Effect of bupivacaine on ATP-dependent potassium channels in rat cardiomyocytes

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Bupivacaine induces fatal arrhythmia when accidentally injected i.v. or overdosed, whereas lidocaine is used as an anti-arrhythmic agent. We have suggested recently that the anti-arrhythmic effect of lidocaine may be explained by suppression of ATP-sensitive potassium (Kₐ₅P) channels. Therefore, it could be argued that different sensitivities of Kₐ₅P channels to both drugs could be a reason for their different arrhythmic and anti-arrhythmic properties. In this study, we have investigated the direct action of bupivacaine on Kₐ₅P channels in cardiomyocytes. The effects of bupivacaine on the cardiac Kₐ₅P channel were investigated using the patch-clamp technique on enzymatically dissociated cardiomyocytes of adult rats. Bupivacaine was applied to the outer side of excised membrane patches using a multiple-barrel perfusion system. Concentration–response curves indicated that bupivacaine blocked the mean current of the Kₐ₅P channels at a half-maximum inhibiting concentration (IC₅₀) of 29 µmol litre⁻¹, similar to that reported for lidocaine (43 µmol litre⁻¹). Binding of bupivacaine influenced the gating of this channel, but did not reduce the conductance of the open channel. Bupivacaine and lidocaine were equipotent in blocking Kₐ₅P channels. However, because of its excessive block of the sodium channel in the inactivated state, block of Kₐ₅P channels by bupivacaine will only enhance its cardiotoxicity.

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The local anaesthetic bupivacaine exerts cardiotoxicity when overdosed or during accidental intravascular injection, leading to conduction disturbances and ventricular arrhythmia.¹ The mechanisms underlying this toxicity have been studied extensively but are still not completely understood. For example, another local anaesthetic, lidocaine, does not show this pronounced cardiotoxicity and is used widely for the treatment of ventricular arrhythmia.² The differences in the cardiac effects were generally explained by more potent block of sodium channels by bupivacaine compared with lidocaine.² However, some potassium channels are also blocked by bupivacaine, which may enhance its pro-arrhythmic effects.³ In addition, we recently showed that lidocaine, at therapeutic concentrations, suppressed currents through ATP-dependent potassium (Kₐ₅P) channels in cardiomyocytes, which could explain the anti-arrhythmic properties of lidocaine.⁴ Thus differences in the effects of lidocaine and bupivacaine on Kₐ₅P channels may account for their opposing cardiac effects. In this study, we used the patch-clamp technique to investigate the direct action of bupivacaine on Kₐ₅P channels of cardiomyocytes. We discuss possible reasons for the pro-arrhythmic and cardio-toxic properties of bupivacaine.

Methods and results

Ventricular myocytes were isolated from adult Wistar rats (n=9) anaesthetized with pentobarbital 30–50 mg kg⁻¹. Details of the isolation have been described previously.⁴ Rats were exsanguinated in accordance with national guidelines. Rectangular cells with clear striations were used in this study. Experiments were performed at 20–22°C.

External solution (Tyrode) contained (in mmol litre⁻¹) NaCl 140.0, KCl 5.6, KH₂PO₄ 0.5, Na₂HPO₄ 0.4, MgSO₄ 0.9, CaCl₂ 1.8 and N-(2-hydroxyethyl)piperazine-N9-(2-ethanesulphonic acid) (HEPES) 10 (pH 7.4 adjusted with NaOH). High potassium external solution (high-Kᵡ) contained (in mmol litre⁻¹) KCl 145, CaCl₂ 1.8, MgCl₂ 1.0 and HEPES 5.0 (pH 7.4 adjusted with KOH). Internal solution for outside-out patches (high-Kᵢ) contained (in
mmol litre$^{-1}$) KCl 145, ethylene glycol tetraacetic acid (EGTA) 10 and HEPES 5 (pH 7.2 adjusted with KOH) and that for inside-out patches (high-K$_o^+$) (in mmol litre$^{-1}$), KCl 145, MgCl$_2$ 1.0, EGTA 5 and HEPES 10 (pH 7.4 adjusted with KOH).

Bupivacaine HCl, K$_2$ATP and glibenclamide (Sigma Chemical Co., St Louis, MO, USA) were added directly to the solutions, which were applied using a multiple-barrel perfusion system. Because of the well known run-down of K$_{ATP}$ channels, patches were washed in control solution after application of each bupivacaine concentration. Mean currents, measured as the area under the current curve divided by the time interval, recorded in control solution just before and after application of a given bupivacaine concentration were averaged. This averaged value was used as the control amplitude of K$_{ATP}$ current to calculate the relative block induced by a given concentration of bupivacaine.

**Current recordings**

Ion channels were investigated using the patch-clamp method, as described previously. Pipettes were pulled from borosilicate glass tube (GC 150F-7.5, Clark Electromedical Instruments, Pangbourne, UK), coated with Sylgard 184 (Dow Corning, Seneffe, Belgium) and fire-polished directly before each experiment. Pipette resistance was 7–9 mega ohms (MΩ). Currents were recorded using an EPC-7 patch-clamp amplifier (List, Darmstadt, Germany), low-pass filtered at 10 kHz, and stored on videotape via a modified PCM-501ES (Sony, Tokyo, Japan) pulsecode modulation unit. For analysis, data were filtered with a 4-pole low-pass Bessel filter, digitized with a Labmaster TM-40 AD/DA board (Scientific Solutions, Solon, OH, USA), and recorded on a personal computer. Commercially available software (pCLAMP 5.0, Axon Instruments; Foster City, CA, USA) was used to calculate the open probability of the channel. The channel was considered open if its amplitude exceeded 50% of its mean amplitude. Most recordings were performed on outside-out patches. In some inside-out patches, the identity of K$_{ATP}$ channels was confirmed by sensitivity to internal ATP.

**Statistical analysis**

Data points of concentration–effect curves were fitted using non-linear least squares procedures, as indicated in the legend to Figure 1E. Values are given as mean (SEM). Statistical analysis, fittings and preparation of the figures were performed with Fig.P 6.0c software (Biosoft, Cambridge, UK).

Figure 1A shows K$_{ATP}$ channel currents recorded from an outside-out patch in external Tyrode solution. Current amplitudes were zero at –80 mV (E$_K$=–82 mV) and became larger with depolarization as the driving force for K$^+$ ions increased. Current amplitude as a function of membrane potential is shown in Figure 1B, demonstrating a single-channel conductance of 23 (2.6) pS (n=5). After substitution of external Tyrode solution (K$^+_{o}$ 5.6 mmol litre$^{-1}$) with high-K$_o^+$ solution (K$^+_{o}$ 145 mmol litre$^{-1}$), reversal potential shifted to 0 mV (n=7; not shown), indicating a high selectivity of the channels to K$^+$ ions. Externally applied glibenclamide 10 µmol litre$^{-1}$, a specific blocker of K$_{ATP}$ channels, completely blocked the channels (n=5; not shown). The sensitivity of the channels to internal ATP was tested using inside-out patches. The pipettes were filled with external Tyrode solution and the bath contained high-K$^+$ solution. ATP 2 mmol litre$^{-1}$ blocked the channels (n=6; Fig. 1C), and therefore we conclude that the channels were K$_{ATP}$ channels.

Ionic currents through K$_{ATP}$ channels in the presence and absence of bupivacaine 300 µmol litre$^{-1}$ are shown in Figure 1D. Bupivacaine reversibly reduced the open probability of the channels but not their unitary conductance. Concentration-dependent reduction of the K$_{ATP}$ current by bupivacaine is shown in Figure 1E. Fitting of the data gave a half-maximum inhibiting concentration (IC$_{50}$) of 29 (1.8) µmol litre$^{-1}$ and the Hill coefficient was 1.0 (0.2), indicating a one-to-one interaction between the channel and bupivacaine molecule (Fig. 1E). For comparison, the concentration–effect curve for lidocaine obtained in our preceding study is also shown (IC$_{50}$=43 µmol litre$^{-1}$).

**Comment**

Potassium channels studied here with a conductance of 23 pS in external Tyrode solution (K$^+$ 5.6 mmol litre$^{-1}$) were suppressed by internally applied ATP 2 mmol litre$^{-1}$ and external glibenclamide, and thus showed properties typical of K$_{ATP}$ channels. Externally applied bupivacaine reversibly reduced K$_{ATP}$ currents in a concentration-dependent manner. The IC$_{50}$ value of 29 µmol litre$^{-1}$ was very similar to that of 43 µmol litre$^{-1}$ reported for lidocaine. However, the toxic concentrations of bupivacaine are 4–50 times lower than those of lidocaine. In particular, the therapeutic range for anti-arrhythmic treatment with lidocaine is 5–20 µmol litre$^{-1}$ and may even reach 100 µmol litre$^{-1}$ after bolus injection, whereas after accidental intravascular injection of bupivacaine, cardiotoxic effects were observed at 10–18 µmol litre$^{-1}$. The toxic effects of bupivacaine are usually explained by depression of cardiac conduction resulting from block of voltage-gated sodium channels. Bupivacaine binds much more potently to sodium channels in the open and inactivated state than in the resting state. In contrast with nerve tissue, this is particularly relevant for cardiomyocytes where sodium channels spend a long time in the inactivated state during the long-lasting action potentials. Opening of K$_{ATP}$ channels was shown to shorten action potential duration in ventricular myocytes. Thus block of K$_{ATP}$ channel activity results in prolongation of the action potential and, as a consequence, may increase the cardiotoxicity of bupivacaine. On the other hand, shortening of the action potential may reduce this. This relation between action potential duration and
Bupivacaine blocks cardiac K\textsubscript{ATP} channels

![Figure 1](image_url)

**Fig 1** Single ATP-dependent potassium (K\textsubscript{ATP}) channel currents in rat cardiomyocytes, and the effect of externally applied bupivacaine on K\textsubscript{ATP} channels. 

\textbf{A}: Original traces of single-channel currents through K\textsubscript{ATP} channels recorded from an outside-out patch at different membrane potentials; Tyrode solution (K\textsubscript{o} 5.6 mmol litre \textsuperscript{-1}) in the bath and high-K\textsubscript{i} (K\textsubscript{i} 145 mmol litre \textsuperscript{-1}) solution in the pipette. Filter frequency was 500 Hz and temperature 20–22°C. The broken line represents the current level of closed channels. 

\textbf{B}: The amplitude of single K\textsubscript{ATP} channel current as a function of voltage. Data points were fitted using linear regression, giving a single-channel conductance of 23 (2.6) pS (n=5). 

\textbf{C}: K\textsubscript{ATP} channels at a potential of 0 mV in control solution and in the presence of ATP 2 mmol litre \textsuperscript{-1} using an inside-out patch. The bath contained high-K\textsubscript{i}° solution. The pipette was filled with Tyrode solution. 

\textbf{D}: Recordings of K\textsubscript{ATP} channels in the presence and absence of bupivacaine 300 µmol litre \textsuperscript{-1}, measured in an outside-out patch. Membrane potential was 0 mV and filter frequency 200 Hz. The moments of solution change are indicated by arrows. 

\textbf{E}: Concentration–effect curve for block of K\textsubscript{ATP} channels by bupivacaine at 0 mV (continuous line). Data were non-linearly fitted with a standard isotherm: 

\[ f(C) = C^n/(C^n + IC_{50}^n) \]

where \( C \) = blocker concentration, \( IC_{50} = 29 \) (1.8) µmol litre \textsuperscript{-1}, the concentration at which the channel open probability was reduced by a factor of two and \( n=1.0 \) (0.2), the Hill coefficient. The broken line indicates the concentration–effect curve for suppression of K\textsubscript{ATP} channels by lidocaine from Olschewski and colleagues. 

Bupivacaine cardiotoxicity could also explain the beneficial effect of K\textsubscript{ATP} openers on bupivacaine-induced cardiotoxicity. According to our present data, bupivacaine, through block of K\textsubscript{ATP} channels, enhances its own cardiotoxicity resulting from strong block of sodium channels and, therefore, unlike lidocaine, cannot exhibit beneficial anti-arrhythmic effects. 

In contrast with bupivacaine, during application of therapeutic concentrations of lidocaine, full action potentials in cardiomyocytes were still recorded, although at the same concentrations, half of the K\textsubscript{ATP} conductance was already blocked. This may decrease the incidence of arrhythmia by reducing the ischaemia-induced heterogeneity of action potential duration. 

In summary, our data suggest that bupivacaine and lidocaine were equipotent in blocking K\textsubscript{ATP} channels. However, because of the different potencies in blocking sodium channels, block of K\textsubscript{ATP} channels by lidocaine may be used for anti-arrhythmic treatment, whereas the same effect by bupivacaine would only enhance its cardiotoxicity.

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**References**


3. Castle NA. Bupivacaine inhibits the transient outward K\textsuperscript{+} current but not the inward rectifier in rat ventricular myocytes. *J Pharmacol Exp Ther* 1990; 255: 1038–46
Olschewski et al.


