Effects of articaine on action potential characteristics and the underlying ion currents in canine ventricular myocytes

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Background. In spite of its widespread clinical application, there is little information on the cellular cardiac effects of articaine. In the present study, the concentration-dependent effects of articaine on action potential morphology and the underlying ion currents were studied in isolated canine ventricular cardiomyocytes.

Methods. Action potentials were recorded from the enzymatically dispersed myocytes using sharp microelectrodes (16 cells from 3 dogs). Conventional patch clamp and action potential voltage clamp arrangements were used to study the effects of articaine on transmembrane ion currents (37 cells from 14 dogs).

Results. Articaine-induced concentration-dependent changes in action potential configuration including shortening of the action potentials, reduction of their amplitude and maximum velocity of depolarization (Vmax), suppression of early repolarization and depression of plateau. The EC50 value obtained for the Vmax block was 162 (± 30) μM. Both the reduction of Vmax and action potential shortening were frequency dependent: the former was more prominent at shorter, while the latter at longer pacing cycle lengths. A rate dependent Vmax block, having rapid offset kinetics [τ = 91 (20) ms], was observed in addition to tonic block. Under voltage clamp conditions, a variety of ion currents were blocked by articaine: Ica [EC50 = 471 (75) μM], Ito [EC50 = 365 (62) μM], IK1 [EC50 = 372 (46) μM], IKr [EC50 = 278 (79) μM], and IKs [EC50 = 326 (65) μM]. Hill coefficients were close to unity indicating a single binding site for articaine, except for IK1.

Conclusions. Articaine can modify cardiac action potentials and ion currents at concentrations higher than the therapeutic range which can be achieved only by accidental venous injection. Since its suppressive effects on the inward and outward currents are relatively well balanced, the articaine-induced changes in action potential morphology may be moderate even in the case of overdose.

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Articaine is one of the most widely applied local anaesthetic drugs in dentistry. Its structure is different to other local anaesthetics since it contains a thiophene ring and an additional ester group allowing rapid tissue hydrolysis. In spite of its widespread clinical use, there is little information available on its cardiac electrophysiological effects. In an early study, 141 μM articaine was shown to decrease the overshoot, amplitude, and velocity of depolarization of rabbit ventricular action potentials. However, no data on cardiac ion currents have been reported with articaine, except for HERG channels expressed in CHO cells, indicating an articaine-induced block at high concentrations. Importantly, in contrast to other local anaesthetics, like bupivacaine, lidocaine, or ropivacaine, cardiac arrest has currently not been reported with articaine. In the present study, we aimed to examine the concentration-dependent effects of articaine on action potential morphology and the underlying ion currents in isolated canine ventricular cardiomyocytes in order to address the question of ‘why articaine is so safe’.
Canine myocytes were chosen because their electrophysiological properties are believed to be most similar to those of humans regarding the distribution and kinetics of transmembrane ion currents.\(^{17,18}\) We found that articaine at concentrations higher than the therapeutic range suppressed a variety of cardiac ion currents, however, since these effects on the inward and outward currents were relatively well balanced, articaine had only moderate influence on action potential morphology.

**Methods**

**Isolation of single canine ventricular myocytes**

Adult mongrel dogs of either sex (n=17) were anaesthetized with i.v. 10 mg kg\(^{-1}\) ketamine hydrochloride (Calypsolvet, SelBruHa Kft., Hungary)+1 mg kg\(^{-1}\) xylazine hydrochloride (Rometar, Alfasan, The Netherlands) according to a protocol approved by the local ethical committee and conforming to the principles outlined in the Declaration of Helsinki. The hearts were quickly removed and placed in Tyrode solution. Single myocytes were obtained by enzymatic dispersion using the segment perfusion technique.\(^{18}\) Briefly, a wedge-shaped section of the left ventricle, was washed with Joklik solution. Portions of the left ventricular wall were cut into small pieces and the cell suspension obtained at the end of the procedure, predominantly from the midmyocardial region of the left ventricle, was washed with Joklik solution. Finally the Ca\(^{2+}\) concentration was gradually restored to 2.5 mM. The cells were stored in Eagles’ Minimum Essential Medium until use.

**Recording of action potentials**

All electrophysiological measurements were performed at 37°C. Rod-shaped viable cells showing clear striation were sedimented in a plexiglass chamber allowing continuous superfusion with oxygenated Tyrode solution. Transmembrane potentials were recorded using 3 M KCl filled sharp glass microelectrodes having tip resistance between 20 and 40 MΩ. These electrodes were connected to the input of an Axoclamp-2B amplifier (Axon Instruments). The cells were paced through the recording electrode at steady cycle length of 1 s using 1 ms wide rectangular current pulses with 120% threshold amplitude. Since the cytosol was not dialyzed, time-dependent changes in action potential duration were negligible for at least 60 min under these experimental conditions.\(^{19}\)

Concentration-dependent effects of articaine were determined in a cumulative manner by applying increasing concentrations of the drug between 1 and 300 μM. Each concentration was superfused for 3 min and the washout lasted for 10 min. These incubation and washout periods were sufficient to develop steady-state drug effects and practically full reversion. When performing frequency-dependent measurements cycle length was set to 5 s and after equilibration at least for 5 min, the cycle length was continuously varied to the shorter values. Action potentials were digitized at 200 kHz using Digidata 1200 A/D card (Axon Instruments) and stored for later analysis.

**Conventional voltage clamp**

The cells were superfused with oxygenated Tyrode solution. Suction pipettes, fabricated from borosilicate glass, had tip resistance of 2 MΩ after filling with pipette solution containing K-aspartate, 100; KCl, 45; MgCl\(_2\), 1; HEPES, 5; EGTA, 10; K-ATP, 3 mM, or alternatively, KCl, 110; KOH, 40; HEPES, 10; EGTA, 10; TEACL, 20; K-ATP, 3 mM, when measuring potassium or calcium currents, respectively (pH 7.2 in both cases). Membrane currents were recorded with the Axopatch-2B amplifier using the whole cell configuration of the patch clamp technique.\(^{20}\) After establishing a high (1–10 GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1.5 V electrical pulses for 1–5 ms. The series resistance was typically 4–8 MΩ before compensation (usually 50–80%). Experiments were discarded when the series resistance was high or substantially increasing during the measurement (in less than 10% of the experiments). Outputs from the clamp amplifier were digitized at 100 kHz under software control (pClamp 6.0, Axon Instruments). Ion currents were normalized to cell capacitance determined in each cell using short hyperpolarizing pulses from −10 to −20 mV. The experimental protocol for each measurement is described where pertinent in the Results section.

**Action potential voltage clamp**

After formation of the ‘gigaseal’, action potentials were recorded in current clamp mode from myocytes superfused with Tyrode solution. The pipette solution was identical to that used for potassium current measurement under conventional voltage clamp conditions. The cells were continuously paced through the recording electrode at a steady stimulation frequency of 1 Hz so as a 1–2 ms gap between the stimulus artifact and the upstroke of the action potential could occur. Ten subsequent action
potentials were recorded from each cell, which were digitized and averaged. This averaged signal was delivered to the same cell at the identical frequency as the command voltage after switching the amplifier to voltage clamp mode. The current trace obtained under these conditions is a horizontal line positioned at the zero level except for the very short segment corresponding to the action potential upstroke. Articaine was applied in a cumulative manner (from 10 to 1000 μM). The profile of the ion currents blocked by articaine was determined by subtracting the pre-drug curve from the post-drug curve. This procedure resulted in composite current profiles containing three distinct current peaks after reversing its polarity: an early outward for $I_{to}$, an inward for $I_{Ca}$, and a late outward for $I_{Kr}+I_{K1}$.

Statistics
Results are expressed as mean (SEM) values. Statistical significance of differences was evaluated using ANOVA followed by Student’s t-test. Differences were considered significant when $P$ was less than 0.05. Curve fitting was performed using ORIGIN (version 6.0).

Drugs
Articaine (Ultracain ampoules, 5 ml, 2%) was purchased from Aventis Pharma Deutschland GmbH (Frankfurt, Germany) and was freshly diluted with Tyrode solution to the indicated final concentration on the day of experiment. Other drugs were obtained from Sigma-Aldrich Co. (St Louis, MI, USA).

Results
Effect of articaine on action potential configuration
Articaine treatment caused concentration-dependent changes in action potential morphology in canine ventricular myocytes, paced at a constant frequency of 1 Hz, including a reduction of the amplitude and maximum velocity of depolarization ($V_{\text{max}}$) of the action potential, shortening of action potential duration, reduction of the amplitude of phase-1 repolarization, and depression of the plateau (Fig. 1). From these effects, the reduction of action potential duration and $V_{\text{max}}$ was statistically significant from concentrations of 10 and 30 μM, respectively. Fitting the $V_{\text{max}}$ data to the Hill equation, an EC$_{50}$ of 162 (30) μM and a Hill coefficient of 1.16 (0.03) were obtained from the average of five myocytes studied. All these effects were readily reversible (within 5 min) after superfusion with articaine-free Tyrode solution. Although articaine failed to induce statistically significant changes in the resting membrane potential, a tendency to depolarization was observed at higher concentrations (100 and 300 μM).

Frequency-dependent properties
Both the reduction of $V_{\text{max}}$ and shortening of action potentials by articaine were frequency dependent (Fig. 2A and B). The former effect was prominent at a fast driving rate, that is when the pacing cycle length was decreased from 5 to 0.5 s (normal frequency-dependence), while the latter was more pronounced at longer cycle lengths displaying features of reverse frequency-dependent action. Both are characteristics of ion channel blocking agents including several local anaesthetics.

Restitution kinetics of $V_{\text{max}}$ and action potential duration were also determined. In these experiments, the myocytes were paced using a train of 20 basic stimuli delivered at a
Cardiac effects of articaine

Effect of articaine on cardiac ion currents measured by conventional voltage clamp

In these experiments, performed under conventional voltage clamp conditions, cumulative concentration-dependent drug-effects were studied between 10 and 1000 μM.

The results are summarized in Figure 3.

L-type calcium current ($I_{Ca}$) was recorded at +5 mV using 200 ms long depolarizations from a holding potential of −40 mV. In these experiments, Tyrode solution was supplemented with 3 mM 4-aminopyridine, 1 μM E 4031 (AU Define), and 30 μM chromanol 293B in order to block K+ currents. Articaine blocked $I_{Ca}$ in a concentration-dependent manner (Fig. 3A). An EC50 value of 471 (75) μM and a Hill coefficient of 1.07 (0.14) were obtained when fitting the results to the Hill equation. Articaine failed to influence the inactivation kinetics of $I_{Ca}$. The time constant of current decay at +5 mV was fitted as a sum of two exponential components. Neither the rapid nor the slow time constant was altered in the presence of 300 μM articaine.

The transient outward current ($I_{to}$) was activated by depolarization to +50 mV from a holding potential of −80 mV and having duration of 200 ms. Before each test pulse, a short (5 ms) depolarization to −40 mV was applied in order to inactivate the fast Na+ current, while the Ca2+ current was blocked with 1 μM nisoldipine. The blocking effect of articaine was characterized with an EC50 of 365 (62) μM and a Hill coefficient of 1.02 (0.04) in the seven myocytes studied (Fig. 3n).

The inward rectifier K+ current ($I_{Kr}$) was studied by applying hyperpolarizations to −135 mV from a holding potential of −80 mV. The steady-state current was determined after 400 ms. $I_{Kr}$ was also blocked by articaine with an EC50 of 372 (46) μM and a Hill coefficient of 1.63 (0.09) in six myocytes. (Fig. 3c).

The rapid component of the delayed rectifier K+ current ($I_{K1}$) was activated by 1 s long depolarizing pulses to +40 mV from a holding potential of −80 mV. $I_{K1}$ was assessed as tail current amplitudes recorded after repolarization to −30 mV. $I_{Ca}$ and $I_{K1}$ were suppressed by 1 μM nisoldipine and 30 μM chromanol 293B, respectively. As shown in Figure 3n, the amplitudes of the $I_{K1}$ current tails were progressively decreased by increasing concentrations of articaine. The EC50 value and Hill coefficient were estimated 278 (79) μM and 0.96 (0.14), respectively, in the average of five cells.

The slow component of the delayed rectifier K+ current ($I_{Ks}$) was also evaluated from tail currents shown in
Fig 3  Concentration-dependent effects of articaine (from 10 to 1000 μM) on ion currents measured under conventional voltage clamp conditions. (A–E) Left panels show superimposed current traces recorded before and after superfusion with 10, 100, and 1000 μM articaine. $I_{Ca}$, $I_{to}$, and $I_{K1}$ were recorded during the test pulse, while in the case of $I_{Kr}$ and $I_{Ks}$ the tail currents, obtained upon repolarization, are depicted. Current values were normalized to the cell capacitance. Results fitted to the Hill equation are presented in the right panels, $n$ indicates the number of cells studied.
The current was activated by 3 s long depolarization to +50 mV, and the amplitude of the tail currents was determined at the holding potential of −40 mV after repolarization. $I_{Ca}$ was inhibited by 1 µM nisoldipine and $I_{Kr}$ was blocked by 1 µM E 4031. The EC$_{50}$ was 326 (65) µM and the Hill coefficient 0.87 (0.07) in the five myocytes examined.

**Effect of articaine on the ion currents under action potential clamp conditions**

The profile of an ion current may be markedly different when compared with conventional voltage clamp and action potential clamp conditions. An advantage of the action potential clamp technique is that the effect of any drug on the net membrane current can be recorded enabling monitoring drug-effects simultaneously on more than one ion current. Furthermore, this technique enables recording of true current profiles flowing during an actual cardiac action potential. In the case of a drug acting on more than one ion current, such as articaine a series of peaks can be detected on the current trace, each of them corresponding to the fingerprint of an individual ion current. Accordingly, the early outward current peak, shown in Figure 4, arises when $I_{to}$ is suppressed, while the inward deflection indicates a blockade of $I_{Ca}$. The late outward current peak, coincident with terminal repolarization of the action potential, is composed of $I_{K1}$ plus $I_{Kr}$ in a ratio of 3:1. Articaine significantly blocked $I_{to}$, $I_{Ca}$, and the late current peak containing both $I_{K1}$ and $I_{Kr}$. Inhibition of these currents increased with increasing articaine concentrations up to 1000 µM and was readily reversible. In contrast to the conventional voltage clamp measurements, where the EC$_{50}$ obtained for $I_{Ca}$ was the highest, 100 µM articaine suppressed the inward current ($I_{Ca}$) more effectively than the late outward current peak.

**Discussion**

**Effects of articaine on action potential morphology are in agreement with the voltage clamp data**

The cellular electrophysiological effects of articaine were first analyzed in this study. The results revealed that articaine suppressed several ion currents in a concentration-dependent manner with the concomitant alterations of action potential morphology. These changes observed in the configuration of the action potential can be deduced from suppression of the various ion currents. Reduction of $V_{max}$ and action potential amplitude are clearly consequences of inhibition of the fast Na$^+$ current ($I_{Na}$). Since $V_{max}$ is an indicator of $I_{Na}$ density and is believed to be linearly related to $I_{Na}$, $I_{Na}$ is the current which was most effectively blocked by articaine, considering the EC$_{50}$ value of 162 µM obtained for the $V_{max}$ block. In addition, the articaine-induced rate-dependent $V_{max}$ block showed extraordinarily fast kinetics with an offset time constant of 91 ms. To the best of our knowledge, this is the fastest time constant reported for $I_{Na}$ blockade in cardiac tissues, suggesting that articaine may have class 1B antiarrhythmic properties according to the Vaughan Williams classification. Suppression of $I_{Na}$ is also likely to be involved in the articaine-induced shortening of action potentials, significant from the relatively low concentration of 10 µM, where articaine failed to significantly diminish $V_{max}$. It is not exceptional that a drug blocks the window Na$^+$ current at sufficiently low concentrations where the fast Na$^+$ current, and consequently $V_{max}$, remains unaffected. The other factor likely to be involved in the articaine-induced shortening of action potentials is the inhibition of $I_{Ca}$. Although articaine blocked $I_{Ca}$ with the relatively high EC$_{50}$ value of 471 µM under conventional voltage clamp conditions, the cellular electrophysiological effects of articaine were first analyzed in this study. The results revealed that articaine suppressed several ion currents in a concentration-dependent manner with the concomitant alterations of action potential morphology. These changes observed in the configuration of the action potential can be deduced from suppression of the various ion currents. Reduction of $V_{max}$ and action potential amplitude are clearly consequences of inhibition of the fast Na$^+$ current ($I_{Na}$). Since $V_{max}$ is an indicator of $I_{Na}$ density and is believed to be linearly related to $I_{Na}$, $I_{Na}$ is the current which was most effectively blocked by articaine, considering the EC$_{50}$ value of 162 µM obtained for the $V_{max}$ block. In addition, the articaine-induced rate-dependent $V_{max}$ block showed extraordinarily fast kinetics with an offset time constant of 91 ms. To the best of our knowledge, this is the fastest time constant reported for $I_{Na}$ blockade in cardiac tissues, suggesting that articaine may have class 1B antiarrhythmic properties according to the Vaughan Williams classification. Suppression of $I_{Na}$ is also likely to be involved in the articaine-induced shortening of action potentials, significant from the relatively low concentration of 10 µM, where articaine failed to significantly diminish $V_{max}$. It is not exceptional that a drug blocks the window Na$^+$ current at sufficiently low concentrations where the fast Na$^+$ current, and consequently $V_{max}$, remains unaffected. The other factor likely to be involved in the articaine-induced shortening of action potentials is the inhibition of $I_{Ca}$. Although articaine blocked $I_{Ca}$ with the relatively high EC$_{50}$ value of 471 µM under conventional voltage clamp conditions.

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**Fig 4** Effects of articaine on ion currents under action potential voltage clamp conditions. Representative records of a command signal (A) and the underlying current traces obtained in the presence of increasing concentrations of articaine (B). The horizontal arrows at left indicate the zero current level for each trace. (C) Suppressive effects of articaine on the inward (representing $I_{Ca}$) and late outward (composed of $I_{K1}$ and $I_{Kr}$) current peaks. Symbols and bars represent mean (SEM) values obtained in four myocytes, asterisks denote significant ($P<0.05$) changes from control.
conditions, action potential clamp experiments revealed that articaine evoked a much larger suppression of the inward than the late outward current peak within the concentration range of 30–100 μM in agreement with its shortening effect. This finding underlines the power of the action potential clamp technique in studying drug effects in cardiac tissues. Beyond the shortening effect, depression of the action potential plateau may also be ascribed to the blockade of $I_{Ca}$. Finally, reduction of the amplitude of early (phase 1) repolarization may be explained by the inhibition of $I_{to}$, as it was demonstrated under both conventional and action potential voltage clamp conditions. Regarding the possible mechanism of the articaine-induced ion channel blockade it can be concluded that the drug likely associates with a single binding site on each channel protein (except for $I_{K1}$) as indicated by the Hill coefficients of close to unity.

Comparison with other local anaesthetics

In the voltage clamp experiments the $EC_{50}$ values obtained with articaine ranged between 200 and 500 μM for the various ion currents which were markedly different from the other two most frequently used local anaesthetics, lidocaine, and bupivacaine. For instance, bupivacaine was shown to suppress $V_{max}$, $I_{to}$, and $I_{Ca}$ more effectively than articaine. One micromolar bupivacaine reduced $V_{max}$ by 26%, $I_{to}$ was blocked with an $EC_{50}$ of 22 μM, and a 22% reduction of $I_{Ca}$ was induced by 10 μM bupivacaine. Similar to articaine, $I_{Na}$ was blocked by lidocaine with $EC_{50}$ values of 95 and 226 μM, whereas the $EC_{50}$ of lidocaine for blocking $I_{Ca}$ was only 27 μM. This value is lower than our $EC_{50}$ obtained with articaine (> one order of magnitude). Unlike articaine, neither bupivacaine nor lidocaine inhibited $I_{K1}$ up to concentrations of 1000 μM. These differences between the effects of articaine, bupivacaine, and lidocaine on cardiac ion currents can be evaluated when comparing them on the basis of their plasma concentration. However, relatively little differences were found in peak plasma concentrations measured in patients anaesthetized with bupivacaine, articaine, and lidocaine: typically values of 3–6, 6–7, and 5–10 μM were obtained, respectively.

Lidocaine is a potent class 1B antiarrhythmic agent having cardiac side-effects including negative inotropy, while bupivacaine was found to be proarrhythmic at higher concentrations. In contrast to bupivacaine or lidocaine, no fatal cardiovascular complication has been reported with articaine. Since the lowest concentration of articaine causing statistically significant changes in our study was higher than the usual peak plasma concentration measured with articaine in patients, it is likely that articaine fails to alter cardiac electrogensis during normal anaesthesia. But what can be anticipated in case of articaine intoxication or overdose caused by accidental venous injection of the drug? Although articaine was shown to interfere with several cardiac ion currents at higher concentrations, its shortening effect on action potential duration was relatively moderate, and more importantly it seemed to saturate between 100 and 300 μM. In agreement with this, the clearest increase in the articaine-induced reduction of the late outward current peak was evident over this concentration range.

Therefore, one may conclude that the more and more pronounced articaine-induced blockade of outward currents, appearing with increasing concentrations of articaine, can prevent the further shortening of action potential duration, which might be arrhythmogenic due to shortening of the refractory period.

This property of articaine predicts less proarrhythmic potency compared with other local anaesthetics in case of accidental overdose.

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