

Bupivacaine inhibits a small conductance calcium-activated potassium type 2 channel (SK2) in HEK 293 cells

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Abstract

Background: Bupivacaine blocks many ion channels in the heart muscle, which could cause severe cardiotoxicity. Small conductance calcium-activated potassium type 2 channels (SK2 channels) are widely distributed in the heart cells and are involved in relevant physiological functions. However, whether bupivacaine can inhibit SK2 channels is still unknown. This study investigated the effect of bupivacaine on SK2 channels.

Methods: The SK2 channel gene was transfected into human embryonic kidney 293 cells (HEK-293 cells) with Lipofectamine 2000. The whole-cell patch clamp technique was used to study the effect of bupivacaine on SK2 channels. The inhibitory effect of various concentrations of bupivacaine on SK2 currents exhibited a non-linear relation, and the half-maximal inhibitory concentration (IC₅₀) value was determined.

Results: Bupivacaine inhibited the SK2 channels reversibly in a dose-dependent manner. The IC₅₀ value of bupivacaine, ropivacaine and lidocaine on the SK2 current was 133.7, 189.3, and 885.8 μ M, respectively. The degree of SK2 current inhibition by bupivacaine was dependent on the intracellular concentration of free calcium.

Conclusions: The results of this study suggested a new inhibitory effect of bupivacaine on SK2 channels. Future studies should be concerned with the effects of SK2 on bupivacaine cardiotoxicity.

Keywords: Bupivacaine, SK2 channel, inhibition, cardiotoxicity, HEK 293.

Background

Bupivacaine is one of the long-acting, lipophilic local anaesthetics (LAs) that is used for analgesia during the perioperative period due to its high analgesic efficacy and long-lasting effect. However, accidental delivery or excessive absorption of bupivacaine into the blood circulation may cause severe arrhythmias or even cardiac arrest[1-3]. The mechanism of bupivacaine cardiotoxicity has not been fully elucidated. It is currently believed that bupivacaine could block sodium[4, 5], L-calcium[6, 7]and potassium channels[8, 9] which may be involved in bupivacaine cardiotoxicity.

Calcium-activated potassium channels are calcium-dependent channels triggered by intracellular calcium[10]. In humans potassium channels can be divided into three categories: large conductance channels, intermediate conductance channels and small conductance channels. Small conductance calcium-activated potassium type 2 channel (SK2) is a type of small conductance potassium channel that is involved in hyperpolarization after the action potential. The SK2 channels are distributed in the atria[11], ventricles[11], atrioventricular nodes[12], and Purkinje cells[13], which play an important role in cardiac conduction. Due to the important role of the SK2 channel in the action potential, its dysfunction may lead to atrial or ventricular arrhythmias[3, 14]. So far, there have been no reports on the effect of

bupivacaine on the SK2 channels. We hypothesised that bupivacaine could directly suppress SK2 currents. There has been no report on the effect of bupivacaine on the SK2 channels.

In this study, we transfected HEK 293 cells with SK2 channels. The patch clamp whole-cell technique was applied to demonstrate that clinical concentrations of bupivacaine could inhibit SK2 currents. Our aim was to demonstrate the capacity of bupivacaine to inhibit SK2 channels and the effect of calcium concentration on its blockade.

Methods

Cell line culture and gene transfection

Human embryonic kidney 293 cells (HEK293 cells) were all purchased from the institute of life sciences of Chinese Academy of Sciences (China). After being harvested by 0.25% trypsin, the cell lines were grown at 37 °C in a 5% CO₂ and 95% air atmosphere and cultured in Dulbecco's Minimal Essential Medium (DMEM) mixed with 10% fetal bovine serum (FBS), 75 µg/mL streptomycin and 75 U/mL penicillin. Before transfection, cells were added into plate with a density of about 2×10^5 cells/cm². Transfection was performed when the confluency reached about 85%. The plasmids (pCDNA3/rSK Ca2) used in this study were obtained from OriGene (USA). We performed all the transfections with Lipofectamine 2000 (Invitrogen, USA) followed the manufacturer's instructions. As described previously, we established stable expression SK2 channel in the HEK293 cells (the cells were named SK2 cells)[15]. Before patch clamp experiment, SK2 cells were seeded about 24 hours in the glass cover.

Drugs and Solutions

Trypsin, FBS, penicillin and streptomycin and DMEM were all obtained from Gibco Invitrogen Corp.(USA); Bupivacaine, ropivacaine and lidocaine were purchased from Sigma-Aldrich (USA). The tyrode's solution was composed of the following, in mM: NaCl 137, KCl 5.4, MgCl₂ 1.8, HEPES 10, and glucose 10, pH was maintained at 7.4 with NaOH. The pipette solution was composed of the following, in mM: MgCl₂ 1.15, EGTA 1, HEPES 10, Potassium gluconate 144, and CaCl₂ (0.25,0.5 or 1.0), pH was maintained at 7.2 with KOH.

Patch-clamp experiments

All experiments were conducted with the patch clamp whole-cell technique[16]. The coverslip containing SK2 cells was placed into an inverted olympus microscope (IX70, Japan) on the cell chamber and perfused with the tyrode solution (the flow rate was about 1 ml/min).

The EPC-10 amplifier (HEKA, Germany) was applied for patch clamp whole-cell technique. A glass electrode having an outer diameter of 1.2 mm was pulled out by the microelectrode puller (P-97, SUTTER, USA) to achieve a resistance of 1.5-3.0 MΩ after adding the pipette solution. Under the microscope, SK2 cells with smooth cell membranes were picked up to record the currents. After giga-seal achievement, we

give a negative pressure to break the membrane of SK2 cells. Voltage stimulation and data recording were performed by a Pulse 8.0 software (HEKA, Germany). All experiments were performed at a temperature of 26°C. The stimulation program of SK2 currents was as follows: holding potential was maintained at -80 mV, the step clamp voltage was given from -120 mV to +40 mV at a step of 10 mV with a 200 ms duration (Figure 1A). The ramp clamp voltage was stimulated from +40 mV to -120 mV with a 300 ms duration (Figure 1B). SK2 cells could produce stable currents at 0 mV; therefore, we used currents at 0 mV for comparisons in the following experiments.

Statistical Analysis

The SPSS software (version 19.0, Chicago, IL, USA) was used to analyse the data. The normality of data was tested using the Shapiro-Wilk test and the normally distributed data was expressed as the mean ± standard deviation. Differences between the two groups were assessed by unpaired Student's t-test, and ANOVA was used for comparisons of multiple groups. The relationship between local anaesthetic concentration and its inhibitory effect on SK2 currents was fitted according to a non-linear model using GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA). A P value < 0.05 was considered to indicate statistical significance.

Results

Characteristics of SK2 currents from SK2 cells

Before the experiment, the whole-cell patch-clamp technique was used to determine the characteristics of SK2 currents. HEK 293 cells transfected with the SK2 gene (the transfected cells were named SK2 cells) could produce representative current tracings, but wild-type HEK293 cells could not (Figure 1A). The SK2 current could also be activated according to the voltage ramp protocol in SK2 cells (Figure 1B). Figure 1C depicts the time course of the SK2 current before and after apamin. The currents were both obtained at 0 mV. Similar results were observed separately in 8 SK2 cells. SK2 cells could produce stable currents at 0 mV; therefore, we used currents at 0 mV for comparisons in the following experiments.

Concentration response relationship of bupivacaine, ropivacaine and lidocaine on the inhibition of SK2 currents

The effect of bupivacaine on SK2 currents was determined on the SK2 cells. As the concentration of bupivacaine increased, the SK2 currents decreased gradually in the SK2 cells (Figure 2A). The concentration response relationship of bupivacaine on the inhibition of SK2 current (at 0 mV) was fitted according to a non-linear model. The half-maximal inhibitory concentration (IC₅₀) value for bupivacaine was 133.7 µmol/L (95% CI 87.33 - 204.6) (Figure 2B). The concentration response relationship of ropivacaine and lidocaine was also fitted according to the same model. The IC₅₀ value for ropivacaine and lidocaine was 189.3 µmol/L (95% CI 153.7 - 233.2) and 885.8 µmol/L (95% CI 499.9 - 1570), respectively (Figure 2C and 2D).

Inhibition of SK2 currents with LAs was reversible

Next we explored whether the inhibitory effect of bupivacaine was reversible. In this part, the SK2 currents from SK2 cells were recorded exposing to 10, 100 and 1000 μM bupivacaine. It showed that SK2 currents measured at membrane potential of 0 mV were completely reversed to the baseline value after washout ($P < 0.05$) (Figure 3).

Modulation of the inhibitory effect of bupivacaine by the calcium concentration

The opening probability of the SK2 channel is related to the intracellular calcium concentration. Here, we tested whether the inhibitory effect of bupivacaine on the SK2 current was affected by the calcium concentration. Figure 4A shows SK2 current traces induced with intracellular free calcium concentrations of 0.25, 0.50 and 1.0 mM. The results showed that the SK2 currents were increased when the calcium concentration reached 1.0 mM ($P < 0.05$). Figure 4B shows that SK2 currents were inhibited by bupivacaine to different levels with exposure to different intrapipette concentrations of free calcium. The results showed that the inhibition of the SK2 current was the least at a calcium concentration of 1.0 mM ($P < 0.05$).

Discussion

The results showed that: (1) Bupivacaine could inhibit the SK2 channels reversibly in a dose-dependent way. (2) The IC_{50} values of bupivacaine, ropivacaine and lidocaine for inhibiting SK2 was 133.7, 189.3, and 885.8 μM , respectively. (3) The intracellular calcium concentration could affect the inhibition of bupivacaine on SK2 current.

After transfection with the SK2 channels, the SK2 cells produced stable SK2 currents, which could be inhibited by apamin. For this reason, the SK2 current is also called apamin-sensitive current[17]. Since the opening of the SK2 channel was mainly dependent on the calcium concentration, we recorded the SK2 current at a voltage of 0 mV. The intracellular free calcium concentration was controlled through the electrode solution, and the extracellular buffer did not contain free calcium. Therefore, the effect of bupivacaine on free calcium in SK2 cells was unlikely to affect its inhibition of the SK2 channel.

Bupivacaine cardiotoxicity results from the blockade of a wide range of myocardial ion channels, the most important being the sodium channel. In this study, we used the whole-cell patch-clamp technique to investigate the effects of LAs on SK2 currents, and measured the IC_{50} of bupivacaine, ropivacaine, and lidocaine. Bupivacaine ranked first in its potency of inhibition of SK2 currents, followed by ropivacaine and lidocaine. Interestingly, this order of potency is consistent with the order of LA cardiotoxicity. In the past, Pedro Martín[18] had studied the inhibitory effect of bupivacaine on large conductance calcium-activated potassium channels in human umbilical artery smooth muscle cells. In his study, clinical concentrations of bupivacaine could block large conductance calcium-activated potassium channels. Additionally, Sbarbaro et al. [19] found that lidocaine could also block SK2 currents on nerve cells. However, lidocaine induced SK2 current blockade only when it exceeded clinical concentrations. It has

been suggested that the blockade of SK2 channels by lidocaine was unlikely to cause clinical effects. However, our study found that the SK2 channels were very sensitive to bupivacaine and ropivacaine. The specific mechanism of this inhibition is still unclear. Since the inhibitory effect of bupivacaine on the SK2 channel could affect different physiological functions, this inhibitory effect of bupivacaine might be taken into account in bupivacaine cardiotoxicity.

We also found that the intracellular calcium concentration could affect the inhibitory effect of bupivacaine on SK2 currents. The SK2 channel proteins are coupled with calmodulin, and calcium binding to calmodulin would affect the conformation and function of the SK2 channels[20]. Since studies have shown that bupivacaine might alter intracellular calcium concentrations, bupivacaine is expected to indirectly regulate the SK2 channel. To eliminate the effects of this process, this study controlled the intracellular calcium concentration in the pipette solution. According to the result of the present study, this concentration-dependent inhibition of SK2 currents suggests that concentration of intracellular free calcium may contribute to the cardiotoxicity of bupivacaine.

Since bupivacaine could inhibit several ion currents in the heart (e.g., sodium channels, L-calcium channels and potassium channels), this study adds SK2 channels to the list of ion channels affected by bupivacaine. SK2 channels could cause arrhythmia depending on their expression levels in cardiomyocytes[21, 22]. SK2 channels could cause arrhythmia when they are expressed too much or too little in cardiomyocytes[23]. SK2 channels also participate in the mitochondrial function[24-27]. Therefore, the effects of SK2 channels on the action potential and mitochondrial function suggest that the blockade of SK2 channels might be involved in the cardiotoxicity of bupivacaine. More experiments are needed to demonstrate this hypothesis.

Limitations

Under normal circumstances, the SK2 channel proteins are coupled with calmodulin, and calcium binding to calmodulin would affect the conformation and function of the SK2 channels[20]. However, because only the SK2 channels were transfected into HEK 293 cells in our study, this effect of calcium could not be observed.

Conclusions

The results of this study suggest a new inhibitory effect of bupivacaine on SK2 channels. Future studies should be concerned with the effects of SK2 on bupivacaine cardiotoxicity.

Abbreviations

SK2 channel: small conductance calcium-activated potassium type 2 channel;

HEK-293 cell: human embryonic kidney 293 cell;

Bupi: bupivacaine;

IC50: half-maximal inhibitory concentration.

Con: control;

Declarations

Ethics approval and consent to participate

Not applicable.

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Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflicts of interest.

Author contributions

1. H.C. Contribution: Design and conduct the study, analyze the data, and write the manuscript.
2. F.X. Contribution: Conduct the study and study data collection.
3. Z.J. Contribution: Conduct the study and write the manuscript.
4. Y.H. Contribution: study data collection.
5. J.C. Contribution: study data collection.
6. S.W. Contribution: study data collection.
7. X.C. Contribution: study data collection.
8. Z.F. Contribution: Design and conduct the study, analyze the data, and write the manuscript.

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Not applicable

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Figures

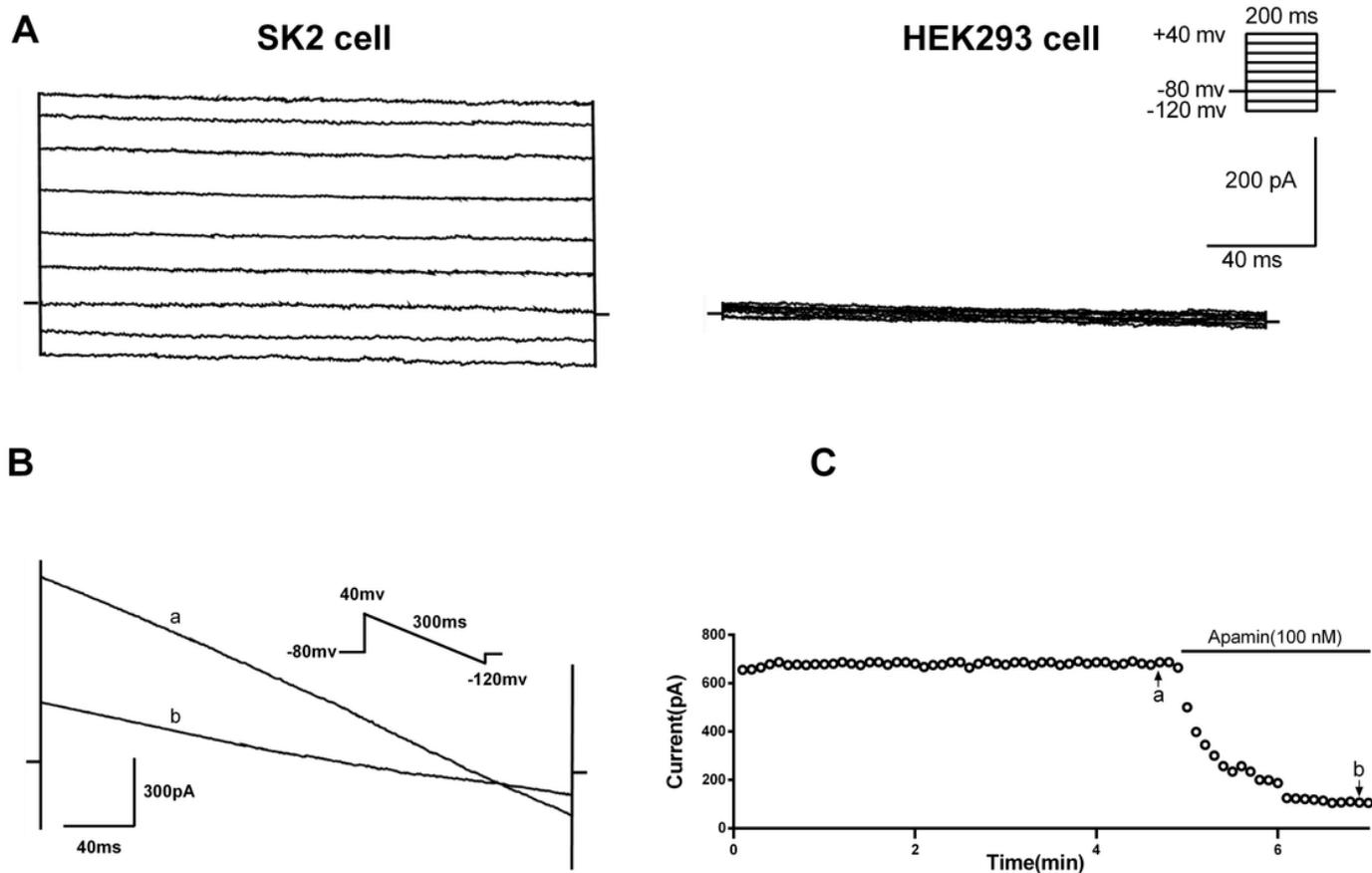


Figure 1

SK2 currents recorded from SK2 cell. (A) Superimposed SK2 current traces was recorded from a SK2 cell and a HEK293 cell. (B) SK2 current traces obtained at time points marked with arrow a and b in (C). The protocol shown in the inset was used to activate SK2 current. (C) Time course of SK2 current in a SK2 cell measured at 0 mV. The current could be inhibited by 100 nM apamin completely and the pipette solution contained 1 mM free calcium. SK2 cell:HEK 293 cell transfected with SK2 genes.

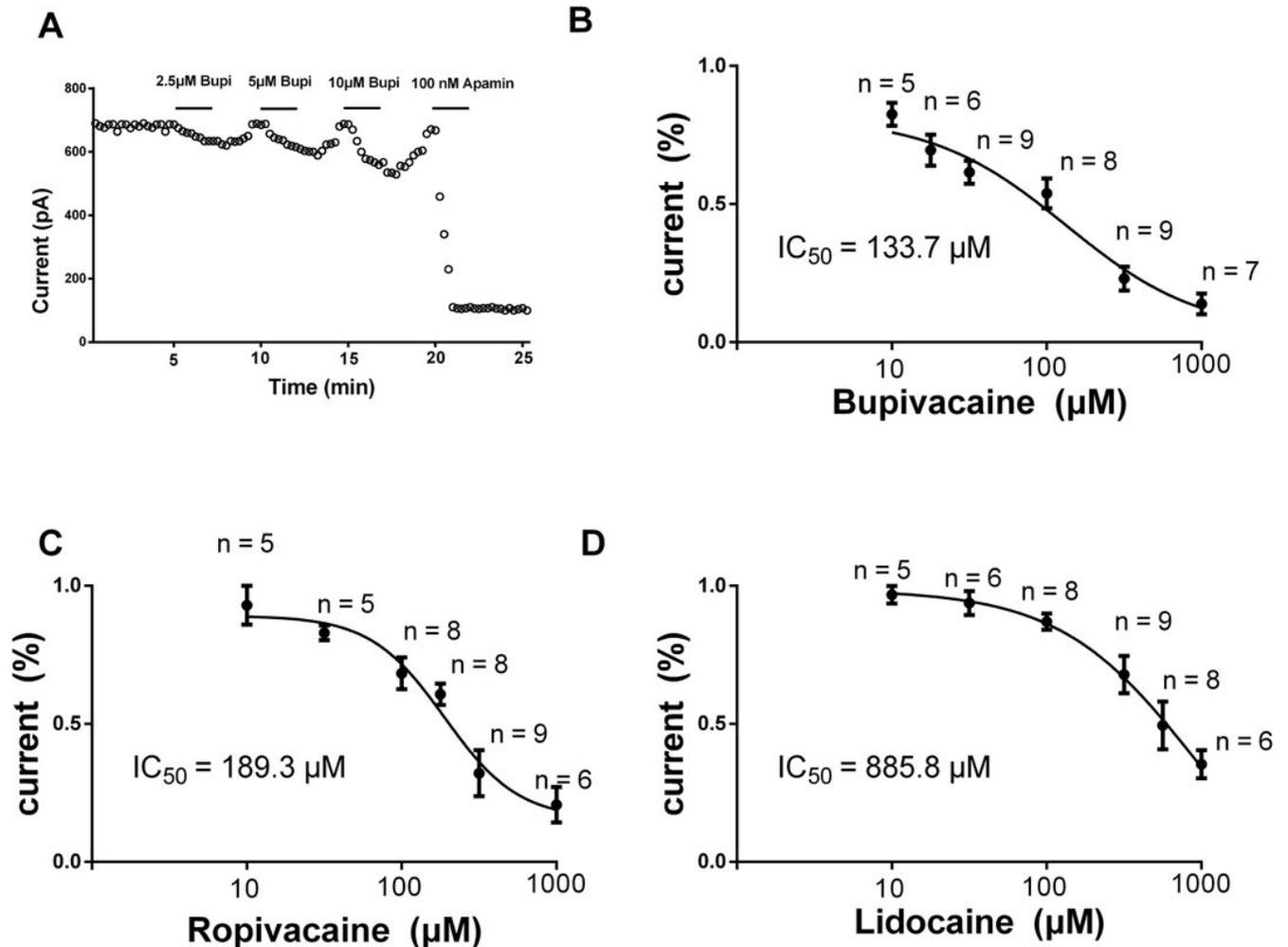


Figure 2

Concentration-dependent inhibitory effects of bupivacaine, ropivacaine and lidocaine on SK2 currents. (A) Time course of SK2 currents obtained at 0 mV in the presence or absence of different bupivacaine concentrations and apamin. (B,C and D) Dose-dependent effects of bupivacaine for inhibiting SK2 current (0 mV) was fitted to a Hill equation to obtain IC_{50} value of bupivacaine, ropivacaine and lidocaine, respectively. The pipette solution contained 1 mM free calcium.

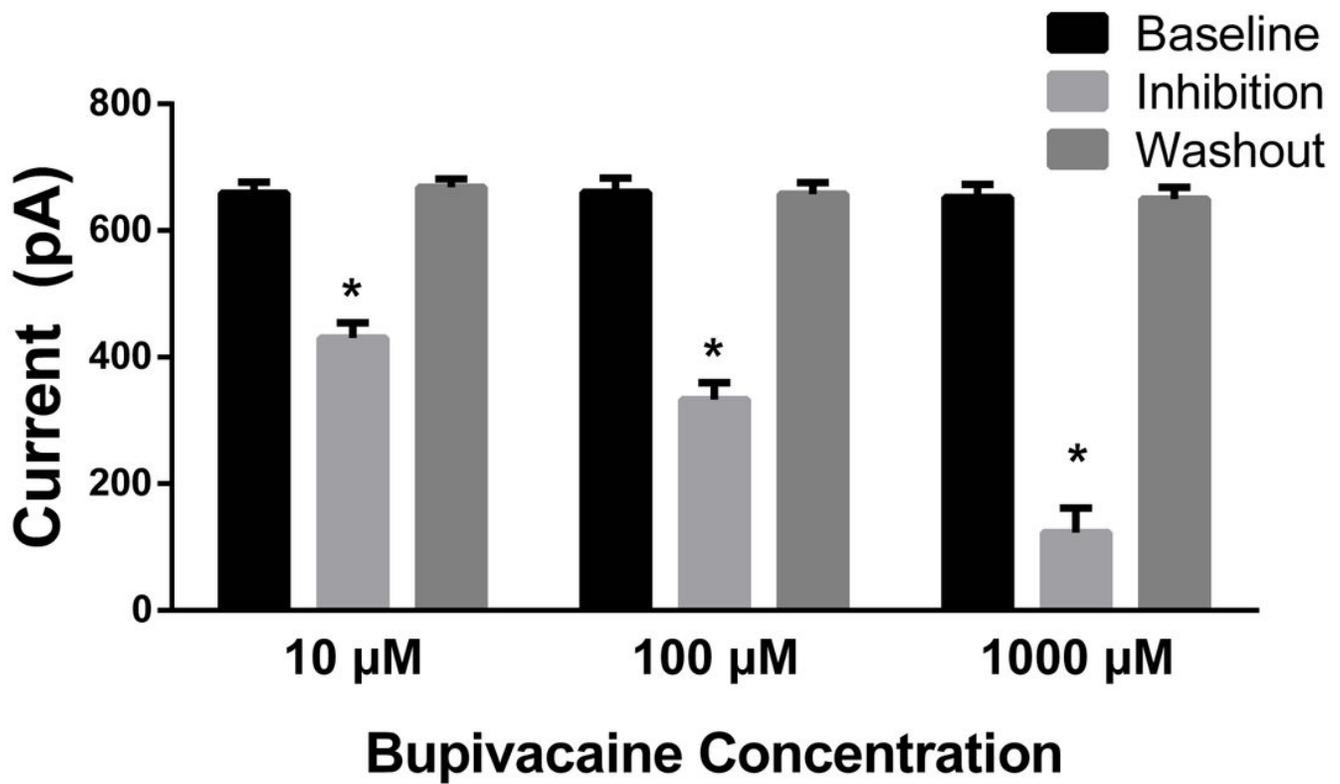


Figure 3

Inhibitory effects of LAS on SK2 currents are reversible (n=7 in each concentration). SK2 currents (0 mV) obtained at baseline, inhibition and washout phases when exposing to 10, 100 and 1000 μM bupivacaine. The intrapipette free calcium concentration was 1 mM. *P < 0.05, compared with baseline value.

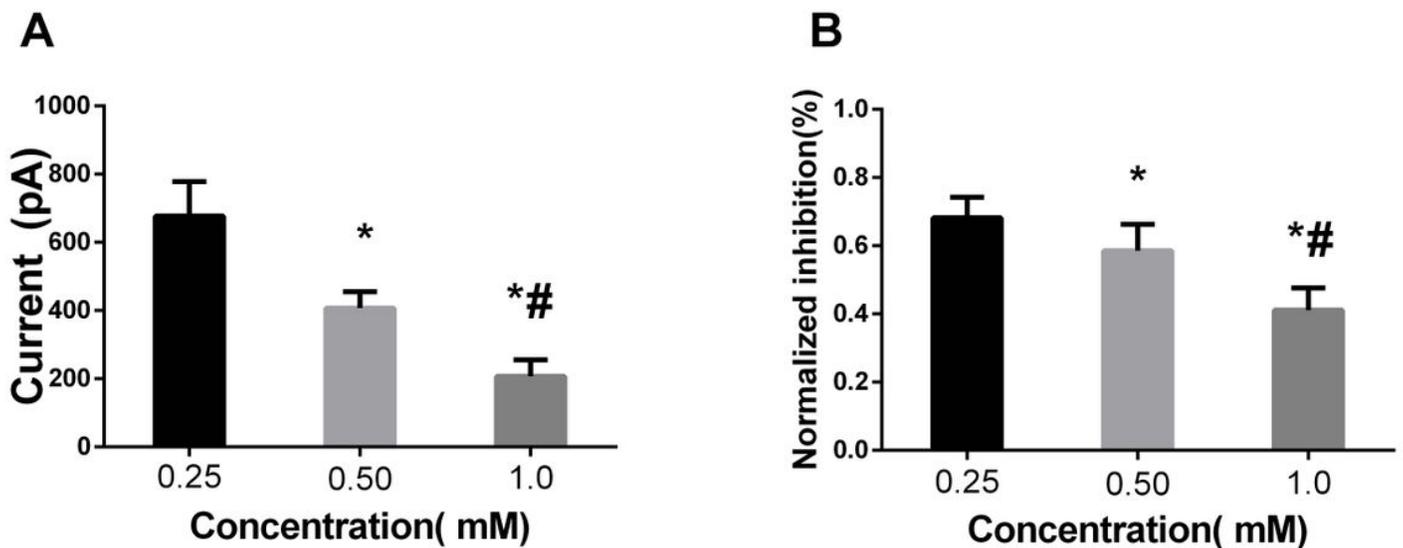


Figure 4

Effect of calcium concentration on the inhibitory effect of bupivacaine. (A) SK2 currents obtained at 0 mV in different concentrations of free calcium (n=8). (B) The degree of inhibition of SK2 currents (0 mV) by 100 μ M bupivacaine when the pipette solution contained different concentrations of free calcium (n=8). *P < 0.05, between 0.25 mM group and 0.5 mM group, #P < 0.05, between 0.5 mM group and 1 mM group.