The Role of Transmembrane Chloride Current in Afterdepolarisations in Canine Ventricular Cardiomyocytes

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Abstract. The physiological role of chloride currents (I_{Cl}) in cardiac cells is poorly understood. The aim of the present study was to reveal the role of I_{Cl} in the genesis of early and delayed afterdepolarisations (EADs and DADs, respectively). First we identified I_{Cl} under action potential voltage clamp conditions as the anthracene-9-carboxylic acid (ANTRA) (0.5 mmol/l)-sensitive current. The ANTRA-sensitive current was large and outwardly directed at the beginning, while it was moderate and inwardly directed at the end of the action potential. Application of ANTRA under current clamp conditions decreased the depth of the incisura, shifted the plateau upwards and lengthened the duration of action potentials.

The effect of ANTRA was studied in three models of afterdepolarisations: the ouabain-induced DAD model, the caesium-induced EAD model, and in the presence of subthreshold concentration of isoproterenol. Preincubation of the cells with 0.5 mmol/l ANTRA failed to induce afterdepolarisations. Ouabain (200 nmol/l) alone caused DADs in 62.5 % of the cells within 15 min. When ouabain was applied in the presence of ANTRA, 60 % of the myocytes transiently displayed EADs before the development of DADs, and all cells developed DADs within 7 min. Isoproterenol (5 nmol/l) alone failed to induce afterdepolarisations. However, 75 % of the cells produced DADs within 6 min when superfused with isoproterenol in the presence of ANTRA. Incubation of the myocytes with 3.6 mmol/l CsCl caused EADs in 71.4 % of the cells within 30 min. Application of CsCl in the presence of ANTRA resulted in immediate depolarisation of the membrane from -79.6 ± 0.4 to -54.2 ± 3.5 mV. Summarizing our results we conclude that the ANTRA-sensitive current is an important mechanism of defence against afterdepolarisations. Suppression of I_{Cl} may thus increase the incidence and accelerate the rate of development of both EADs and DADs.

Key words: Cardiomyocytes — Chloride currents — Afterdepolarisations — Isoproterenol — Ouabain

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Introduction

Afterdepolarisations, defined as subthreshold depolarising afterpotentials of the cell membrane following an action potential (AP), have been proposed as the mechanism responsible for genesis of cardiac arrhythmias (Priori and Corr 1990; Fozzard 1992; Szabo et al. 1994). Afterdepolarisations are divided into two types according to their relationship to the preceding AP. Early afterdepolarisations (EADs) develop before the completion of repolarisation, whereas delayed afterdepolarisations (DADs) arise from the resting potential level of the fully repolarised cell. Since generation of both EADs and DADs requires a preceding AP, afterdepolarisations are frequently referred to as triggered activities (Fozzard 1992). Both types of afterdepolarisations were studied extensively during the last two decades; however, the underlying ionic mechanisms are still not fully elucidated. DADs are seen at various conditions in both atrial and ventricular cells exposed to catecholamines, digitalis or low extracellular sodium (Wit and Rosen 1992). It is generally believed that the prerequisite for DAD generation is an increased calcium load to the cell, and consequently to the sarcoplasmic reticulum (SR), resulting in spontaneous oscillatory release of calcium from the overloaded SR. Transient elevation of the myoplasmic calcium is responsible for activation of a current, called transient inward current (I_{TI}) which, in turn, leads to membrane depolarisation. I_{TI} was characterised first as a non-selective cation current (Kass et al. 1978; Cannell and Lederer 1986; Ehara et al. 1988; January and Fozzard 1988; Han and Ferrier 1992). Later, the involvement of the electrogenic Na^+/Ca^{2+} exchange current was suggested by several investigators (Kimura et al. 1986; Mechmann and Pott 1986; Callewaert et al. 1989; Šimurda et al. 1992; Janvier and Boyett 1996). Recently, the calcium-activated chloride current was proposed as the charge carrier for I_{TI} in rabbit (Szigeti et al. 1998) and in dog (Zygmunt et al. 1998) myocardium.

EADs are typically observed when low pacing rates are associated with prolonged APs usually due to suppression of delayed rectifier potassium currents I_{Kr} and I_{Ks}, or alternatively, due to increased density of I_{Ca} or I_{Na} during the plateau of the AP (January et al. 1988; January and Riddle 1990). The assumed mechanism for generation of EADs is as follows. The initial depolarisation activates I_{Ca}, which rapidly inactivates during the early plateau. The sustained plateau of a lengthened AP or augmentation of I_{Ca} and I_{Na} might establish conditions necessary for reactivation of I_{Ca}. Reactivation of I_{Ca} is supposed to be responsible for interruption of the normal repolarisation processes and generation of EADs (Fozzard 1992; Szabo et al. 1994). Since I_{Cl} has been shown to contribute to repolarisation in canine ventricular myocytes (Zygmunt 1994; Collier et al. 1996), one should attribute a protective role to this current against EADs.

Beta-adrenergic activation or isoproterenol was shown to activate several ionic currents, such as I_{Ca}, I_{Cl}, and I_{K} in mammalian ventricular cells (Wang et al. 1997; Zygmunt et al. 1998; Wallis et al. 2001; Lei at al. 2002; Sah et al. 2002) in addition to increasing the calcium load of the SR (Priori and Corr 1990; Zeng and Rudy 1995). Therefore, submicromolar concentrations of isoproterenol are widely

applied to induce both EADs and DADs (Priori and Corr 1990; Szabo et al. 1994; Zeng and Rudy 1995), however, nanomolar concentrations of isoproterenol fail to initiate afterdepolarisations. In spite of their apparent inefficacy, these very low concentrations were found to facilitate the effects of other agents suitable to evoke EADs or DADs (unpublished observation).

The present work was designed to study the role of the anthracene-9-carboxylic acid (ANTRA)-sensitive component of I_{Cl} in the generation of different types of afterdepolarisations. APs and ANTRA-sensitive currents were recorded in cardiac myocytes enzymatically dissociated from canine left ventricle. Afterdepolarisations were induced by isoproterenol, ouabain and caesium. We found that suppression of I_{Cl} by ANTRA may increase the incidence and accelerate the rate of development of both EADs and DADs.

Materials and Methods

Isolation of single canine ventricular myocytes

Adult mongrel dogs of either sex were anaesthetised with intravenous injections of 10 mg/kg ketamine hydrochloride (Calypsolvet) plus 1 mg/kg xylazine hydrochloride (Rometar). The hearts were quickly removed in deep anaesthesia and placed in Tyrode solution. The entire investigation conformed to the guidelines for the care and use of laboratory animals published by the U.S. National Institutes of Health, as well as to the principles outlined in the Declaration of Helsinki, and was approved by the local ethical committee.

Single ventricular myocytes were obtained by enzymatic dispersion using the segment perfusion technique (Bányász et al. 2001). Briefly, a wedge-shaped section of the ventricular wall supplied by the left anterior descending coronary artery was dissected, cannulated and perfused with oxygenated Tyrode solution containing (in mmol/l): NaCl 144, KCl 5.6, $CaCl_2$ 2.5, $MgCl_2$ 1.2, HEPES 5, and glucose 11 at pH = 7.4. Perfusion was maintained until the removal of blood from the coronary system and then switched to a nominally Ca^{2+}-free Joklik solution (Minimum Essential Medium Eagle, Joklik Modification, Sigma Chemicals, St. Louis, MO, USA) for 5 min. This was followed by 30 min perfusion with re-circulated Joklik solution supplemented with 1 mg/ml collagenase (Type II, Worthington Chemical Co. Lakewood, NJ, USA) and 0.2% bovine serum albumin (Fraction V, Sigma Chemicals, St. Louis, MO, USA) containing 50 μmol/l Ca^{2+}. Portions of the left ventricular wall were cut into small pieces and the cell suspension obtained at the end of the procedure was washed with Joklik solution and the Ca^{2+} concentration was gradually increased to 2.5 mmol/l. The cells were stored in Minimum Essential Medium Eagle supplemented with (in mmol/l): taurine 20, pyruvic acid 2, ribose 5, allopurinol 0.1, $NaHCO_3$ 26, and NaH_2PO_4 1.5 at 14℃ until use (for periods not longer than 24 hours).

Recording of APs and afterdepolarisations

APs were recorded from Ca^{2+}-tolerant myocytes superfused with modified Krebs solution containing (in mmol/l): NaCl 120, KCl 5.6, $CaCl_2$ 2.5, $MgCl_2$ 1.1, NaH_2PO_4 1.1, $NaHCO_3$ 24 and glucose 11. The solution was equilibrated with carbogen (5 % CO_2 + 95 % O_2) at a temperature of 37 ℃ and the pH was adjusted to 7.4. Transmembrane potentials were recorded using glass microelectrodes filled with 3 mol/l KCl and having tip resistance between 20 and 40 MΩ. These electrodes were connected to the input of an Axoclamp-2B amplifier (Axon Instruments, Union City, CA, USA). The cells were continuously paced through the recording electrode at a steady cycle length of 1000 ms using 1 ms wide rectangular current pulses with 120 % threshold amplitude. APs were digitised at 100 kHz using Digidata 1200 A/D card (Axon Instruments, Union City, CA, USA) and stored for later analysis.

The effect of a 20 min superfusion with 0.5 mmol/l ANTRA on AP configuration was studied in 8 myocytes. The effect of ANTRA pretreatment (0.5 mmol/l) on the incidence of afterdepolarisations was investigated as follows. In the control experiments the effects of isoproterenol (5 nmol/l, $n = 6$), ouabain (200 nmol/l, $n = 8$) or CsCl (3.6 mmol/l with a concomitant reduction of KCl to 2 mmol/l, $n = 7$) alone was tested on myocytes for 20–40 min. These protocols were repeated in another sets of myocytes in the presence of ANTRA ($n = 8$, $n = 5$ and $n = 5$, respectively). The application of ANTRA started 10 min before challenging the cells with the above agents. APs were recorded continuously, the first appearance of the EADs or DADs was monitored, and the fraction of cells displaying EADs or DADs was plotted as a function of time generating thus a probability function for the incidence of EAD or DAD (pEAD or pDAD, respectively). Finally, these curves were fitted to a two-state Boltzmann function.

Recording of ANTRA-sensitive current using AP voltage clamp

The whole-cell configuration of the ruptured patch clamp technique was used (Hamill et al. 1981). The cells were superfused with oxygenated Tyrode solution. The patch electrodes were prepared from borosilicate glass, having tip resistance of 2 MΩ when filled with the pipette solution, containing (in mmol/l): K-aspartate 100, KCl 45, $MgCl_2$ 1, EGTA 10, K-ATP 3, and HEPES 5 at pH = 7.4. Careful suction was applied to help gigaseal formation and the subsequent disruption of the membrane patch. Axoclamp-2B amplifier was used in current clamp or continuous single electrode voltage clamp mode. The output filter was set to 10 kHz. Digidata 1200 A/D-D/A converter operated under a pClamp 6.0 software (Axon Instruments, Union City, CA, USA) was used to collect data and to deliver command pulses. Ionic currents were normalised to cell capacitance (142 ± 5.4 pF on average).

In these AP voltage clamp studies, the AP waveform was first recorded from 5 cells, undergoing steady-state stimulation at a cycle length of 1 s in the current clamp mode, and stored on the hard disk. This record was transformed to a com-

mand file using home-made software, then delivered as the command voltage in the voltage clamp mode. In this case the current trace was a horizontal line at the zero level (Doerr et al. 1990). Application of 0.5 mmol/l ANTRA for 2 min dissected the ANTRA-sensitive current (presumably I_{Cl}) with an inverse polarity. In our graph this ANTRA-sensitive current was inverted so as to appear as the current flows normally, i.e., first as an outwardly, then as an inwardly directed current.

Statistics

Results are expressed as mean ± S.E.M. values. The statistical significance of differences was evaluated using one-way ANOVA followed by Student's *t*-test when more than two groups were analysed. In the case of two groups, Student's *t*-test for paired or unpaired data was applied. Differences were considered significant when p was less than 0.05.

Results

Effect of ANTRA on the AP configuration and transmembrane ion current

Superfusion of the myocytes with ANTRA (0.5 mmol/l) caused characteristic alterations in the AP configuration in Krebs solution: it decreased the depth of the incisura, shifted the plateau towards more positive potentials, and lengthened AP duration (APD) (Fig. 1A). This latter effect varied in magnitude when measured at various levels of repolarisation, i.e. APD_{20} was increased from 56 ± 11 to 87 ± 20 ms (31 ± 9 ms increase, $55 \pm 9\%$), APD_{50} from 152 ± 13 to 212 ± 16 ms (60 ± 12 ms increase, $40 \pm 8\%$), and APD_{90} from 231 ± 16 to 308 ± 25 ms (77 ± 13 ms increase, $33 \pm 5\%$). All these changes were statistically significant ($p < 0.05$) in the 8 myocytes studied. Although the absolute magnitude of the lengthening increased from APD_{20} to APD_{90}, the relative change was most pronounced at the level of APD_{20}, suggesting that the major effect of ANTRA developed during the early plateau. No significant effect of ANTRA on the resting potential, AP amplitude, or maximum rate of depolarisation was observed during the 20 min of exposure. Since the effects of ANTRA on AP morphology reached their steady-state levels by the 10^{th} min of superfusion (Fig. 1B), this period of pretreatment was applied later in the experiments studying effects of ANTRA on afterdepolarisations.

A representative record of the ANTRA-sensitive current obtained under AP voltage clamp conditions displays a transient positive peak of outward current during the early plateau followed by a smaller negative peak of inward current coinciding with the terminal repolarisation (Fig. 1C). The peak amplitude of the outward current was significantly greater than that of the inward current (1.15 ± 0.39 pA/pF *versus* 0.53 ± 0.29 pA/pF). This difference was even larger when the outward and inward change transfers were compared (23.2 ± 9.5 fC/pF *versus* 9.5 ± 5.8 fC/pF, $p < 0.05$, $n = 5$, Fig. 1D). These data are congruent with the results obtained on APs, as suppression of a predominantly outward current is expected to increase AP duration.

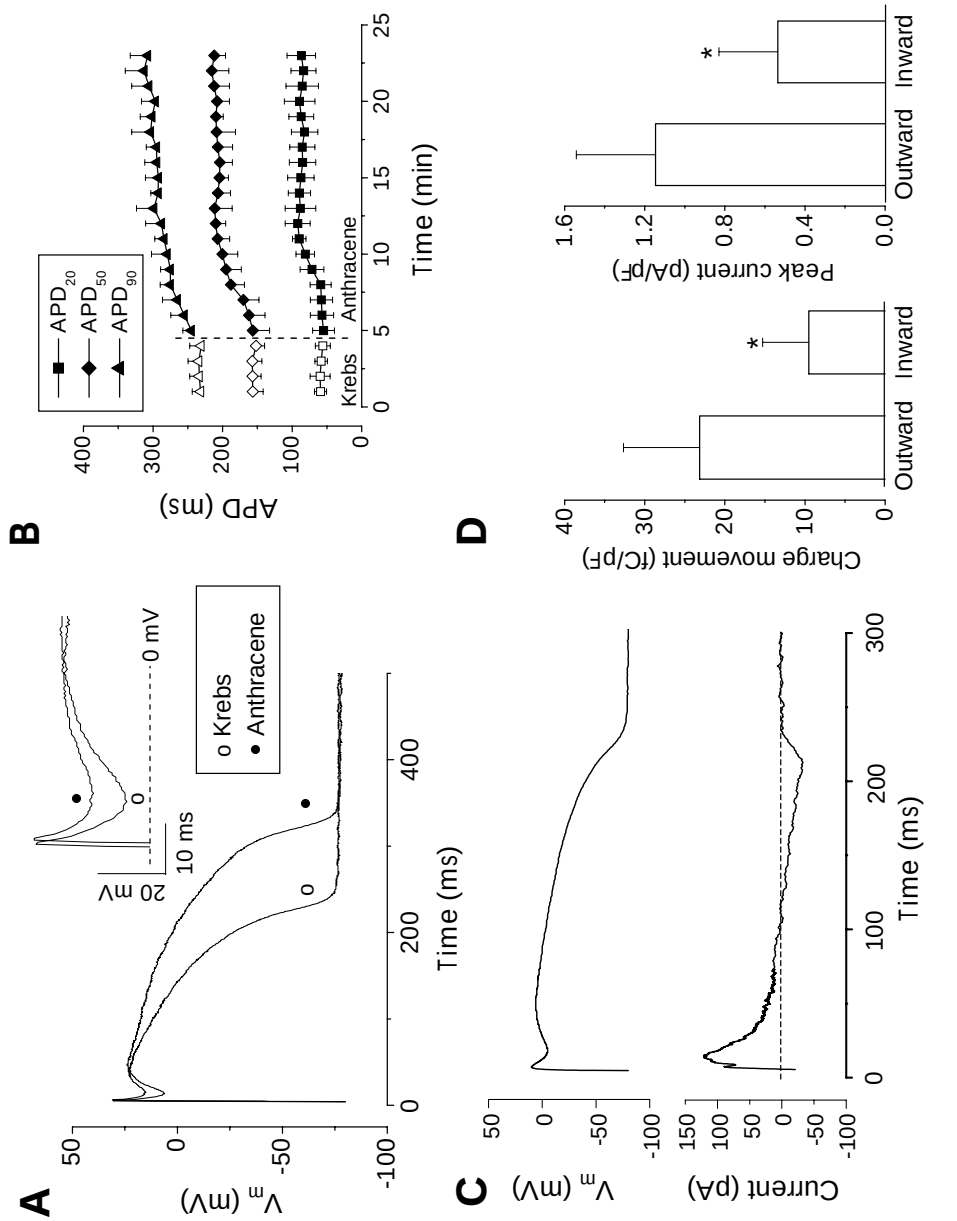

Figure 1. Effect of 20 min superfusion with 0.5 mmol/l ANTRA on AP morphology (**A**), and APD (**B**) measured at 20%, 50% and 90% levels of repolarization (APD_{20}, APD_{50} and APD_{90}, respectively, $n = 8$) in canine ventricular myocytes paced at 1 Hz. Events of the early plateau are enlarged in the inset. **C**. ANTRA-sensitive current (I_{Cl}) recorded under action potential voltage clamp conditions, and displayed on the time scale of the action potential. Dashed line indicates zero current. **D**. Comparison of peak current amplitudes and the net charge carried by I_{Cl} into inward and outward directions during an action potential ($n = 5$). Symbols and bars represent mean ± S.E.M. values, asterisks denote the level of significance ($p < 0.05$).

Effect of ANTRA pretreatment on the incidence of afterdepolarisations

The effect of ANTRA was studied in 3 models of afterdepolarisations: the ouabain-induced DAD model, the caesium-induced EAD model, and in the presence of a subthreshold concentration of isoproterenol. Preincubation of the cells with 0.5 mmol/l ANTRA failed to induce either type of afterdepolarisations during the 20 min of exposure ($n = 8$).

Application of ouabain (200 nmol/l) alone resulted in development of DADs in 5 of the 8 cells studied (62.5%) within 15 min (Fig. 2A). Action potential duration was determined from records taken before superfusion with ouabain and also in the presence of ouabain in each cell just prior to development of DADs. Ouabain increased APD_{90} from 240 ± 25 to 264 ± 19 ms (lengthening of 24 ± 7 ms, $p < 0.05$, $n = 8$). When ouabain was applied in the presence of ANTRA (ANTRA pretreatment started 10 min before addition of ouabain), 3 of the 5 myocytes (60%) displayed transiently EADs at the 2^{nd} min of the ouabain treatment before the development of DADs (Fig. 2B). However, the incidence of EADs fell back to zero by the 5^{th} min. Independent of the transient appearance or absence of EADs, all the 5 cells developed DADs within 7 min in the presence of ANTRA + ouabain. The ouabain-induced prolongation of APD_{90} (determined just before the first occurrence of the afterdepolarisation) was 20 ± 2 ms in the 2 cells failing to display EADs, a value comparable to that obtained without ANTRA. In contrast, a much greater prolongation of APD_{90} (94 ± 13 ms) was obtained in the 3 cells developing first EADs and later DADs. The timing of the incidence of afterdepolarisations is summarised in Fig. 2C. The transient appearance of EADs, obtained with the conventional DAD-inducer ouabain in the presence of ANTRA, suggests that the generation of EADs and DADs may share common mechanisms. Actually, we have never observed both types of these afterdepolarisations simultaneously.

Application of CsCl (as equimolar replacement of KCl) is often used to study EADs. Caesium is known to suppress I_K currents, which results in extreme lengthening of the AP (January and Riddle 1990). Indeed, superfusion of myocytes with 3.6 mmol/l CsCl alone increased APD_{90} significantly from 246 ± 11 to 389 ± 24 ms in our experiments ($p > 0.05$, $n = 7$), and evoked EADs in 5 of the 7 cells (71.4%) within 30 min, whereas application of CsCl in the presence of ANTRA resulted in immediate depolarisation of the membrane (within less than 2 min) from -79.6 ± 0.4 to -54.2 ± 3.5 mV ($p < 0.05$, $n = 5$). Restoration of the normal resting potential or development of afterdepolarisations was never observed from this strongly depolarised membrane potential level (Fig. 3).

A subthreshold concentration of isoproterenol (5 nmol/l) – when applied alone – shortened APD_{90} significantly (from 230 ± 6 to 209 ± 6 ms, 21 ± 4 ms of shortening, $p < 0.05$, $n = 6$) and shifted the plateau potential markedly towards more positive voltages. Afterdepolarisations were not observed at this very low concentration. Application of isoproterenol following pretreatment with ANTRA failed to shorten APD_{90} any more (237 ± 10 *versus* 236 ± 13 ms, not significant, $n = 8$), but the elevation of plateau was still evident, and 6 of the 8 cells (75%) developed DADs

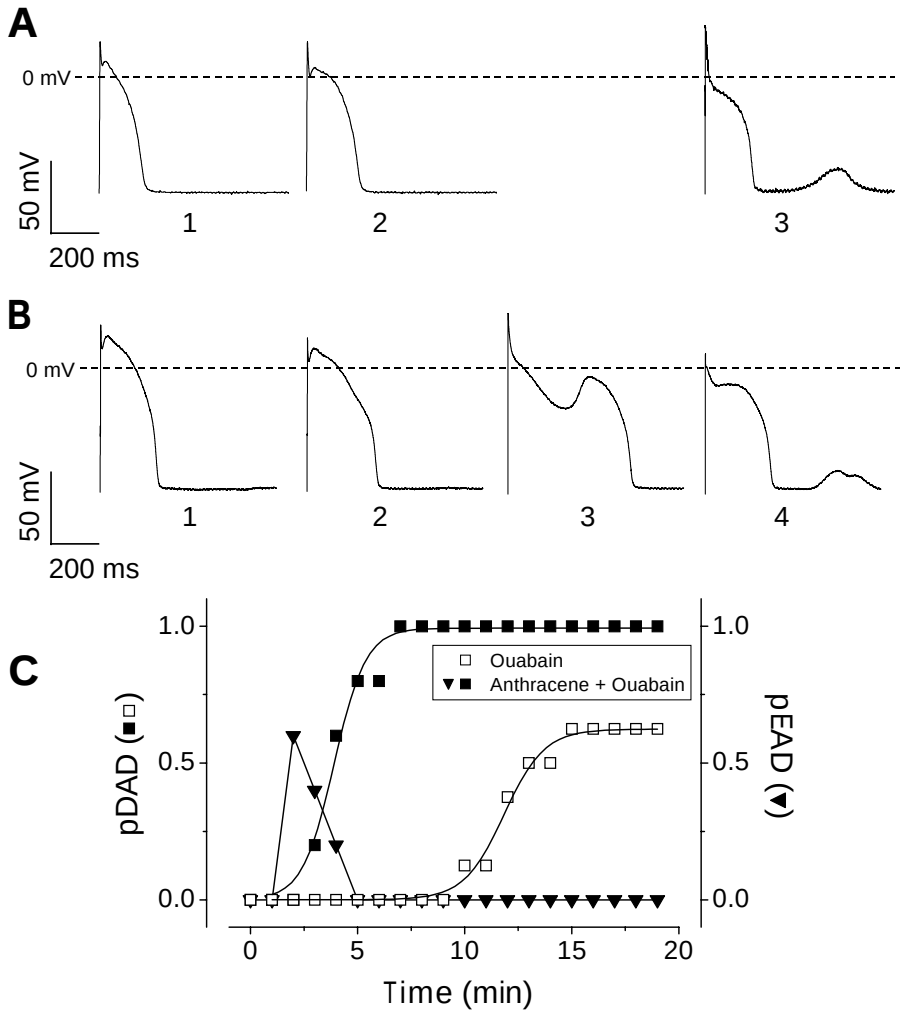

Figure 2. Effect of ANTRA pretreatment on the incidence of ouabain-induced afterdepolarisations. **A**. Effect of 200 nmol/l ouabain alone. Control record in Krebs solution (1) followed by APs obtained before (2) and after (3) development of DAD in the presence of ouabain. **B**. Effect of ouabain in the presence of ANTRA. Control record in ANTRA (1) followed by an AP taken before development of ouabain-induced afterdepolarisations in the presence of ANTRA (2). Representative EAD and DAD, induced by ouabain in the presence of ANTRA, are displayed in records (3) and (4), respectively. **C**. Probability of incidence of afterdepolarisations (pDAD and pEAD) induced by ouabain in the absence (open symbols, $n = 8$) and presence of ANTRA (filled symbols, $n = 5$). ANTRA was applied 10 min before addition of ouabain. pDAD data were fitted to a two-state Boltzmann function yielding 50 % probabilities for DAD at 11.8 and 3.9 min, respectively, in the absence and presence of ANTRA.

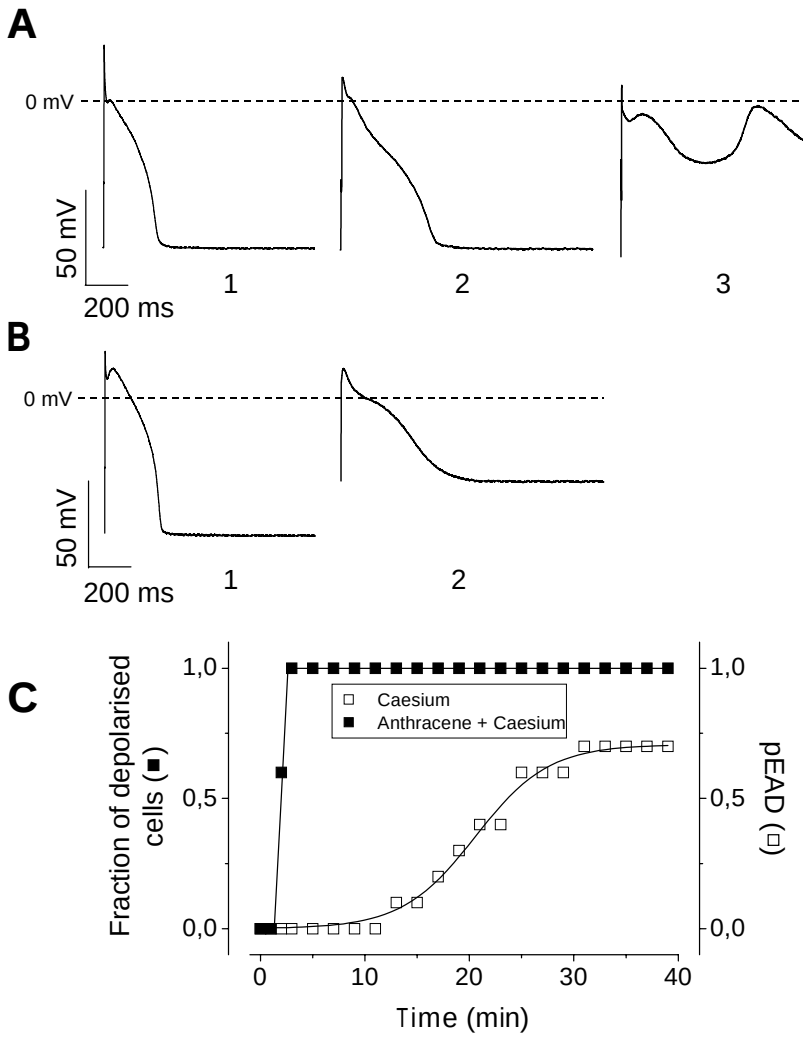

Figure 3. Effect of ANTRA pretreatment on the incidence of caesium-induced EADs. **A**. Effect of 3.6 mmol/l caesium alone. Control record in Krebs (1) followed by APs obtained before (2) and after (3) development of EAD in the presence of caesium. **B**. Effect of caesium in the presence of ANTRA. Control record in ANTRA (1) followed by an abortive slow-response type AP (2) arising from depolarised membrane potential at the 2nd min of caesium superfusion. **C**. Probability of incidence of EADs (caesium alone, open symbols, $n = 7$) and depolarisation (caesium + ANTRA, filled symbols, $n = 5$). ANTRA was applied 10 min before the addition of caesium. The 50% probabilities were obtained at 20.6 and 2 min, respectively, in the absence and presence of ANTRA.

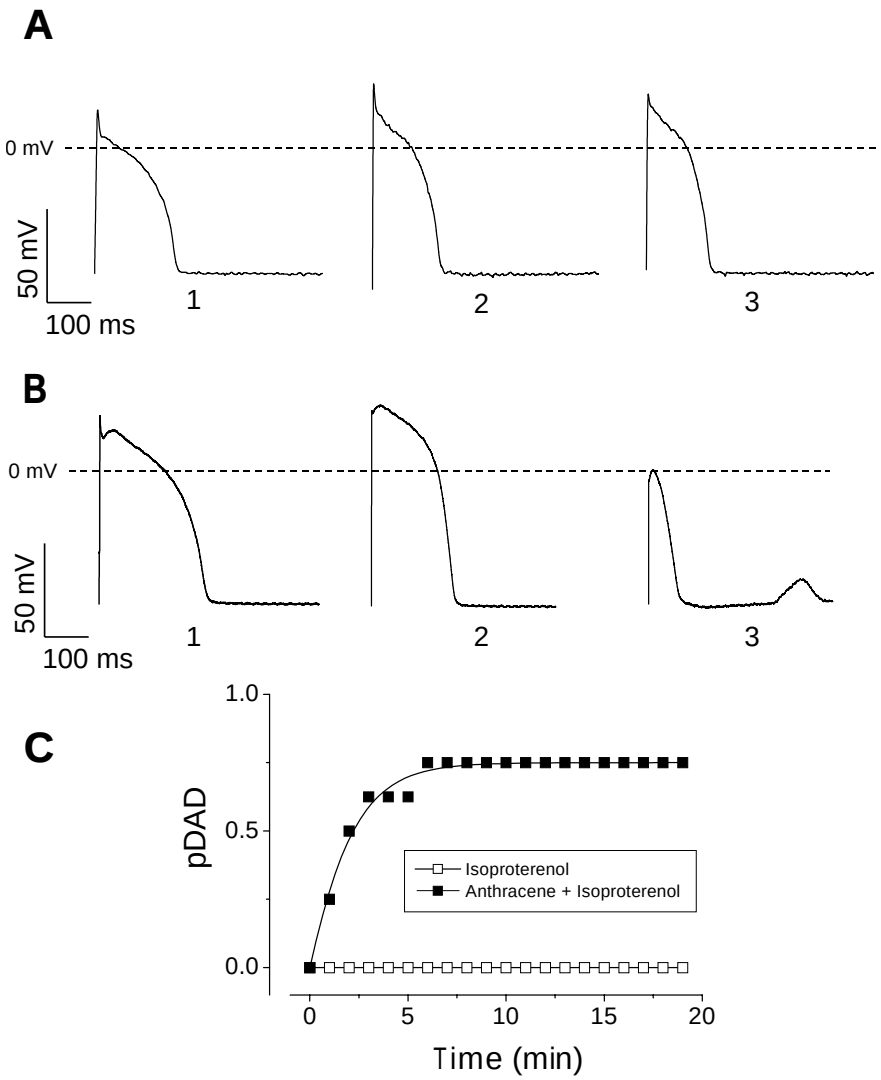

Figure 4. The enhancing effect of ANTRA pretreatment on the incidence of DADs induced by isoproterenol. **A**. The absence of afterdepolarisations in the presence of 5 nmol/l isoproterenol alone. Control record in Krebs (1) followed by APs obtained after superfusion with isoproterenol (records 2 and 3). **B**. Effect of isoproterenol in the presence of ANTRA. Control record in ANTRA (1) followed by APs taken before (2) and after (3) development of isoproterenol-induced DAD. **C**. Probability of incidence of DAD induced by isoproterenol in the absence (open symbols, $n = 6$) and presence of ANTRA (filled symbols, $n = 8$). ANTRA was applied 10 min before the addition of isoproterenol. The 50 % probability for incidence of DADs was obtained at 1.6 min in the presence of ANTRA.

within 6 min (Fig. 4). EADs were not observed with 5 nmol/l isoproterenol even in the presence of ANTRA, in contrast to the well-known EAD-inducing effect of higher, submicromolar concentrations of isoproterenol (Priori and Corr 1990; Szabo et al. 1994; Zeng and Rudy 1995).

Discussion

In this study we were first to visualise the ANTRA-sensitive current (which is mediated predominantly by chloride ions) flowing during the AP in canine ventricular cells. The current was large and outward at the time of the early plateau but inwardly directed around terminal repolarisation. The outward peak of this current clearly contributes to formation of the incisura of the AP, (an effect analogous to that of the transient outward potassium current), since 0.5 mmol/l ANTRA halved the depth of the incisura. These results are congruent with those of others obtained with conventional voltage clamp protocols (Zygmunt 1994; Szigeti et al. 1998). Since the charge carried by the outward component of the ANTRA-sensitive current was more than twice as large as the inward component, the net effect of the current is to facilitate repolarisation – primarily during the incisura and the crest of the dome of APs. This is a very critical period of time regarding determination of AP configuration. It is now generally believed that EADs result from the imbalance of inward and outward ionic currents during the AP plateau. In these terms, inhibition of a repolarising current (by ANTRA in our case) can promote the generation of EADs. Indeed, our results show that EADs transiently appeared in ouabain-treated cells in the presence of ANTRA in spite of the fact that neither ANTRA nor ouabain alone were able to evoke EADs at concentrations applied by us. This effect of ANTRA was even more pronounced in the experiments with caesium, where the simultaneous suppression of two repolarising currents (I_K by Cs, and I_{Cl} by ANTRA) caused a rapid and irreversible depolarisation of the membrane to a second stable level (Gadsby and Cranefield 1977).

The enhancing effect of ANTRA pretreatment on the incidence of DADs requires more explanation since the calcium-dependent I_{Cl} was implicated to contribute to development of DADs as a charge carrier in mammalian cardiac preparations (Szigeti et al. 1998; Zygmunt et al. 1998). Our results obtained with ouabain and isoproterenol clearly show that the incidence of DADs was increased in the presence of ANTRA, probably due to suppression of a predominantly outward current. This, in turn, suggests that I_{Cl} may provide a mechanism of defence against both types of afterdepolarisations. However, the question arises, what is the possible mechanism of defence against DADs, which are known to be due to calcium overload (Priori and Corr 1990; Wit and Rosen 1992; Szabo et al. 1994). The ANTRA-induced changes in AP configuration, namely the prolongation of APs and elevation of their plateau may increase calcium entry, and what is more important, may limit the efflux of calcium through the Na^+/Ca^{2+} exchanger, resulting thus in calcium overload of the SR, which is a prerequisite for generation of DADs.

It is also worthy of speculation why the appearance of EADs was transient in myocytes exposed to ouabain in the presence of ANTRA. Following from the nature of afterdepolarisations, once an EAD or DAD has developed, its incidence should continuously increase; in other words, spontaneous disappearance of an afterdepolarisation (either EAD or DAD) has never been reported previously. Furthermore, EADs and DADs have never been observed simultaneously in ouabain-treated myocytes. These interesting observations can be interpreted if one assumes that genesis of EADs and DADs share common mechanisms (e.g., both can be modulated by the calcium content of the SR), but their appearance during or after the AP depends on the actual balance of inward and outward membrane currents (Szabo et al. 1994). Further studies are required to elucidate this point.

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References

Bányász T., Magyar J., Körtvély Á., Szigeti Gy., Szigligeti P., Papp Z., Mohácsi A., Kovács L., Nánási P. P. (2001): Different effects of endothelin-1 on calcium and potassium currents in canine ventricular cells. Naunyn Schmiedeberg's Arch. Pharmacol. **363**, 383—390

Callewaert G., Cleemann L., Morad M. (1989): Caffeine-induced Ca^{2+} release activates Ca^{2+} extrusion *via* Na^{+}-Ca^{2+} exchanger in cardiac myocytes. Am. J. Physiol. **257**, C147—152

Cannell M. B., Lederer W. J. (1986): The arrhythmogenic current ITI in the absence of electrogenic sodium-calcium exchange in sheep cardiac Purkinje fibres. J. Physiol. (London) **374**, 201—219

Collier M. L., Levesque P. C., Kenyon J. L., Hume J. R. (1996): Unitary Cl^{-} channels activated by cytoplasmic Ca^{2+} in canine ventricular myocytes. Circ. Res. **78**, 936—944

Doerr T., Denger R., Doerr A., Trautwein W. (1990): Ionic currents contributing to the action potential in single ventricular myocytes of the guinea pig studied with action potential clamp. Pflügers Arch. **416**, 230—237

Ehara T., Noma A., Ono K. (1988): Calcium activated non-selective cation channel in ventricular cells isolated from adult guinea-pig hearts. J. Physiol. (London) **403**, 117—133

Fozzard H. A. (1992): Afterdepolarizations and triggered activity. Basic Res. Cardiol. **87**, (Suppl. 2) 105—113

Gadsby D. C., Cranefield P. F. (1977): Two levels of resting potential in cardiac Purkinje fibers. J. Gen. Physiol. **70**, 725—746

Hamill O. P., Marty A., Neher E., Sakmann B., Sigworth F. J. (1981): Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. Pflügers Arch. **391**, 85—100

Han X., Ferrier G. R. (1992): Ionic mechanisms of transient inward current in the absense of Na^{+}-Ca^{2+} exchange in rabbit cardiac Purkinje fibres. J. Physiol. (London) **456**, 19—38

January C. T., Fozzard H. A., (1988): Delayed afterdepolarizations in heart muscle: mechanism and relevance. Pharmacol. Rev. **40**, 219—227

January C. T., Riddle M. J. (1990): Early afterdepolarizations: Mechanism of induction and block; A role for L-type Ca^{2+} current. Circ. Res. **64**, 977—990

January C. T., Riddle M. J., Salata J. J. (1988): A model for early afterdepolarizations: Induction with the Ca^{2+}-channel agonist Bay K 8644. Circ. Res. **62**, 563—571

Janvier N. C., Boyett M. R. (1996): The role of Na^{+}-Ca^{2+} exchange current in the cardiac action potential. Cardiovasc. Res. **32**, 69—84

Kass R. S., Lederer W. J., Tsien R. W., Weingart R. (1978): Role of calcium ions in transient inward currents and aftercontractions induced by strophantidin in cardiac Purkinje fibres. J. Physiol. (London) **281**, 187—208

Kimura J., Noma A., Irisawa H. (1986): Na^{+}-Ca^{2+} exchange current in mammalian heart cells. Nature **319**, 596—597

Lei M., Cooper P. J., Camelliti P., Kohl P. (2002): Role of the 293b-sensitive, slowly activating delayed rectifier potassium current, I_{Ks}, in pacemaker activity of rabbit isolated sino-atrial node cells. Cardiovasc. Res. **53**, 68—79

Mechmann S., Pott L. (1986): Identification of Na^{+}-Ca^{2+} exchange current in single cardiac myocytes. Nature **319**, 597—599

Priori S. G., Corr P. B. (1990): Mechanisms underlying early and delayed afterdepolarizations induced by catecholamines. Am. J. Physiol. **258**, H1796—1805

Sah R., Ramirez R. J., Backx P. H. (2002): Modulation of Ca^{2+} release in cardiac myocytes by changes in repolarization rate: role of phase-1 action potential repolarization in excitation-contraction coupling. Circ. Res. **90**, 165—173

Šimurda J., Šimurdová M., Bravený P., Šumbera J. (1992): A contraction-related component of slow inward current in dog ventricular muscle and its relation to Na^{+}-Ca^{2+} exchange. J. Physiol. (London) **456**, 49—70

Szabo B., Kovacs T., Lazzara R. (1994): Role of calcium loading in early afterdepolarizations generated by Cs^{+} in canine and guinea pig Purkinje fibers. J. Cardiovasc. Electrophysiol. **6**, 796—812

Szigeti G., Rusznak Z., Kovacs L., Papp Z. (1998): Calcium-activated transient membrane currents are carried mainly by chloride ions in isolated atrial, ventricular and Purkinje cells of rabbit heart. Exp. Physiol. **83**, 137—153

Wallis W., Cooklin M., Sheridan D. J., Fry C. H. (2001): The action of isoprenaline on the electrophysiological properties of hypertrophied left ventricular myocytes. Arch. Physiol. Biochem. **109**, 117—126

Wang Z., Mitsuiye T., Rees S. A., Noma A. (1997): Regulatory volume decrease of cardiac myocytes induced by ß-adrenergic activation of the Cl^{-} channel in guinea pig. J. Gen. Physiol. **110**, 73—82

Wit A. L., Rosen M. R. (1992): Afterdepolarizations and triggered activity: distinction from automaticity as an arrhythmogen mechanism. In: The heart and cardiovascular system (Eds. H. A. Fozzard, E. Haber, R. B. Jennings, A. M. Katz and H. E. Morgan), pp. 2148—2149, Raven Press, New York, USA

Zeng J., Rudy Y. (1995): Early afterdepoarizations in cardiac myocytes: mechanism and rate dependence. Biophys. J. **68**, 949—964

Zygmunt A. C. (1994): Intracellular calcium activates a chloride current in canine ventricular myocytes. Am. J. Physiol. **267**, H1984—1995

Zygmunt A. C., Goodrow R. J., Weigel C. M. (1998): I_{NaCa} and $I_{Cl(Ca)}$ contribute to isoproterenol-induced delayed afterdepolarizations in midmyocardial cells. Am. J. Physiol. **275**, H1979—1992

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