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Effects of dipfluzine on delayed afterdepolarizations and triggered activity induced by ouabain in guinea pig papillary muscles

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KEY WORDS dipfluzine; ouabain; papillary muscles; microelectrodes; electrophysiology

ABSTRACT

AIM: To investigate the effects of dipfluzine (Dip) on delayed afterdepolarizations (DADs) and triggered activity (TA) induced by ouabain and high Ca^{2+} in guinea pig papillary muscles. **METHODS:** Stable and reproducible DADs and TA in guinea pig papillary muscles were induced by ouabain (1 μmol/L) and high Ca^{2+} (5.4 mmol/L). DADs and TA were recorded using intracellular glass microelectrode technique. **RESULTS:** (1) DADs and TA were markedly inhibited by pretreatment with Dip (10, 30 μmol/L). The amplitude and duration of DADs were reduced by Dip (30 μmol/L) from 10.5 mV±2.2 mV and 230 ms±19 ms to 3.6 mV±0.3 mV and 152 ms±14 ms, respectively, and the induced time of DADs was prolonged from (21±5) to (66±11) min. TA was not observed. (2) Dip (10, 30 μmol/L) had significant therapeutic effects on DADs and TA. The amplitude and duration of DADs were reduced by Dip (30 μmol/L) from 10.4 mV±1.2 mV and 218 ms±22 ms to 3.3 mV±0.6 mV and 159 ms±26 ms. The occurrence of TA was also abolished. **CONCLUSION:** Dip has inhibitory effects on DADs and TA induced by ouabain and high Ca^{2+} in guinea pig papillary muscles, which might be related to alleviation of intracellular calcium overload through inhibiting calcium channel and/or calcium release from sarcoplasmic reticulum. The effects of Dip on DADs and TA might produce anti-arrhythmic effects.

INTRODUCTION

Delayed afterdepolarizations (DADs) are oscillations of membrane potential and occur after complete repaolarization of the cardic action potential, which can cause triggered activity and cardiac arrhythmias[1]. To expose cardiac tissue to higher concentrations of cardiac glycosides is the prototypical experimental method used to induce DADs. Dipfluzine (Dip), a novel calcium antagonist of diphenylpiperazines with similar structure to flunarizine, can decrease overshoot, action potential amplitude (APA), maximal rate of depolarization in phase 0 (V_{max}), duration of plateau phase (PPD), and durations of 50 % and 90 % repolarization of action potential (APD_{50}, APD_{90}) in partially depolarized guinea pig papillary muscles[2], and reduce APA, V_{max}, velocity of diastolic (phase 4) depolarization, and rate of pacemaker firing in rabbit sinoatrial node pacemaker cells[3]. Dip can also exert preventive effects on early afterdepolarization and TA induced by isoproterenol in guinea pig papillary muscles[4]. However, the effects of Dip on DADs and TA induced by ouabain in guinea pig papillary muscles have not yet been elucidated. The present

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study was to investigate these effects.

MATERIALS AND METHODS

Materials Guinea pigs of either sex weighing 300-350 g, provided by Experimental Animal Center of Hebei Province (Grade II, Certificate № 04064), were stunned by heavy blow on the heads. The papillary muscles were excised from right ventricle in cold (0-4 ℃) oxygenated Krebs-Henseleit (K-H) solution (NaCl 118, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ 1.2, $NaHCO_3$ 25, and glucose 10 mmol/L, pH 7.3-7.4), and then mounted to the silicon rubber placed on the bottom of perfusing chamber (3 mL) by stainless steel needles. The preparation was perfused (4 mL/min) with K-H solution (35.5-36.0 ℃) gassed with a mixture of 95 % O_2 and 5 % CO_2.

Methods The preparation was stimulated through a bipolar electrode at a control basic cycle length (BCL) of 600 ms (1 ms rectangular pulse and 1.5 times threshold intensity) from the stimulator (SEN-3201, Nihon Kohden, Japan). The transmembrane action potentials (APs) were recorded by a glass microelectrode filled with KCl 3 mol/L (a tip resistance of 10-30 MΩ), coupled to a high input impedance amplifier (MEZ-8201, Nihon Kohden, Japan). The amplified signals were fed to the A/D convertor and processed by a microcomputer, which collected the transmembrane potential signals and analyzed the parameters of resting potential (RP), APA, V_{max}, APD_{50}, APD_{90}, PPD, DADs, and triggered activity (TA).

Experimental protocols APs were recorded after an equilibration time of 60 min. The experiments consisted of 3 groups: (1) electrophysiological effects of ouabain (1 μmol/L) and high Ca^{2+} (5.4 mmol/L) on the APs of guinea pig papillary muscles. (2) prophylactic effects of Dip on the development of DADs and TA . This part of the experiment were divided into 6 groups at random : (a) control group, the preparation was perfused with K-H solution containing ouabain 1 μmol/L and Ca^{2+} 5.4 mmol/L; (b) solvent control group, the preparation was perfused with K-H solution containing ethanol 3 mL/L for 10 min followed by perfusion with K-H solution containing ethanol 3 mL/L, ouabain 1 μmol/L, and high Ca^{2+} 5.4 mmol/L; (c) Dip treated groups, the preparation was perfused with K-H solution containing Dip (3, 10, or 30 μmol/L) for 10 min followed by perfusion with K-H solution containing Dip (3, 10, and 30 μmol/L), ouabain 1 μmol/L, and high Ca^{2+} 5.4 mmol/L; (d) flunarizine (Flu) treated group, the tissue was perfused with K-H solution containing Flu 10 μmol/L for 10 min, and then perfused with K-H solution containing Flu 10 μmol/L, ouabain 1 μmol/L, and high Ca^{2+} 5.4 mmol/L. (3) the therapeutic effects of Dip on DADs and TA. The papillary muscles of the guinea pig were first exposed to ouabain 1 μmol/L and high Ca^{2+} 5.4 mmol/L. After stable DADs had been induced, solvent (ethanol, 3 mL/L), Dip (3, 10, and 30 μmol/L), or Flu 10 μmol/L was then applied to the perfusate.

Using a program designed by our university, the following parameters of DADs and TA were analyzed automatically. (1) amplitude of DADs: the amplitude from the onset of DADs to the peak of DADs; (2) duration of DADs: the time from the onset of DADs to the end of DADs; (3) induced time of DADs: the time from the beginning of perfusion with K-H solution containing ouabain and high Ca^{2+} to the occurrence of first delayed afterdepolarization ($\geqslant$ 2.0 mV); (4) incidence of TA.

Drugs Dip (purity >99.85 %, synthesized by Department of Chemistry, Peking University) was dissolved in water-free ethanol as stock solution (10 mmol/L) and diluted to the desired final concentrations (3, 10, and 30 μmol/L). The volume concentrations of ethanol in external solution were 0.3, 1, and 3 mL/L, respectively. Flu obtained from Henan Xichuan Pharmaceutical Factory was dissolved in distilled water. Ouabain was provided by Sigma Co and dissolved in K-H solution to 1 μmol/L.

Statistics All data were expressed as mean±SD. The differences of parameters of APs were analyzed by paired *t* test. Independent *t* test was used for the analysis of amplitude and duration of DADs. Rank sum test was used for the analysis of induced time of DADs. Chi-square test was used for the analysis of incidence of TA.

RESULTS

Effects of ouabain and high Ca^{2+} on APs of guinea pig papillary muscles APs were elicited with BCL of 600 ms. RP, APA, APD_{50}, APD_{90}, V_{max}, and PPD were -85.2 mV±2.8 mV, 117 mV±4 mV, 108 ms±8 ms, 151 ms±16 ms, 185 V/s±9 V/s and 80 ms±6 ms, respectively. Ouabain and high Ca^{2+} decreased these parameters (P<0.01, Tab 1). The parameters were recorded 30 min after perfusion with K-H solution con-

taining ouabain and high Ca^{2+} (Tab 1). The effects reached steady state about 5 min before recording. The effects of ouabain and high Ca^{2+} were reversible following washout with K-H solution for 60 min.

Stability and characteristics of DADs induced by ouabain and high Ca^{2+} in guinea pig papillary muscles In the pilot experiment, ouabain 0.2, 0.5, 1.0, and 2.0 μmol/L were used to induce DADs. Ouabain induced DADs at higher concentration (2 μmol/L) but not at lower concentrations (0.2 and 0.5 μmol/L). These DADs, in many cases, developed into sustained TA accompanied by an irreversible reduction in RP, APA, V_{max}, APD_{50}, APD_{90}, and PPD. Thus an intermediate concentration of ouabain (1 μmol/L) was chosen to produce stable DADs and TA. The amplitude of DADs reached its peak magnitude within 17-25 min and lasted for at least 20 min. Elevation of extracellular Ca^{2+} concentrations increased the amplitude of DADs and made the model stabler[5].

Prophylactic effects of Dip on DADs and TA induced by ouabain and high Ca^{2+} Ouabain 1 μmol/L and high Ca^{2+} 5.4 mmol/L induced DADs in all of 6 preparations tested, out of which 5 preparations (83 %) were accompanied by TA. The amplitude, duration and induced time of DADs were 10.5 mV±2.2 mV, 230 ms±19 ms, and 21 min±5 min (Fig 1A). Dip 10 and 30 μmol/L greatly decreased the amplitude, duration, and induced time of DADs and abolished TA (*vs* the control group, $P<0.01$, Fig 1B). Flu had similar effects on DADs and TA to Dip at the equal concentration (10 μmol/L, $P>0.05$, Fig 1C, Tab 2).

Therapeutic effects of Dip on DADs and TA induced by ouabain and high Ca^{2+} DADs induced by ouabain and high Ca^{2+} remained stable for at least 20 min. After recording several control DADs (Fig 2A), Dip was added to the perfusate at the concentrations of 3, 10, and 30 μmol/L. The amplitude and duration of DADs were decreased by Dip 10 and 30 μmol/L

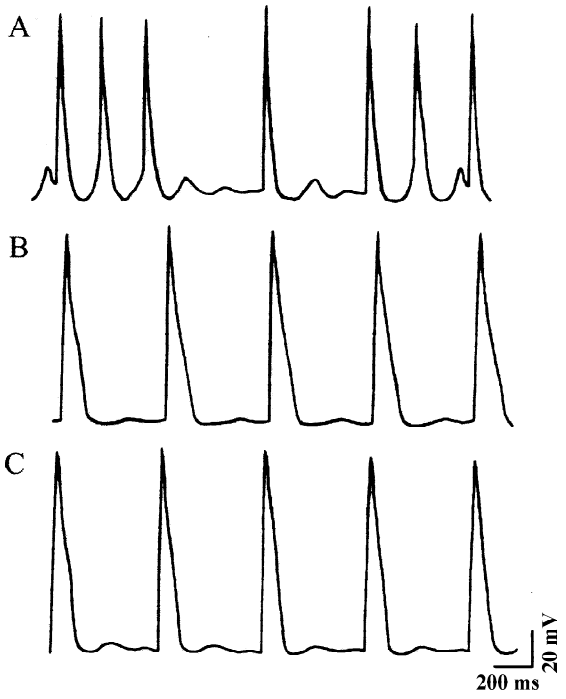

Fig 1. Original record showed prophylactic effects of dipfluzine (Dip) and flunarizine (Flu) on DADs and TA induced by ouabain and high Ca^{2+} (Oua-H-Ca^{2+}) in guinea pig papillary muscles (basic cycle length 600 ms). A: control (Oua-H-Ca^{2+}), DADs and TA were evoked; B: Dip 30 μmol/L+Oua-H-Ca^{2+}; C: Flu 10 μmol/L+Oua-H-Ca^{2+}.

(Fig 2B), and inhibitory effects of Dip on DADs reached its maximum in about 20 min. TA was not observed, possibly due to the decrease in amplitude of DADs. Flu had similar effects to Dip at the equal concentration (10 μmol/L, $P>0.05$, Tab 3, Fig 2C).

DISCUSSION

The present study demonstrated that the amplitude and duration of DADs induced by ouabain and high Ca^{2+} were greatly attenuated by Dip either before or after DADs had been elicited; TA was abolished by Dip

Tab 1. Effects of ouabain 1 μmol/L and high Ca^{2+} 5.4 mmol/L (Oua-H-Ca^{2+}) on action potentials of guinea pig papillary muscles . BCL=600 ms. n=6. Mean±SD. [a]$P>0.05$, [c]$P<0.01$ *vs* control.

Groups	RP/mV	APA/mV	V_{max}/V· s^{-1}	APD_{50}/ms	APD_{90}/ms	PPD/ms
Control	-85.2±2.8	117±4	185±9	108± 8	151±16	80±6
Oua-H-Ca^{2+}	-73.6±1.0[c]	107.3±1.8[c]	164±4[c]	48± 9[c]	107± 8[c]	30±8[c]
Washout	-85±3[a]	117±5[a]	184±9[a]	107±11[a]	150±19[a]	79±9[a]

Tab 2. Prophylactic effects of dipfluzine (Dip) on delayed afterdepolarizations (DADs) and triggered activity (TA) induced by ouabain and high Ca^{2+} (Oua-H-Ca^{2+}) in guinea pig papillary muscles. *n*=6. Mean±SD. $^{a}P>0.05$, $^{c}P<0.01$ *vs* Oua-H-Ca^{2+}. $^{d}P>0.05$ *vs* Dip 10 mmol/L. The concentration of solvent (ethanol) was mL/L.

Groups	Dose /μmol·L^{-1}	Induced time of DADs/min	Amplitude of DADs /mV	Duration of DADs /ms	Incidence of TA /%
Oua-H-Ca^{2+}	1.0	21±5	10.5±2.2	230±19	83
Solvent	3	23±3 ^{a}	10.5±1.1^{a}	241±17^{a}	83^{a}
Dip	3	20±4 ^{a}	10.1±1.7^{a}	219±26^{a}	83^{a}
Dip	10	41±8 ^{c}	5.7±0.5^{c}	167±10^{c}	0^{c}
Dip	30	66±11 ^{c}	3.6±0.3^{c}	152±14^{c}	0^{c}
Flu	10	41±7 ^{cd}	5.8±0.5^{cd}	171± 9^{cd}	0^{cd}

Tab 3. Therapeutic effects of dipfluzine (Dip) on delayed afterdepolarizations (DADs) and triggered activity (TA) induced by ouabain 1 mmol/L and high Ca^{2+} 5.4 mmol/L (Oua-H-Ca^{2+}) in guinea pig papillary muscles. *n*=6. Mean±SD. $^{a}P>0.05$, $^{c}P<0.01$ *vs* Oua-H-Ca^{2+}. $^{d}P>0.05$ *vs* Dip 10 mmol/L. The concentration of solvent (ethanol) was mL/L.

Groups	Dose / μmol L^{-1}	Amplitude of DADs/mV	Duration of DADs/ms	Incidence of TA/%
Oua-H-Ca^{2+}	1.0	10.4±1.2	218±22	83
Solvent	3	11.2±2.2^{a}	225±24^{a}	83^{a}
Dip	3	9.8±1.2^{a}	205±33^{a}	83^{a}
Dip	10	6.0±0.8^{c}	175±15^{c}	0^{c}
Dip	30	3.3±0.6^{c}	159±26^{c}	0^{c}
Flu	10	5.5±0.5^{cd}	167±13^{cd}	0^{cd}

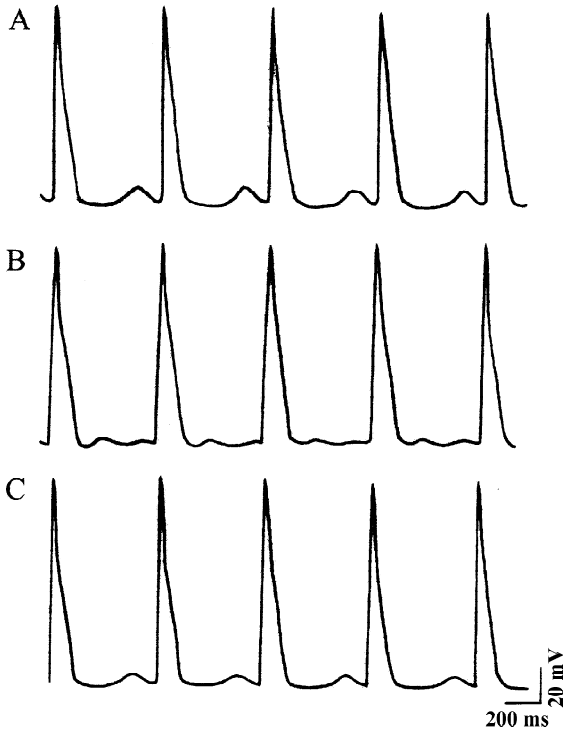

Fig 2. Original record showed therapeutic effects of dipfluzine (Dip) and flunarizine (Flu) on DADs and TA induced by ouabain and high Ca^{2+} (Oua-H-Ca^{2+}) in guinea pig papillary muscles (basic cycle length 600 ms). A: control (Oua-H-Ca^{2+}); B: Oua-H-Ca^{2+}+Dip 30 mmol/L; C: Oua-H-Ca^{2+}+Flu 10 mmol/L.

treatment. Flu had the similar effects to Dip.

It is well known that DADs develops through an oscillatory membrane current occurring after complete repolarization. This current was believed to be a transient inward current (I_{ti}). I_{ti} appears to be either a nonspecific cation current or a current generated by electrogenic Na-Ca exchange, and it is attributed to intracellular calcium overload and spontaneous oscillatory release of calcium from the sarcoplasmic reticulum[1]. In the present study, we developed a stable and reproducible DAD model in guinea pig papillary muscles by using ouabain and elevated extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$). In the case of ouabain, inhibition of Na, K-ATPase results in an intracellular calcium overload[6]; whereas elevated $[Ca^{2+}]_o$ could directly raise intracellular Ca^{2+} concentrations, enhancing the effects of ouabain[5].

It has been reported that Flu, a homologue of Dip, could block L-type and T-type calcium channels in guinea pig ventricular myocytes[7]. At the same time, Flu had direct effect on sarcoplasmic reticulum(SR), eg, the ryanodine-sensitive calcium release channel or SR Ca^{2+}-ATPase[8]. We observed that Dip had similar effects on DADs and TA to Flu. So we hypothesized that Dip realized its action on DADs and TA through the similar mechanism. In fact, our laboratory demonstrated by whole cell patch-clamp technique that Dip had inhibitory effects on both L-type calcium channel[9] and calcium release from SR[10]. This might be the underlining mechanism of effects of Dip.

In summary, Dip has inhibitory effects on DADs and TA induced by ouabain and high Ca^{2+} in guinea pig papillary muscles, which might be related to alleviation of intracellular calcium overload through inhibiting calcium channel and/or calcium release from SR. The effects of Dip on DADs and TA might produce anti-

arrhythmic effects.

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