CARDIOPROTECTIVE ACTION OF AMILORIDE, A POTASSIUM SPARING DIURETIC DRUG, IN CANINE VENTRICULAR MUSCLE

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Abstract: Electrophysiological and mechanical effects and alteration of intracellular Ca^{2+} concentration in canine ventricular muscle by amiloride, a potassium sparing diuretic drug, were examined, using conventional microelectrode technique and fura-2 Ca^{2+}-sensitive fluorescent dye. Amiloride (50 μM) depressed the action potential amplitude by 8.2±1.4 % (n= 8, P<0.05) and the maximum rate of depolarization by 16.2±2.0 % (n= 8, P<0.01). In addition. the action potential duration was prolonged by 26.7±3.4 % (n= 6, P<0.05) at 30 μM, and the resting potential was depolarized by 11.8±1.7 % (n= 6, P<0.05) at 0.5 mM amiloride. In contrast, amiloride (0.5 to 1 mM) significantly increased the contractile force by 8 to 30 % (n= 8), but tended to decrease it at lower concentrations (30 μM to 0.1 mM). The positive inotropic effect was not affected by propranolol (0.1 μM), a β-adrenoceptor blocker. In fura-2 loaded ventricular myocytes, amiloride (1 mM) initially elevated cellular Ca^{2+} level ($[Ca]_i$) by 24.5±2.9 % (n= 6, P<0.01), and during the application, the $[Ca]_i$ level declined. These results indicate that amiloride possesses complex cardiac (protective) actions: electrical inhibitory and mechanical stimulatory actions, accompanied with the elevation of cellular Ca^{2+} concentration.

Index Terms

amiloride, potassium sparing diuretic drug, action potentials, contractile force, intracellular Ca^{2+} concentration

INTRODUCTION

Amiloride, a potassium-sparing diuretic drug, possesses many actions on cardiac and smooth muscles, as well as on renal tubule. In cardiac muscles, amiloride prolongs the action potential duration (APD), and reduces the amplitude of action potential (APA) and the maximum rate of rise of depolarization ($\dot{V}_{max}$)[1),2),3),4)]. These actions would produce antiarrhythmic actions. Amiloride causes a positive or negative inotropic effect, depending on extracellular Ca^{2+} concentration[4),5),6)]. In rabbit sino-atrial (SA) node cells, amiloride inhibited the Ca^{2+} current (I_{Ca}), the delayed rectifying K^+ current (I_K), and the hyperpolarization-activated inward current (I_h)[4)]. In addition, it has been reported that amiloride has inhibitory actions on Na^+-H^+ and Na^+-Ca^{2+} exchanges[7),8),9)].

The aim of the present study is to examine electrophysiological and mechanical effects of amiloride on canine ventricular muscle. The effect on the contractile force is complex and its mechanism is still unclear. Thus, to elucidate the mechanism underlying the positive inotropic effect, change in intracellular Ca^{2+} concentration ($[Ca]_i$) was measured using fura-2 Ca^{2+}-

sensitive fluorescent dye.

METHODS

Electrical and mechanical experiments

Mongrel dogs of either sex, weighing 7-10 kg, were used. The preparations were made as reported previously[4),10)]. In brief, right ventricular muscles (2-3 mm in diameter) obtained from dog heart were placed in an organ bath and superfused with Tyrode solution at a temperature of 36℃. One end of the preparation was fixed on the paraffin base of the bath, and the other end was connected to a force displacement transducer (Nihon Kohden SB-1T) using a fine nylon thread. Field stimulation at frequencies of 60 beats/min with pulses of 1-2 ms duration and twice the voltage threshold in strength was used. The action potential, recorded by using a standard glass microelectrode technique, and the contractile force were registered on an oscilloscope (Nihon Kohden VC-11), and photographed (Nihon Kohden RLG-6201). The Tyrode solution (NaCl 137, KCl 4.0, $MgCl_2$ 1.0, $CaCl_2$ 1.8, NaH_2PO_4 0.4, $NaHCO_3$ 12.0 and glucose 5.0 in mM) was bubbled with 95 % O_2 and 5 % CO_2. The pH was adjusted to 7.4 with NaOH. Drug used was amiloride hydrochloride dihydrate (Merk, Sharp and Dohme Research Lab., Munich, Germany).

Since solutions in the bath were exchanged within 1-2 min and the effects of drugs reached a completely steady state within 5-6 min, the data were obtained about 7-8 min after changing to the new solution. Values were represented as mean±SEM. Differences of the mean values were analysed by Student t-test for paired data, and $P<0.05$ was considered significant.

Measurement of cellular Ca^{2+} concentration

Isolated single ventricular myocytes by collagenase (Type I, Sigma Chemical Co., MO, U. S. A.) were incubated for 30 min with the acetoxy-methyl-ester of 0.3 μM fura-2 (fura-2/AM) (Dojin Chemical Co., Kumamoto, Japan) at 30℃. The short loading period was to reduce the fluorescence contribution of the sarcoplasmic reticulum (SR) (Williams et al., 1987). The $[Ca]_i$ in cells loaded with fura-2 was monitored as a change in the ratio of fluorescent intensities at 380-nm and 340-nm excitations[11)]. The fluorescence was quantified with a silicon-intensified target (SIT) camera and a digital imaging system (Hamamatsu Photonics Argus 100, Hamamatsu, Japan).

RESULTS

Effects on the action potentials and the contractile force

Electrophysiological and mechanical effects of amiloride were examined. Figure 1 A shows the action potentials, the maximum rate of depolarization ($\dot{V}_{max}$), and the contractile force. Amiloride (0.1 to 1 mM) prolonged the action potential duration at 75 % repolarization (APD_{75}) and depressed the $\dot{V}_{max}$, significantly (Fig. 1 B-D). The effects were concentration-dependent. The average values are summarized in Table 1. The contractile force was decreased at 0.1 mM or lower, whereas it was increased at 0.5 and 1 mM. The increase was not modified by propranolol (0.1 μM). The resting potential (RP) was significantly depolar-

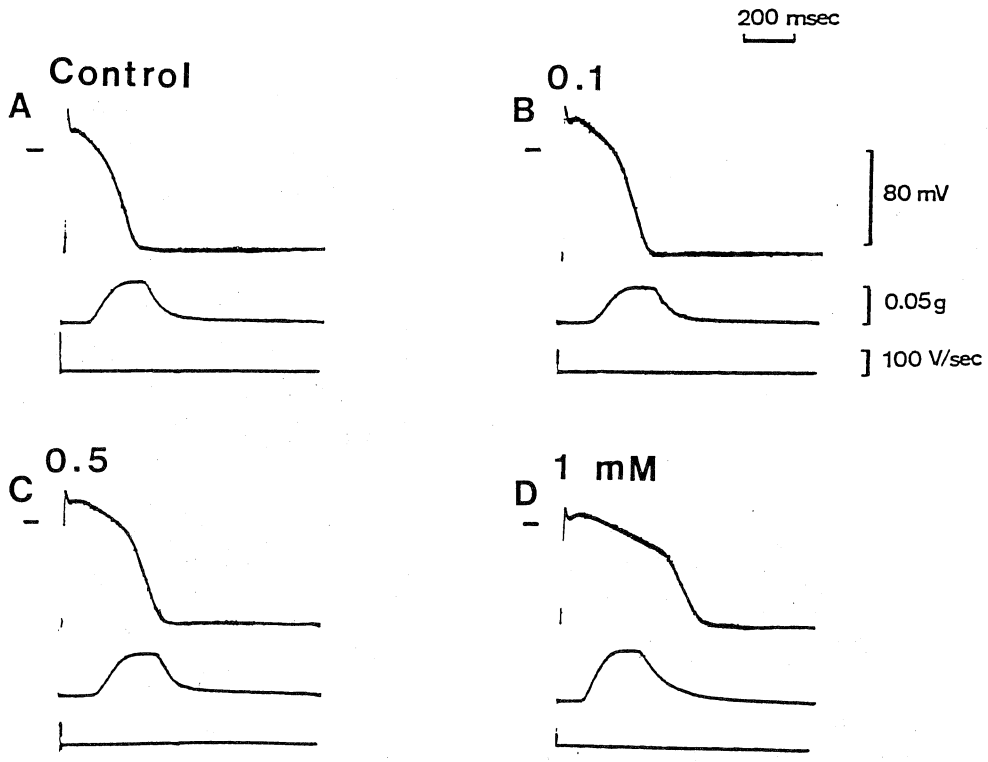

Fig. 1. Effects of amiloride on the action potentials and the contractile force in canine ventricular muscle. Each panel shows the action potential, the contractile force, and the maximum rate of depolarization. A : Control. B-D : Amiloride applications (0.1, 0.5 and 1 mM). Brief line before the action potential trace represents zero mV.

Table 1. Amiloride actions on the action potential parameters and the contractile force in canine ventricular muscle

	n	RP (mV)	APA (mV)	$\dot{V}_{max}$ (V/s)	APD_{75} (ms)	T (mg)
Control	8	−83±2	112±5	210±12	185±6	52±3
Amiloride						
5 μM	6	−82±2	108±3	199±6	202±5	52±6
30 μM	6	−81±1	106±4	185±4*	235±7*	50±4
50 μM	8	−82±2	103±2*	176±6**	239±7*	49±6
0.1 mM	8	−81±2	100±6*	168±10**	246±5**	51±3
0.5 mM	8	−78±1*	96±3*	153±9**	265±8***	56±5*
1 mM	8	−76±1*	92±4**	109±11**	389±14**	68±4**

Values represent means±SEM. * : $P<0.05$, ** : $P<0.01$, *** : $P<0.001$, with respect to control value. RP : resting potential, APA : action potential amplitude, $\dot{V}_{max}$: maximum rate of depolarization, APD_{75} : action potential duration (75 % repolarization), T : contractile force.

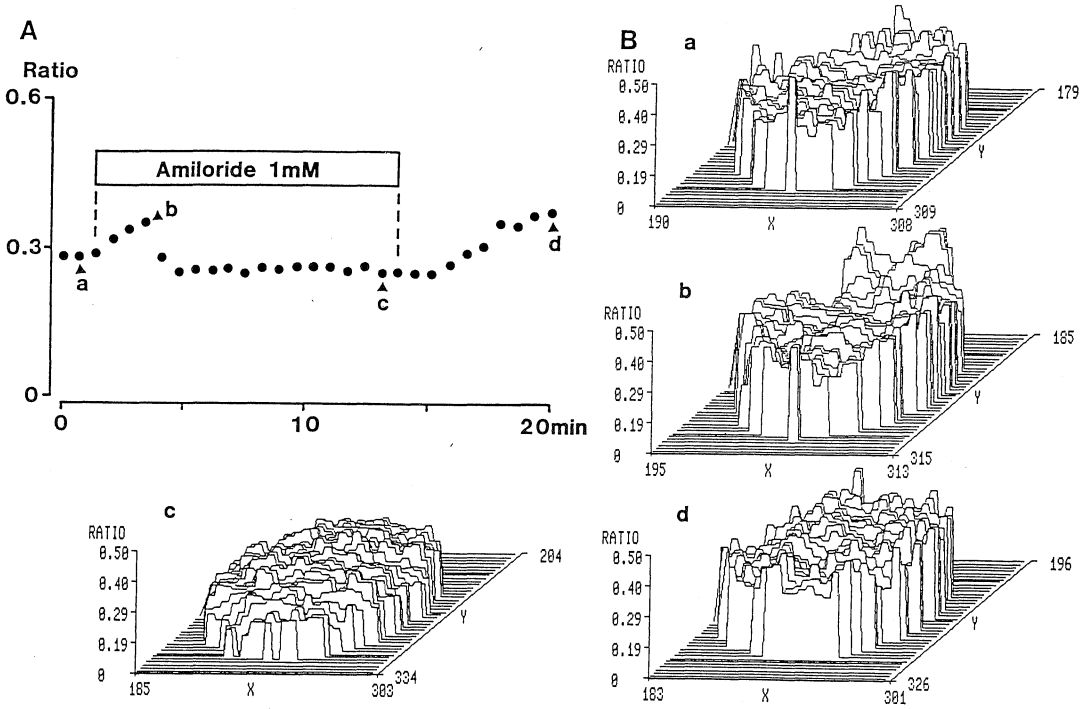

Fig. 2. Change in the cytosolic Ca^{2+} concentration in a canine ventricular myocyte. Ratio of fluorescence intensities of the cytoplasm measured at 380-nm and 340-nm excitations represents in fura-2 (a Ca^{2+}-sensitive fluorescent dye)-loaded single cardiomyocyte. A : Ratio of 380/340 nm in the absence and presence of amiloride (1 mM). B : Stereographs of intracellular Ca^{2+} concentration before (a), during (b and c) and after (d) amiloride application (1 mM), as indicated by triangles in panel A.

ized at over 0.5 mM.

Effects on cellular Ca^{2+} concentration

Time-dependent change in $[Ca]_i$ during exposure to amiloride was examined using fura-2 fluorescent dye. Amiloride (1 mM) initially elevated $[Ca]_i$ level, but then the $[Ca]_i$ decreased almost to control value, as shown in Fig. 2A. The average increased values were $7.4\pm2.6\%$ ($n=5$, $P>0.05$) at 0.1 mM, $11.8\pm3.6\%$ ($n=5$, $P<0.05$) at 0.5 mM, and $24.5\pm2.9\%$ ($n=5$, $P<0.01$) at 1 mM. The secondary decrease in $[Ca]_i$ during amiloride application (1 mM) was $6.1\pm3.2\%$ ($n=5$, $P>0.05$), as compared with control value. After washing out, the $[Ca]_i$ level elevated again (rebound). The rebound increase was $56.6\pm7.1\%$ ($n=4$, $P<0.01$). Then, the $[Ca]_i$ level declined to control value about 15 min after the start of wash out. Figure 2B shows the stereographs from a cell of different stages before, during and after amiloride (1 mM) application.

DISCUSSION

The present study in canine ventricular muscle showed that amiloride (1) inhibited the $\dot{V}_{max}$ and depressed the APA, (2) depolarized the RP, (3) produced a profound prolongation of APD,

and (4) decreased the contractile force at low concentrations, but increased it at high concentrations, and (5) that in isolated single ventricular muscle cells, amiloride actually elevated the $[Ca]_i$ level.

Amiloride is a widely used potassium-sparing diuretic drug, and also has various actions on cardiac muscles[4),5),12),13),14)]. As mentioned in the Introduction, amiloride could produce potent antiarrhythmic actions due to I_{Na} inhibition (class I antiarrhythmic action) and APD prolongation (class III antiarrhythmic action), and depress spontaneous activity due to decrease in pacemaker currents (class V antiarrhythmic action)[15),16)]. The electrophysiological and mechanical actions of amiloride on cardiac muscles, which is a cardioprotective action, are discussed below.

On the cardioprotective actions

Amiloride has been reported to reduce digitalis sensitivity of the heart by a direct cardiac action[2),17)] as well as by altering the pharmacokinetics of digoxin[18),19)]. The protection for heart muscle against toxic effects is due to the reduction (or inhibition) of cellular Ca^{2+} overload via Na^+-K^+ pump inhibition induced by digitalis[20),21),22)], and thereby the onsets of rhythm disturbances and cardiac arrest would be delayed or eliminated. The protective actions would result from the I_{Na} (or $\dot{V}_{max}$) inhibition and the profound APD prolongation. The I_{Na} inhibition could not only depress the excitability of the membrane, but also indirectly decrease the $[Ca]_i$ level through Na^+-Ca^{2+} exchange[20),21),23)]. On the other hand, the APD prolongation simultaneously accompanies a lengthening of the refractory period, resulting in inhibition and abolishment of premature beat and re-entry arrhythmia. Since amiloride inhibits I_{Ca} and I_h currents in the SA node cells[4)], amiloride has class IV and V antiarrhythmic actions. In addition, amiloride depolarizes the RP due to decrease in K^+ conductance, which may contribute to the depressions in the Na^+ and Ca^{2+} channel activities.

On the inotropic action

Amiloride at low concentrations decreased the contractile force, but at high concentrations increased it. The biphasic inotropic effect of amiloride has already been shown in guinea-pig papillary and rat left atrial muscles[6),12)], and it might be dependent on $[Ca]_i$ level. At high extracellular Ca^{2+} concentration ($[Ca]_o$), amiloride enhanced the contractile force, whereas at low $[Ca]_o$, amiloride decreased it. The change in the amiloride actions at different $[Ca]_o$ level would be dependent on $[Ca]_i$ level. The $[Ca]_i$ level would be regulated by complex mechanisms (i. e. Na^+-Ca^{2+} and Na^+-H^+ exchanges, and Ca^{2+}-pump). In the present experiments ($[Ca]_o$ was 1.8 mM), however, the contractile force was not significantly decreased at low concentrations of amiloride. This suggests that it may be not enough for the exchange systems to be activated at low concentrations.

Amiloride inhibits $\dot{V}_{max}$ and I_{Na} current[4),24),25)]. In SA node pacemaker cells, amiloride inhibited all the currents (I_{Ca}, I_K, and I_h)[4)]. These inhibitions of ionic currents would produce negative inotropic effect. The I_{Na} inhibition induced by class I antiarrhythmic drugs, like lidocaine or procainamide and TTX, produces the negative inotropic effect[20),21),26)]. Decline of cellular Na^+ concentration leads to a decrease in $[Ca]_i$ level through Na^+-Ca^{2+} exchange.

Also, the I_{Ca} inhibition decreases the contractile force[4),26),27)].

On the other hand, amiloride at high concentrations enhanced the contractile force. Since the positive inotropic effect was no mediated through β-adrenoceptor stimulation, this might be due to the APD prolongation. The prolongation can enhance the contractile force[4)]. Actually, amiloride increased the $[Ca]_i$ level, but transiently (see Fig. 2). Amiloride caused the concentration-dependent positive inotropic effect, but the $[Ca]_i$ was decreased even during the APD prologation. Therefore, these results suggest that the positive inotropic effect may not be due to the APD prolongation.

Amiloride possesses inhibitory actions on Na^+-Ca^{2+} and Na^+-H^+ exchanges[7),9),28)]. In cardiac muscle cells, these exchange systems and Ca^{2+}-pump are the major mechanisms for Ca^{2+} efflux[29)]. The pump has high affinity and low capacity, whereas the Na^+-Ca^{2+} exchange is a low affinity and high capacity system for regulation of $[Ca]_i$[30),31),32)]. The direction and level of operation of Na^+-Ca^{2+} exchange are determined by the electrochemical gradient for Na^+ and the membrane potential[23),32)].

During washing out, the $[Ca]_i$ level elevated further (rebound), and finally reverted to control level. It is considered that, when the cellular high Ca^{2+} concentration is decreased suddenly, the depressed uptake and release of Ca^{2+} during the Ca^{2+} overload were transiently potentiated, and were returned to regular actions[20)].

In the present experiments, therefore, the $[Ca]_i$ level would be elevated mainly by the inhibitions of exchange systems, although the mechanism is so complex. At 1 mM, amiloride initially increased $[Ca]_i$ and then, the $[Ca]_i$ level decreased. These results suggest that amiloride at high concentrations would directly and indirectly inhibit the exchanges to exclude Ca^{2+}, resulting in induction of the $[Ca]_i$ elevation. The secondary decline in $[Ca]_i$ during exposure to amiloride would be due to the other mechanisms, if Na^+-Ca^{2+} and Na^+-H^+ exchanges were inhibited. Other possibilities for the $[Ca]_i$ modulation by amiloride are: (1) a direct modulation of Ca^{2+}-pump to extrude Ca^{2+}, and (2) a stimulation of inositol turnover (α_1-adrenoceptor stimulation produces positive inotropic effect)[27)]. Further experiments are required to elucidate these possibilities.

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