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Ionic mechanisms responsible for the antiarrhythmic action of dehydroevodiamine in guinea-pig isolated cardiomyocytes

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1 Dehydroevodiamine alkaloid (DeHE), an active ingredient of a Chinese herbal medicine Wu-Chu-Yu (*Evodiae frutus*), has been shown to decrease aterial blood pressure in experimental animals and prolong action potential duration in cardiac cells. The aim of the present study was to explore the ionic basis of its possible antiarrhythmic effects.

2 Guinea-pig atrial and ventricular myocytes were isolated enzymatically and the ionic currents were recorded under whole-cell patch-clamp with single suction pipettes.

3 DeHE at a concentration of 0.1 μM inhibited reversibly the time-dependent outward K current (delayed rectifier, I_k) and the Na-dependent inward current (I_{Na}).

4 In low-K (1 mM) and high-Ca (9 mM) solution, DeHE also depressed the delayed afterdepolarizations (DAD) and the transient inward current (I_{ti}) induced by 2 μM strophanthidin. On the other hand, DeHE occasionally induced early afterdepolarizations and slow response action potentials at a depolarized level.

5 At higher concentrations (1 μM and above), the L-type Ca current ($I_{Ca,L}$) was moderately inhibited.

6 The present findings indicate that DeHE may depress triggered arrhythmias in Ca-overloaded guinea-pig cardiac myocytes through its inhibitory actions on I_{Na}, I_{ti} and, to a smaller extent, I_{Ca}. DeHE may also exert class III antiarrhythmic effect through a reduction of outward K currents (I_k) across the sarcolemma.

Keywords: Dehydroevodiamine; action potential; ionic currents; myocytes; atrium; ventricle; triggered activity; whole-cell patch-clamp

Introduction

Dehydroevodiamine (DeHE), a quinazolinocarboline alkaloid isolated from a Chinese folk medicine Wu-Chu-Yu (*Evodiae frutus*) (Chen *et al.*, 1981), has been shown to induce hypotension and bradycardia in rats (Yang *et al.*, 1988; 1990). Recent studies on rabbit sinoatrial tissues in our laboratory (Lin *et al.*, 1990) have shown that the negative chronotropic effect of this compound was associated with a prolongation of action potential duration (APD) and a reduction of maximum diastolic potential and diastolic slope. In ventricular papillary muscles, DeHE at higher concentrations ($\geqslant$ 0.3 μM) induced a development of early afterdepolarization (EAD) but consistently decreased force of contraction only at concentrations higher than 3 μM (Lin *et al.*, 1990). The aims of the present experiments were to investigate the ionic mechanisms responsible for the APD prolongation and to explore the possible antiarrhythmic effect induced by DeHE on guinea-pig cardiac cells with whole-cell voltage-clamp techniques.

Methods

Single atrial and ventricular myocytes of the guinea-pig, weighing 300–500 g, were used. The methods described by Mitra & Morad (1985) and Tytgat *et al.* (1990a) for cell dissociation, whole-cell patch-clamp recording, and superfusion and internal perfusion of the cells were used in the present study. In brief, guinea-pigs were killed by a blow on the neck and the heart was quickly removed and mounted on a Langendorff perfusion column. The aorta was cannulated and the heart was perfused with a solution containing collagenase and protease at 37°C. After isolation, the cardiomyocytes were bathed at room temperature (20–25°C) in a superfusate containing (in mM): NaCl 137.6, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.5, HEPES 11.5 and glucose 22. The pipette solution contained KCl 120, $MgCl_2$ 6, Na_2ATP 5, HEPES 10, EGTA 5, and $CaCl_2$ 0.15, adjusted to pH 7.2 with 1 N KOH. To record action potential, the myocytes were occasionally paced through a recording micropipette by use of suprathreshold depolarizing stimuli of 2 ms duration provided by a Grass S88 stimulator.

For measurement of ionic currents, single-electrode chopped clamp technique was used by means of an Axoclamp 2-A amplifier (Axon Instruments, Calif, U.S.A.). Data acquisition and analysis were controlled by pCLAMP software (Axon Instruments). The cells were allowed to stabilize in the bath at room temperature for at least 30 min before experiments. The time-dependent outward K currents (I_k) of atrial and ventricular cells were activated by pulses from a holding potential of −70 mV to a conditioning potential of +20 mV of varying duration (0.3–1.8 s), and outward tail currents were measured at −30 mV (see Roden *et al.*, 1989).

For determination of L-type calcium current ($I_{Ca,L}$) of the ventricular cell, a holding potential (V_h) of −50 mV was used to inactivate T-type calcium current ($I_{Ca,T}$) (Tytgat *et al.*, 1990a). Currents were produced at clamped potentials from −40 to +60 mV in 10 mV steps for 250 ms every 2 s. To measure accurately $I_{Ca,L}$ with no contamination of other ionic currents, the cells were bathed in K- and Na-free Tris solution containing (in mM): tris (hydroxymethyl) aminomethane 137.6, $MgCl_2$ 1, CsCl 20, $CaCl_2$ 5.5 and glucose 27.5. The standard internal solution in the micropipette (2–5 MOhm

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resistance) contained (in mM) CsCl 125, MgATP 5, EGTA 15, tetraethylammonium Cl 20, and HEPES 10, adjusted to pH 7.2 with 1 N CsOH. $I_{Ca,L}$ was measured as the difference between peak inward current and the current at the end of the 250 ms pulse. There was almost invariably a run-down of the $I_{Ca,L}$ (Belles *et al.*, 1988), especially during the initial 15 min of internal cell perfusion. Thus experiments were started after a 15 min period of internal perfusion when a further decline of $I_{Ca,L}$ with time had become about 10% for a 30–60 min period. To determine the effects of DeHE, the drug was added to the Tris bath solution at concentrations ranging from 0.1 to 3 μM. For measurement of the Na-dependent inward current (I_{Na}), the experiments were performed in a very low $[Na]_o$ (2 or 12 mM) in K-free Tris solution following the method of Tytgat *et al.* (1990b).

To evaluate the depressant effects of DeHE on triggered activity, the cells were bathed in low-K (1 mM), high-Ca (9 mM) HEPES solution plus 2 μM strophanthidin. The cells were dialysed with a pipette solution containing (in mM): KCl 120, Na_2ATP 5, HEPES 10, EGTA 0.5, $MgCl_2$ 1, $CaCl_2$ 0.01 and pH 7.2 (see Enous *et al.*, 1990). The holding potential was set to −44 mV. A 1.2 s depolarizing pulse to +20 mV was applied every 6 s for 10 steps. Each depolarizing step elicited a time-dependent I_{Ca}. On returning to V_h, a series of transient inward currents (I_{ti}, see Kass *et al.*, 1978) were observed. After eliciting the I_{ti} for 10–15 min, DeHE (0.1– 1 μM) was added to the superfusate and changes in amplitudes of the first I_{ti} on repolarization were measured from the first to the tenth step.

The dehydroevodiamine chloride (DeHE) was synthesized chemically following the methods described in the literature (Shapiro & Abramvitch, 1955; Abramovitch & Shapiro, 1956; Pachter & Suld, 1960; Pachter *et al.*, 1960). Other chemicals were obtained from Sigma Chemicals (U.S.A.).

Values are expressed as mean ± s.e.mean. Student's *t* test was used to compare test and control values. The values obtained before, during, and after DeHE exposure were evaluated by one way analysis of variance (ANOVA). *P* values less than 0.05 were regarded as being statistically significant.

Results

Effects on action potential duration

DeHE prolonged the action potential duration (APD) in both atrial and ventricular myocytes as illustrated in Figure 1. At a constant driving frequency of 1 Hz, 0.1 and 0.3 μM DeHE increased APD at 50% repolarization (APD_{50}) of an atrial myocyte by 22 and 41%, respectively (Figure 1a and b). In ventricular myocytes (Figure 1c and d), the percentage increases in APD_{50} induced by DeHE were relatively smaller (22 and 26% by 0.3 and 1 μM DeHE, respectively). The APD returned to control value within 10 min of washout. Results obtained from 7 atrial and 12 ventricular myocytes are summarized in Table 1.

Effects on time-dependent outward K current

To investigate the role of delayed rectifier (I_k) in the DeHE-induced prolongation of action potential, depressant effects of DeHE on I_k were determined. Figure 2b shows an example in an atrial myocyte. DeHE (0.1 μM) reduced the outward current tail, measured at −30 mV after varying duration (0.3–1.8 s) depolarizing pulses, by about 50% in 8 min (right upper section of Figure 2b). Increasing the concentration of DeHE to 0.3 μM reduced further the I_k tail to about 25% of the control value (left lower section of Figure 2b). I_k returned to 60% of the control value after washout in DeHE-free perfusate for 20 min (right lower Figure 2b). In ventricular myocyte (Figure 2c, right upper and left lower panels), 0.1 and 1 μM DeHE reduced the I_k tail to a similar extent (50% and 20%, respectively, of the control value) as that observed in atrial myocyte. However, the I_k tail returned to near control value after 20 min washout (right lower Figure 2c). Figure 2d and e summarize the results of 9 experiments in atrial myocytes and 13 experiments in ventricular myocytes, respectively.

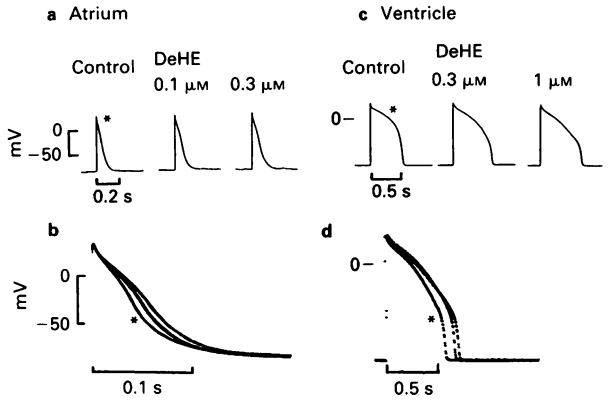

Figure 1 Prolongation of action potential duration induced by dehydroevodiamine (DeHE) in guinea-pig atrial (a and b) and ventricular myocytes (c and d). Upper panels (a and c) show the chart recordings of action potential before (Control, asterisk) and during 10th min superfusion with DeHE (0.1 and 0.3 μM in a, 0.3 and 1 μM in c). The oscillascope traces of action potential before (asterisk) and during DeHE exposure were superimposed in (b) and (d). Duration of each drug exposure was 10 min. The myocytes were paced through a recording micropipette by use of 2 ms duration depolarizing stimulus at 1 Hz in left panels and at 0.3 Hz in right panels.

Table 1 Prolongation of action potential duration (APD) induced by dehydroevodiamine (DeHE) in guinea-pig cardiomyocytes

	Atrium (change in %)			*Ventricle* (change in %)		
Condition	n	APD_{50}	APD_{90}	n	APD_{50}	APD_{90}
DeHE 0.1 μM	5	13 ± 4	31 ± 11*	7	9 ± 3	9 ± 3
DeHE 0.3 μM	4	23 ± 6*	40 ± 8*	6	14 ± 3*	12 ± 3*
Wash	4	5 ± 5	13 ± 6	3	4 ± 4	14 ± 14
DeHE 1 μM	6	28 ± 5*	54 ± 11*	9	20 ± 6*	24 ± 10*
Wash	5	21 ± 10*	35 ± 18*	7	14 ± 12	11 ± 11

The atrial and ventricular cells were superfused in normal HEPES-Tyrode solution. APD_{50} and APD_{90}, action potential duration measured at 50% and 90% repolarization, respectively. Values are means ± s.e. of the percentage changes in APD as compared with the control values before drug exposure. *$P < 0.05$, significantly different from control by one way ANOVA. The control absolute values of APD_{50} and APD_{90} before drug exposure were 100 ± 10 and 169 ± 27 ms, respectively, for 7 atrial myocytes. Those values for 12 ventricular myocytes were 475 ± 44 and 547 ± 51 ms, respectively.

Effects on inwardly rectifying K current

To study whether DeHE also blocked the inward rectifier I_{k1}, the effects of DeHE on steady state K current seen at potentials negative to −60 mV were analyzed. A series of hyperpolarizing voltage clamp pulses in 10 mV steps were applied from a V_h of −50 mV. Results showed that 0.1–1 μM DeHE reduced I_{k1} in three myocytes, but increased it slightly in another three cells and did not change it in the remaining two cells. Thus DeHE may serve as a tool for the pharmacological separation of the delayed rectifier and the inward rectifier.

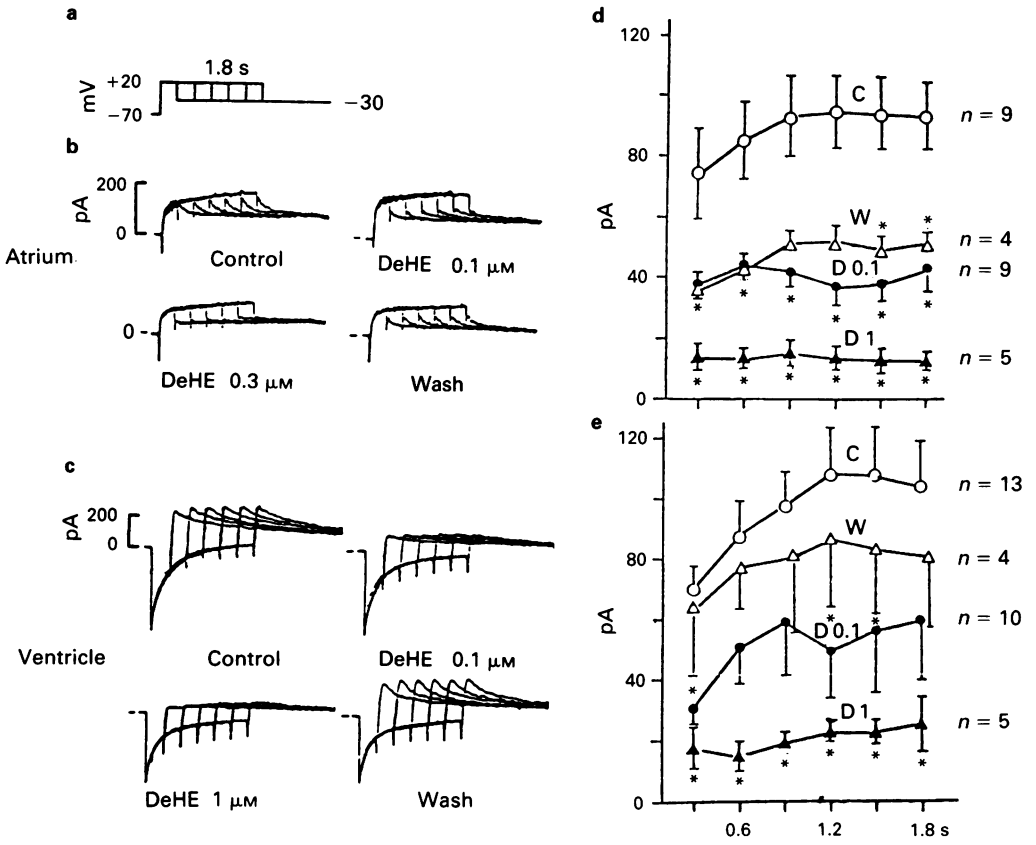

Figure 2 Depressant effect of dehydroevodiamine (DeHE) on delayed rectified (I_k) in guinea-pig atrial (b and d) and ventricular myocytes (c and e). The membrane was held at −70 mV and was conditioned to +20 mV for various durations (0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 s) and then repolarized to −30 mV as indicated in (a). Upper (b) panels show the current traces of a single atrial myocyte before (control) and during 8th min of DeHE (0.1 μM) superfusion. Lower (b) panels show the current traces during 0.3 μM DeHE exposure and after washout of DeHE for 20 min (Wash). Note changes in the amplitude of tail of I_k on repolarization after each conditioning step. In (c) traces obtained from a single ventricular myocyte before (Control) and during 5th min of DeHE (0.1 μM) superfusion are shown in upper panels. The lower panels show traces during 1 μM DeHE exposure and after washout in DeHE-free superfusate for 20 min (Wash). Panels (d) and (e) summarize the time-dependent depressant effect of DeHe on I_k in atrial and ventricular myocytes, respectively. *n* indicates number of cells. $^*P<0.05$, significantly different from control value (C) before drug exposure by one way ANOVA. D 0.1 and D 1, exposure to 0.1 and 1 μM DeHE, respectively, for 10 min. W, washout in DeHE-free superfusate for 20–30 min.

Effects on inward Ca current

The prolongation of APD_{50} induced by DeHE could also be due to an enhanced Ca influx, in addition to an inhibited outward K current. Therefore, the effect of DeHE on the L-type Ca current ($I_{Ca,L}$) was also determined in K- and Na-free Tris solution in the presence of CsCl. As shown in Figure 3, I_{Ca} were elicited in the ventricular myocyte by a series of depolarizations from a holding potential of −50 mV. The peak I_{Ca} was elicited by a clamp step to 0 mV and its amplitude was reduced by 13% and 24% after exposure to 1 and 3 μM DeHE, respectively, for 10 min. The depressant effect of DeHE on I_{Ca} was not reversible after 20 min washout. Lower concentrations of DeHE did not induce a consistent effect on I_{Ca}. Table 2 summarizes results obtained from 18 experiments with 0.1–1 μM DeHE.

Effect on Na-dependent inward current

Separation of the inward Na current (I_{Na}) and T-type Ca current ($I_{Ca,T}$) was obtained by the different current method of Tytgat *et al.* (1990b) in the presence and absence of 2 or 12 mM $[Na]_o$. As shown in Figure 4a (column 1), step depolarizations were applied from a holding potential of −90 or −50 mV and the different current obtained in the absence of Na_o (Figure 4b, column 1) is assumed to be $I_{Ca,T}$. The difference current in the presence of Na_o contains a Na

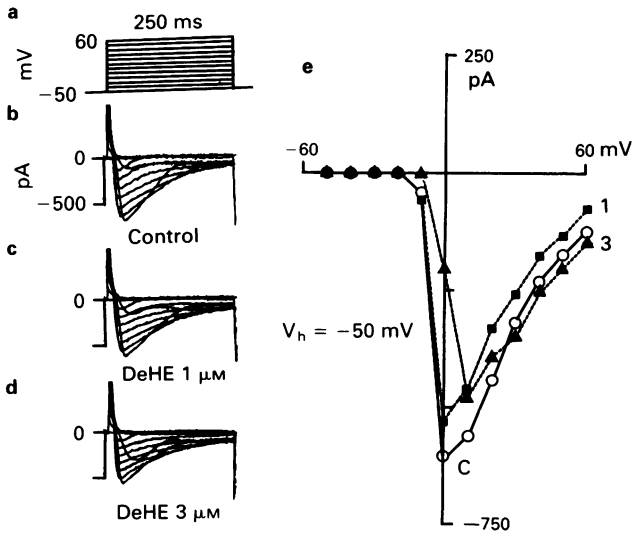

Figure 3 Ca currents recorded from a single ventricular cell in the absence (Control, b) and presence of 1 and 3 μM dehydroevodiamine (DeHE) (c and d, respectively). Currents were recorded in K- and Na-free external and internal solutions. Holding potential (V_h) was −50 mV and voltage step potentials were from −40 to +60 mV in 10 mV steps as indicated in (a). In (c) is shown the current-voltage relationship of peak inward Ca currents before (C, ○), during 1 μM (■) and 3 μM DeHE (▲) exposure for 10 min.

Table 2 Inhibition of L-type Ca current ($I_{Ca,L}$) induced by dehydroevodiamine (DeHE)

Condition	n	$I_{Ca,L}$ (change in %)
DeHE 0.1 μM	9	−8 ± 4
DeHE 0.3 μM	6	−18 ± 5
DeHE 1 μM	18	−19 ± 4*
Wash 10 min	13	−27 ± 7*
Wash 30 min	13	−32 ± 7*

The guinea-pig ventricular myocytes were superfused in Tris solution. All experiments were started after 15 min period of internal perfusion. Values are means ± s.e. of the percentage reduction of $I_{Ca,L}$ as compared with the control values before drug exposure. *$P<0.05$, significantly different from control by one way ANOVA. In the absence of DeHE, the decline of $I_{Ca,L}$ with time (run-down) was about 10% for 30–60 min.

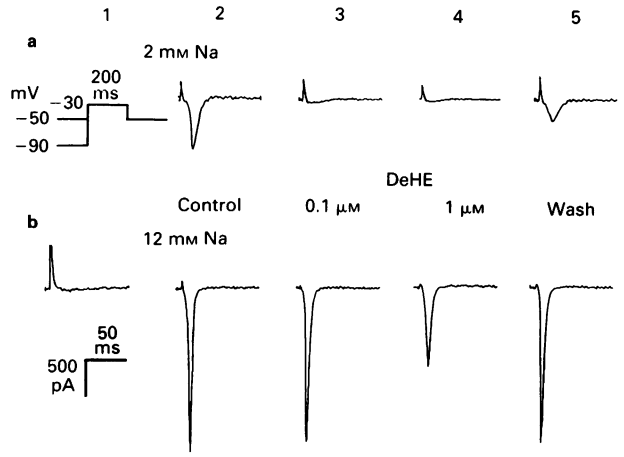

Figure 4 Depressant effect of dehydroevodiamine (DeHE) on Na-dependent inward current. Column 1 in (a) illustrates the experimental protocol for separation of T-type and L-type Ca current in Na-free, tris (hydroxymethyl)-aminomethane. Step depolarizations from a holding potential of either −90 or −50 mV to −30 mV were applied. The difference current in Na-free Tris (T-type Ca current) is displayed in column 1 (b). The difference current (fast inward Na current) in 2 mM Na Tris (a panels) or 12 mM Na Tris (b panels) before (Control, column 2), during 0.1 μM (column 3) and 1 μM DeHE exposure (column 4), and after washout (column 5) for 20 min (a) or 4 min (b) are illustrated. Traces in (a) and (b) were obtained from two different myocytes.

current in addition to $I_{Ca,T}$. Subtracting the difference current in the absence of Na from that in the presence of Na gives the Na-dependent component of the current (Figure 4, column 2). In the presence of 2 mM $[Na]_o$ (Figure 4a), 0.1 and 0.3 μM DeHE induced a strong depressant effect on I_{Na} to 10% and 8%, respectively, of control value. Also the I_{Na} remained depressed (36% of control value) after 20 min washout in DeHE-free superfusate. In the presence of 12 mM $[Na]_o$ (Figure 4b), however, 0.1 μM DeHE reduced only slightly the I_{Na} and 1 μM DeHE reduced it to 48% of the control value. The I_{Na} recovered to 90% of control value after 4 min washout. Table 3 summarizes results obtained from 5 experiments with 2 mM $[Na]_o$ and another 5 experiments with 12 mM $[Na]_o$.

Effects on afterpotentials

Since DeHE reduces both the I_{Na} and the I_{Ca}, it should be able to depress the delayed afterdepolarization (DAD) and the triggered activity (Cranefield, 1977; Kass *et al.*, 1978)

Table 3 Percentage inhibition of dehydroevodiamine (DeHE) on Na-dependent current (I_{Na})

Condition	*2 mM $[Na]_o$* I_{Na} (Δ%)	*12 mM $[Na]_o$* I_{Na} (Δ%)
DeHE 0.1 μM	−41 ± 12*	−10 ± 3
DeHE 0.3 μM	−54 ± 11*	−32 ± 8*
DeHE 1 μM	−68 ± 12*	−48 ± 16*
Wash	−41 ± 7*	−28 ± 10*

The guinea-pig ventricular myocytes were superfused in 2 or 12 mM $[Na]_o$ Tris solution. Values are means ± s.e. of the percentage inhibition of I_{Na} as compared with the control values before drug exposure. Number of preparations = 5. *$P<0.05$, significantly different from control by one way ANOVA.

developed in myocytes overloaded with calcium ion. Figure 5 illustrated the generation of DADs in a ventricular myocyte paced by a train of depolarizing stimuli at 0.67 Hz (left panels) or 1.25 Hz (right panels) in a low-K and high-Ca superfusate containing 2 μM strophanthidin. At a pacing frequency of 0.67 Hz, 0.1 μM DeHE suppressed completely the DADs within 10 min (Figure 5b, left panel), while APD was reduced presumably due to opening of a Na-activated K channel in the presence of Na pump blockade (Luk & Carmeliet, 1990) induced by strophanthidin. These effects remained after 22 min washout in DeHE-free superfusate. At the higher pacing frequency (1.25 Hz), the DADs were larger in amplitude and shorter in cycle length. The depressant effect of DeHE was weaker even at a concentration of 0.3 μM.

In 6 myocytes, the average resting potential was −111 ± 2 mV before pacing. At ending of pacing (0.67–1.25 Hz) for 10–30 beats, the MDP and the amplitude of DAD were −107 ± 2 mV and 13 ± 4 mV, respectively. DeHE (0.1 μM) barely changed the MDP (−106 ± 2 mV) but reduced the amplitude of DAD to 5 ± 1 mV. In another myocyte (Figure 6), two triggered action potentials were generated after termination of electrical drive (Figure 6a). Exposure to 0.1 μM DeHE reduced the number of triggered action potentials by half (Figure 6b). After washout in DeHE-free superfusate, the number of triggered action potentials increased to four as

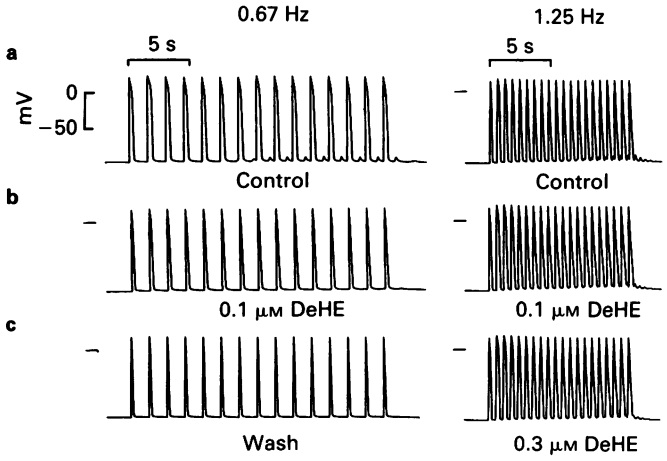

Figure 5 Depressant effect of dehydroevodiamine (DeHE) on delayed afterdepolarizations induced by a train of electrical stimulations with different pacing frequency (0.67 Hz in left panels and 1.25 Hz in right panels). The myocytes were superfused in low-K (1 mM) and high-Ca (9 mM) solution plus 2 μM strophanthidin. Traces of action potential in (a) and (b) were recorded before (Control) and during superfusion with 0.1 μM DeHE for 10 min, respectively. The left panel (c) was recorded after washout in DeHE-free superfusate for 22 min and the right panel (c) during 0.3 μM DeHE superfusion.

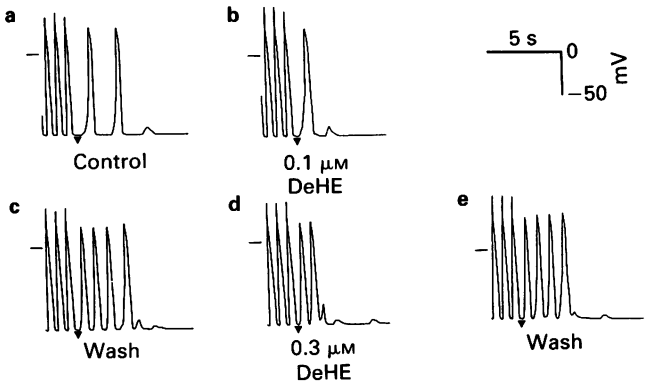

Figure 6 Depressant effect of dehydroevodiamine (DeHE) on triggered action potentials induced by superfusing the ventricular myocyte in low-K and high-Ca solution plus 2 μM strophanthidin. In each panel, a train of 30 depolarizing stimuli was applied but only the last 3 driven action potentials are illustrated. Electrical stimulation was terminated at (▼). Panels (a) and (b) show the traces before (Control) and during superfusion with 0.1 μM DeHE for 10 min. After washout in DeHE-free superfusate (c), the myocyte was exposed to 0.3 μM DeHE for 10 min (d). Panel (e) shows that, after washout of 0.3 μM DeHE for 5 min, the driven action potentials were followed by 4 triggered action potentials and 2 delayed afterdepolarizations.

the effects of strophanthidin progressed (Figure 6c). Exposure to 0.3 μM DeHE depressed the triggered activity again (Figure 6d).

In contrast to the depressant effect on DAD and triggered activity, DeHE should be able to potentiate the development of early afterdepolarization (EAD) through its inhibitory action on outward K current as quinidine did (Roden & Hoffman, 1985). Figure 7 shows a ventricular myocyte paced by a train of depolarizing stimuli at 2 Hz in the low-K and high-Ca superfusate. The repolarization process was inhibited and action potential duration was increased in the presence of 0.1 μM DeHE (Figure 7b). When the concentration of DeHE was increased to 0.3 μM, the myocyte failed to repolarize completely and, after termination of electrical drive, a series of slow response action potentials (EADs) were induced at a depolarized level (near −60 mV) (Figure 7c). The

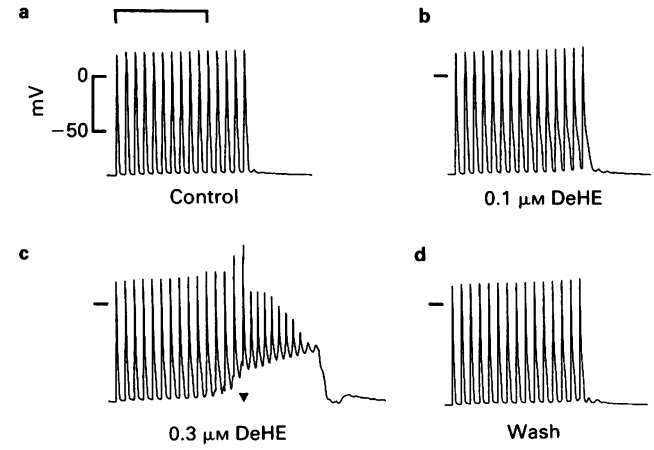

Figure 7 Inhibition of repolarization process and induction of early afterdepolarizations in ventricular myocyte superfused in low-K and high-Ca solution plus 2 μM strophanthidin. The myocyte was paced with a train of 15 depolarizing stimuli at 2 Hz. Panel (a) was recorded before (Control) and (b) during the 10th min of superfusion with to 0.1 μM dehydroevodiamine (DeHE). Panel (c) shows that, during the 6th min of superfusion with 0.3 μM DeHE, a series of slow responses and EADs were induced at a depolarized level after termination of electrical drive (at ▼). Panel (d) was recorded after washout in DeHE-free solution for 15 min.

effect was reversible after 15 min washout (Figure 7d). It is important to note that the same myocyte had been paced at 1 Hz and generated DADs of about 8 mV in amplitude. DeHE (0.1 μM) suppressed the DAD but did not reduce the MDP or induce EAD (traces not shown).

In 4 myocytes, the resting potential before pacing was −110 ± 2 mV and the MDP at end of pacing (1–2 Hz) for 20–30 beats was −106 ± 2 mV. In the presence of 0.1–1 μM DeHE, the resting potential before pacing was about the same (−112 ± 2 mV) but during pacing the MDP was reduced gradually to a level of −79 ± 4 mV at end of the 11–30th beat and was followed by EADs which lasted for 9 ± 4 s.

Effect on transient inward current

It has been demonstrated that generation of transient inward currents (I_{ti}) is responsible for the development of DAD and the subsequent triggered action potentials (Kass *et al.*, 1978). Thus effects of DeHE on I_{ti} were investigated in ventricular myocytes superfused with low-K and high-Ca solution containing 2 μM strophanthidin. As shown in Figure 8a, a 1200 ms depolarizing pulse from a V_h of −44 mV to +20 mV was applied every 6 s for 10 steps. Each depolarizing step induced a slow inward Ca current (I_{si}) and on repolarization to V_h a series of I_{ti}. Only traces obtained from the first and tenth step (arrow) were superimposed in Figure 8b. Figure 8c shows the time-dependent reduction in I_{ti} induced by 0.1 μM DeHE in all ten steps. It is evident that 0.1 μM DeHE reduced significantly the amplitude of I_{ti} after 2 min of DeHE exposure and more so after a longer period of exposure. On the other hand, I_{si} was only slightly inhibited after 10 min exposure. The inhibition on both currents was not reversible even after washout in DeHE-free superfusate for 30 min. The time course of changes in I_{si} and I_{ti} induced by 0.1 μM DeHE in this preparation are illustrated in Figure 8d. Table 4 summarizes results obtained from 8 experiments.

Discussion and conclusions

The present findings in guinea-pig myocytes provide evidence for the depressant actions of DeHE on the Na-dependent inward current (I_{Na}) and the transient inward current (I_{ti}) generated under conditions of Ca overloading induced by a cardiotonic steroid in low-K and high-Ca superfusate. The changes in ionic currents are correlated with depression of arrhythmogenic delayed afterdepolarization (DAD) and triggered action potential. Thus in addition to the hypotensive effect reported in the literature (Yang *et al.*, 1988; 1990), DeHE may exert an antiarrhythmic action on triggered activity.

It has been well documented that DADs, aftercontractions and triggered action potentials (Cranefield, 1977) could be induced by cardiotonic agents such as digitalis (Vassalle, 1986), catecholamines and methylxanthines (Lin *et al.*, 1985; Hou *et al.*, 1989; Satoh & Vassalle, 1989), especially in the presence of high-Ca and/or low-K (Lin & Vassalle, 1980). These electromechanical activities were indications of intracellular Ca overload and could be depressed by either Ca or Na channel blockers. The I_{ti} (or I_{os}) is the underlying ionic mechanism responsible for the DAD and the subsequent triggered action potentials (Kass *et al.*, 1978; Lin *et al.*, 1986). The fact that 0.1–0.3 μM DeHE induced significant inhibitory actions on I_{ti} but not on I_{Ca} suggests that DeHE has a selective effect on transport of Na across sarcolemma. This concept is supported by our findings that 0.1–0.3 μM DeHE reduced markedly the I_{Na} measured by using the difference current method (Tytgat *et al.*, 1990b) in the absence and presence of 2 mM or 12 mM $[Na]_o$. Also the depressant effect of DeHE was greater and lasted longer in the presence of 2 mM $[Na]_o$ than in 12 mM $[Na]_o$ (Figure 6 and Table 3), in agreement with the antagonistic action of DeHE on Na transport. A decrease in Na influx should

result in a decrease in intracellular Na ion activity (Vassalle & Lee, 1984). The fall in intracellular Na would in turn decrease cellular Ca through the Na-Ca exchange mechanism (Mullins, 1979; Kimura *et al.*, 1987; Beuckelmann *et al.*, 1989) and, as a consequence, suppress the I_{ti} and DAD induced by the cardiotonic steroid (Kass *et al.*, 1978). How-

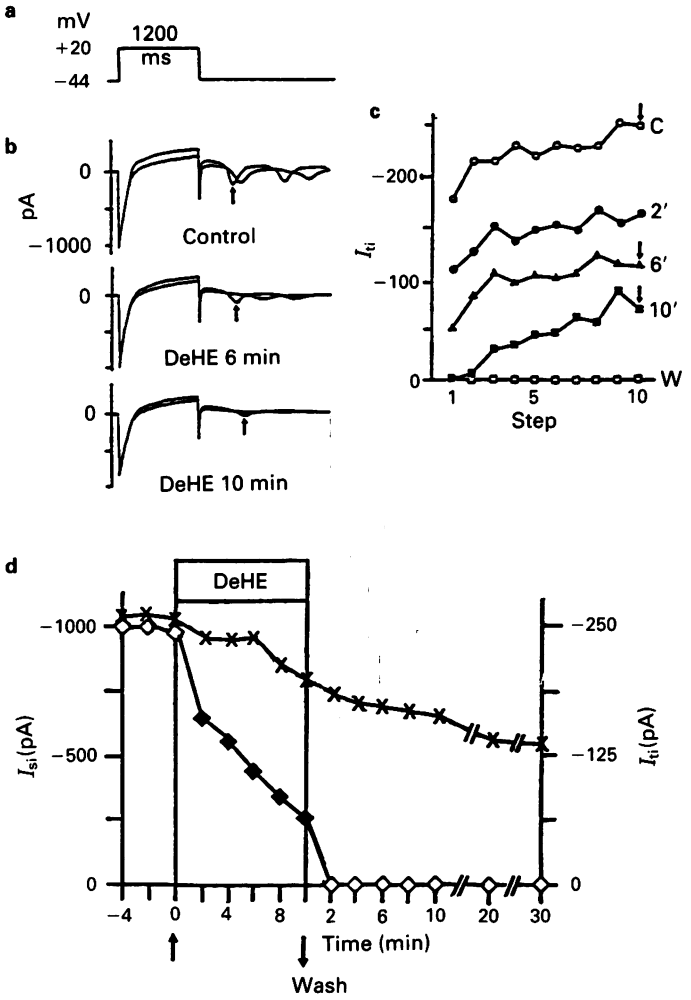

Figure 8 Depressant effect of dehydroevodiamine (DeHE, 0.1 μM) on transient inward current (I_{ti}) of a single ventricular myocyte induced by 2 μM strophanthidin in low-K and high-Ca superfusate. Panel (a) shows the experimental protocol. The holding potential was −44 mV. A 1200 ms depolarizing pulse to +20 mV was applied every 6 s for 10 steps. In (b), I_{ti} induced by the first and the tenth step (arrow) were superimposed before (Control) and during exposure to DeHE for 6 min (middle panel b) and 10 min (lower panel b). Panel (c) shows the amplitudes of the first I_{ti} on repolarization from first to the tenth steps. Curve C (open (○) circles) indicates the values before DeHE exposure; (●) (2'), (▲) (6') and (■) (10') indicate time-dependent reduction in I_{ti} during DeHE superfusion. W indicates that the value of I_{ti} decreased to baseline after washout of DeHE for 2 min. Panel (d) shows time course of changes induced by 0.1 μM DeHE in slow inward Ca current (I_{si}) on depolarization and transient inward current (I_{ti}) on repolarization. Left ordinate scale gives amplitudes of I_{si} (X) and right ordinate values of I_{ti} (◆) in pA. DeHE was added between the two vertical lines. Abscissa scale gives time in min. Upward arrow (time 0) indicates beginning of DeHE exposure while downward arrow shows washout of DeHE.

Table 4 Percentage inhibition of slow inward Ca current (I_{si}) and transient inward current (I_{ti}) induced by dehydroevodiamine (DeHE)

		Step 1		*Step 5*		*Step 10*	
Condition	n	I_{si} (Δ%)	I_{ti} (Δ%)	I_{si} (Δ%)	I_{ti} (Δ%)	I_{si} (Δ%)	I_{ti} (Δ%)
DeHE							
2 min	8	−6 ± 2	−29 ± 13*	−12 ± 7	−24 ± 5*	−7 ± 1	−33 ± 7*
5 min	8	−14 ± 5	−56 ± 10*	−10 ± 4	−57 ± 7*	−12 ± 3*	−55 ± 8*
10 min	8	−27 ± 9*	−78 ± 10*	−15 ± 5*	−77 ± 7*	−17 ± 4*	−78 ± 7*
Wash							
10 min	6	−37 ± 11*	−86 ± 13*	−25 ± 8*	−86 ± 6*	−24 ± 8*	−71 ± 10*
20 min	4	−34 ± 9*	−100 ± 0*	−33 ± 11*	−100 ± 0*	−39 ± 9*	−87 ± 11*
30 min	4	−41 ± 8*	−100 ± 0*	−41 ± 10*	−100 ± 0*	−41 ± 8*	−100 ± 0*

The ventricular myocytes were superfused in high-Ca and low-K solution containing 2 μM strophanthidin. Values are means ± s.e. of the percentage reduction in I_{si} or I_{ti} as compared with control values before DeHE (0.1 μM) exposure. *$P<0.05$, significantly different from control by one way ANOVA.

ever, as indicated by the findings of our previous experiments on rabbit sinoatrial tissues and high-K depolarized guinea-pig ventricular tissues (Lin *et al.*, 1990), the inhibitory action on transmembrane Ca current is also important for the depressant effect of DeHE. In human multicellular atrial preparations, DeHE (0.1–0.3 μM) also depressed the Ca-dependent automatic rhythm and the slow response action potential generated at a depolarized level (MDP near −40 mV). Thus a reduction of Ca influx may be involved in the depression of DAD and aftercontraction induced by 2.5 μM adrenaline in human atrial tissues (unpublished observations). Further experiments using human atrial myocytes are required to clarify this point.

In multicellular guinea-pig and rabbit ventricular muscle fibres (Lin *et al.*, 1990), DeHE at low concentrations (0.1–0.3 μM) has been shown to increase the APD. The prolongation of APD could be due to either an enhanced Ca influx or an inhibited K conductance. The marked depressant effect of I_k and the lack of enhancement of I_{Ca} observed in the present experiments suggest that the depressed K conductance was responsible for the increase in APD. An increase in APD and refractory period could exert a protective effect against cardiac tachyarrhythmias as class III antiarrhythmic drugs did (Singh & Nademanee, 1985). On the other hand, DeHE at concentrations of 1 μM and above consistently induced arrhythmogenic EAD in rabbit ventricular tissues as reported in our previous study (Lin *et al.*, 1990). In the guinea-pig myocytes, 0.1–1 μM DeHE also could induce EAD in the presence of low-K. This effect was correlated to an inhibition of I_k and similar to that induced by quinidine in canine Purkinje fibres (Roden & Hoffman, 1985).

In conclusion, DeHE at low concentrations (0.1–0.3 μM) inhibited markedly the I_{Na} and depressed the arrhythmogenic DADs and I_{ti} induced by cardiotonic steroid in low-K and high-Ca superfusate. DeHE also may exert class III antiarrhythmic effect through a reduction of the time-dependent outward K current (Figure 2) and the subsequent prolongation of APD (Figure 1) and effective refractory period.

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