complexity[5]. Our previous work showed that the mouse atrial fiber was easy to induce stable EAD, while the mouse ventricular cells was not[6]. In the heart of rabbit and guinea pig, there were many differences in the properties of ionic currents, especially potassium currents, between atrial and ventricular cells[7,8]. The aim of this paper is to find out if there is any difference in the induction of EAD by CsCl, a blocker of potassium channels[9-11], between mouse atrial and ventricular fibers.

MATERIALS AND METHODS

Swiss albino mice (n=15) of either sex weighing 18.8±s 3.5 g were killed by cervical dislocation. A papillary muscle from left ventricle or a small piece (1 mm×1 mm) of right atrium was fixed in a glass bath mounted on the stage of an inverted microscope (Nikon Diaphot) and superfused with Tyrode's solution (33±10 ℃) gassed with 95 % O_2+ 5 % CO_2.

The Tyrode's solution contained the following components: NaCl 137.0, KCl 3.0, $CaCl_2$ 2.7, $NaHCO_3$ 12.0, NaH_2PO_4 1.8, and glucose 11.0 mmol·L^{-1}; (pH 7.2—7.4). CsCl 500 mmol·L^{-1} was stored and added into the superfusate.

Conventional glass microelectrodes filled with KCl 3 mol·L^{-1} having resistance of 15—30 MΩ were used to record transmembrane action potentials (AP)

and EAD with a microelectrode amplifier (MEZ-8201, Nihon Koden), a dual-beam oscilloscope (SBR-1, Santou Electronic Co) and a polygraph (RM 6000, Nihon Koden). To induce action potentials (AP) and EAD, square wave pulses (1 ms duration, 1—2 mA) were applied at different CL by an electric stimulator (SEN 3201, Nihon Koden).

The results were analyzed with paired t test.

RESULTS

The most pronounced effect of CsCl on myocardium was the prolongation of APD. After 5—10 min exposure to CsCl, APD increased in both ventricular and atrial fibers (Tab 1) without obviously affecting the resting potentials (RP) and the action potential amplitude (APA, from 110±6 mV to 106 ±4 mV in atrium and from 105±7 mV to 104±5 mV in ventricle, both $P>0.05$).

Tab 1. Effects of CsCl on duration of action potential (APD) of mouse atrium ($n=8$) and ventricle ($n=7$). $\bar{x}\pm s$. $^bP<0.05$, $^cP<0.01$ *vs* control.

Cycle length /ms	APD of atrium/ms Control	APD of atrium/ms 5 mmol·L^{-1}	APD of ventricle/ms Control	APD of ventricle/ms 3 mmol·L^{-1}
500	131±18	188±21^c	166±24	220±29^c
1000	153±24	220±40^c	180±25	229±25^c
2000	163±22	266±57^c	200±22	250±29^c
3000	176±31	351±135^c	217±29	283±72^b
5000	193±38	473±235^c	227±31	310±78^b

During superfusion of normal Tyrode's solution, the parameters of action potential of atrial and ventricular fibers were unchanged with the increasing of CL from 500 to 5000 ms except the prolongation of APD (Tab 1). EAD was easily induced in both preparations on the basis of elongation of APD. After 5—15 min exposure of the myocardium to CsCl 3 mmol·L^{-1} EAD occured in 5/7 ventricular preparations while at the same concentration it hardly appeared in the atrial fibers. When the concentration of CsCl in the superfusion increased to 5 mmol·L^{-1}, EAD appeared in 4/8 atrial preparations. The effects of CsCl on APD and EAD were abolished after washing.

Fig 1 showed two typical types of EAD induced in atrial and ventricular fibers, *ie*, the second plateau type which is formation of a second plateau in phase 3 with a prominence only; and the triggered burst type which is occurrence of triggered action potentials in phase 3.

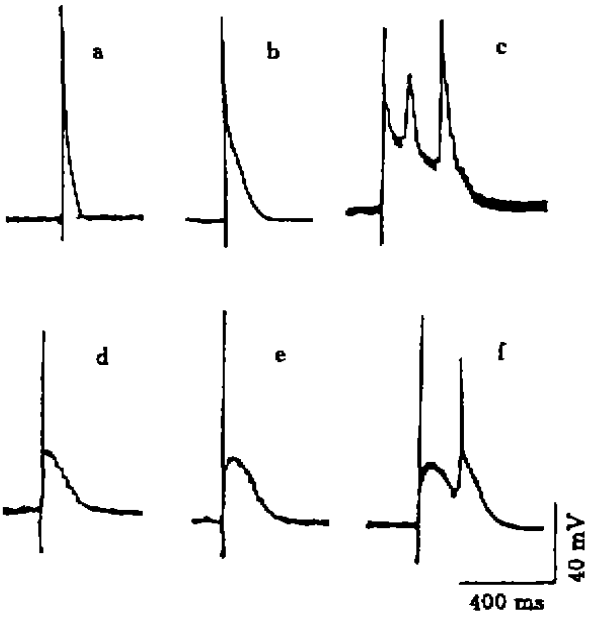

Fig 1. Induction of early afterdepolarizations by CsCl. a) Normal action potential in mouse atrial and ventricular fibers. b) Duration of action potential increased after CsCl. c) Triggered bursts at long cycle length in atrium. d) A second plateau in ventricle. e) A second plateau with a prominence in ventricle. f) A triggered burst in ventricle.

In atrial fibers (Fig 2), at short CL under the treatment of CsCl a second plateau occasionally appeared, but most of the atrial EAD occurred at long CL and belonged to the type of triggered burst which demonstrated the property of CL-dependence as described before[12]: *eg*, the longer the CL, the longer the duration of triggered bursts (TBD) or the

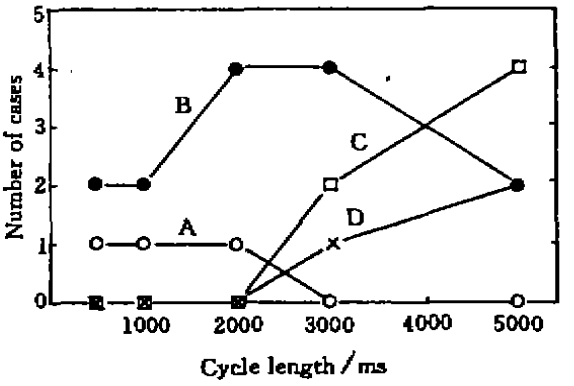

Fig 2. Generation of early afterdepolarizations (EAD) after CsCl in mouse atrial and ventricular fibers. A) second plateau type of EAD in atrium. B) second plateau type of EAD in ventricle. C) triggered burst type of EAD in atrium. D) triggered burst type of EAD in ventricle.

larger the number of triggered bursts (TBN, Fig 3).

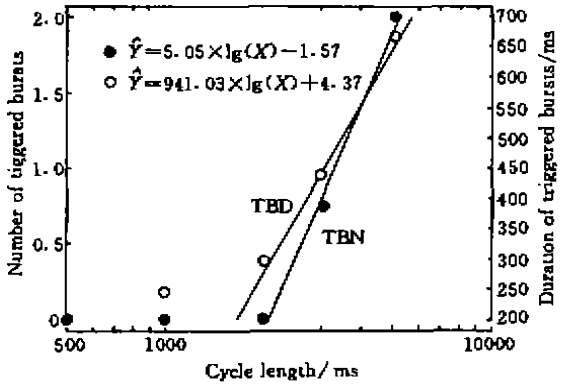

Fig 3. Relationship between number and duration of triggered bursts and cycle length in CsCl-induced early afterdepolarizarions in mouse atrial fibers.

In contrast, in ventricular fibers (Fig 2) triggered activities appeared occasionally, randomly, and instably, but the second plateau with a prominence was easily induced even at the short CL. As the CL increased in more preparations showed this type of EAD and sometimes the amplitude of the prominence increased too. However, the relationship between the CL and the number of the EAD cases or the amplitude of the prominence on the second plateau could not definitely be described. The take-off potential (TOP) of the first triggered burst or of the prominence varied from −30 to −60 mV. In the 3 preparations which appeared both the second plateau type and the triggered burst type EAD, the TOP of the second plateau type was 6−12 mV more positive than that of the triggered burst type.

DISCUSSION

CsCl is one of the widely-used agents in the induction of EAD, but the inducing concentration and the inducing time were varied in different reports[12,14]. Our study showed that in mouse cardiac myocardium, especially the ventricular fibers, the inducing concentration was comparatively lower and the inducing time shorter than those seen in other species. The possible explanations include: 1) There are some differences in potassium currents between the mouse atrium and ventricle, for example, the I_{K1} in the ventricular fibers may be larger than that in the atrial fibers as in other mammals[7]; 2) The properties of potassium channels may be different. It was reported that CsCl might blocked the outward potassium currents at an extracellular site in some kinds of cells but at an intracellular site in other kinds of cells[10]. The specific mechanism for the effect of cesium on the mouse remains to be elucidated.

The second major difference between the effect of CsCl on the mouse atrium and ventricle was the inducing types of EAD with the second plateau type appeared mainly in the ventricular fibers and the triggered burst type in the atrial fibers. Unlike the EAD induced in the Purkinje fibers[13] which occurred at two levels of membrane potential-triggered action

potentials at higher membrane potentials (−50 to −70 mV) under the superfusion of the Tyrode's solution with K^+ 2 mmol·L^{-1} and oscillations at lower membrane potentials (−3 to −30 mV) under the superfusion of the normal Tyrode's solution, the TOP of both EAD types in the mouse atrium and ventricle was in the same range of membrane potential (−30 to −60 mV), though in the same preparation the TOP of the triggered burst was a little more negative than that of the second plateau. Our results suggested that in the mouse myocardium the appearance of EAD was not only dependent on the TOP. The difference between the atrial fibers and the ventricular fibers may also play an important role in the appearance of EAD. Multiple ionic currents could participate in the generation of EAD[15]. Since CsCl is primarily a blocker of potassium channels, it is reasonably suggested that there lies some differences in potassium channels between the mouse atrial and ventricular fibers.

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氯化铯诱发小鼠心房肌和心室肌早后去极化的比较

R365.2

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目的：研究钾通道阻断剂氯化铯（CsCl）诱发小鼠心房肌和心室肌早后去极化（EAD）的异同。**方法**：以游离的小鼠左室乳头状肌和右房组织条为标本，细胞内微电极记录跨膜动作电位(AP)和EAD。**结果**：CsCl可显著延长小鼠心房肌和心室肌的动作电位时程并诱发出EAD。EAD的起始电位均在−30至−60 mV

之间，但 CsCl 作用的有效浓度不同（心室肌为3 mmol·L^{-1}；心房肌为5 mmol·L^{-1}），且心房肌的 EAD 多表现为触发发放型（6/9）并具频率依赖性，心室肌的 EAD 多表现为平台突起型(14/17)而无频率依赖性. 结论：低浓度 CsCl 在小鼠心室肌较心房肌更易诱发 EAD 且二者表现形式不同，提示小鼠心房肌和心室肌的钾通道可能不同.

关键词 铯；动作电位；心肌 小鼠

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Influence of 3,4′,5-trihydroxystibene-3-β-mono-*D*-glucoside on vascular endothelial epoprostenol and platelet aggregation

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AIM: To study the relationship between the inhibiting effect on platelet aggregation and the enhancing effect on epoprostenol (PGI_2) released from vascular endothelium with 3,4′,5 - trihydroxystibene - 3 - β - mono - *D* - glucoside (polydatin, Pol). **METHODS**: After having been incubated with Pol, the incubating medium was withdrawn from the bottles with newborn umbilical vein endothelial cells (VEC group, trypsin digesting method) and added to the platelets (washing method). The medium withdrawn from the bottles without VEC was designated as control group. Reduction of platelet aggregation rates (PAR, turbidity method) and changes of 6-ketoprostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane B_2 (TXB_2) (radioimmunoassay method) in the supernatant of the aggregated platelets induced by thrombin were scrutinized. **RESULTS**: PAR in the control group showed no reduction, whereas PAR reduction (－10 ± 10) and 6-keto-$PGF_{1\alpha}$ increase (108 ± 30 ng·L^{-1}) in the VEC group treated 10 min with Pol 0.41 mmol·L^{-1} (*vs* that of distilled water, ie, 2 ± 12 and 54 ± 20 ng·L^{-1}) occurred. **CONCLUSION**: Increase of PGI_2 from VEC by Pol was involved in its (Pol's) inhibition effect of platelet aggregation.

KEY WORDS 3,4′,5-trihydroxystibene-3-β-mono-*D*-glucoside; polydatin; umbilical veins; vascular endothelium; platelet aggregation; thromboxane A_2; thromboxane B_2; epoprostenol; 6-ketoprostaglandin $F_{1\,alpha}$

Polydatin (Pol), a crystal extracted from the root and stem of *Polygonum cuspidatam* Sieb *et* Zucc[1], inhibited rabbit platelet aggregation *in vitro* and *in vivo*[2]. Pol 5 or 10 mg·kg^{-1} iv could inhibit rabbit arterial thrombosis induced by endothlial damage[3] (to be published). It was desirable to investigate whether the influence of Pol on the function of platelet in the microenvironment where arterial thrombosis took place depended on the release of epoprostenol (prostacyclin, PGI_2) from vascular endothelial cells (VEC).

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