



Research article

Scorpion Venom peptide, AGAP inhibits TRPV1 and potentiates the analgesic effect of lidocaine

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ABSTRACT

The current study was designed to test the hypothesis that BmK AGAP (AGAP) potentiates the analgesic effect of lidocaine. The chronic constrictive injury was performed on 72 rats to induce a rapid onset and long-lasting pain. The rats were randomly assigned to one of six groups; Group A (n = 12) received an intrathecal administration of saline, Group B (n = 12) received an intrathecal injection of lidocaine, Group C (n = 12) received an intrathecal administration of AGAP, Group D, E, and F (n = 12 each) received an intrathecal administration of lidocaine 0.005 mg/ml + AGAP 25, 50, 100 µg/kg respectively. The von Frey filaments were used to assess mechanical allodynia. Nav1.7 and TRPV1 currents were recorded by the whole-cell aspiration patch-clamp technique, and KCNQ2/3 currents were recorded by the whole-cell drilling patch-clamp technique. The whole-cell aspiration patch-clamp technique showed that AGAP inhibited TRPV1 and KCNQ2/3 currents and increased the analgesic effect of lidocaine. AGAP may have a synergistic effect with lidocaine which demonstrates a potential therapeutic approach for optimizing post-operative analgesia.

1. Introduction

Despite the many benefits of the existing local anaesthetics, study reports show a dose and time-dependent toxicity to a variety of tissues such as the heart and the nerves. The incidence of toxicity and other complications associated with local anaesthetics use can occur at the clinical concentration level and vary with the anaesthetic techniques, type of surgery, and patient factor [1, 2, 3]. Analgesics such as morphine and fentanyl are added to local anaesthetics to reduce the total required dose of local anaesthetic, prolong sensory block, minimise central nervous system effects, and optimise perioperative analgesia [4].

Scorpion *Buthus martensii Karsch* (BmK) venom is a natural compound that contains mixtures of peptides that have analgesic properties. Wang *et al* were the first to purify the BmK analgesic peptide from the venom [5]. Since then, more BmK analgesic peptides including AGAP have been purified for pain management in animal models [6]. The scorpion

peptide, AGAP is a long-chain scorpion peptide with a molecular mass of 7142 Da and 66 amino acids' residues [7]. Animal studies have demonstrated some analgesic effects of AGAP [5, 6]. However, a survey of the literature suggests that little is known about the effects of AGAP use as an anaesthetic agent with lidocaine. This study, therefore, aimed to examine the effects of AGAP with lidocaine during intrathecal use.

2. Materials and methods

2.1. Animals

Adult male SD rats weighing between 180 and 200 g were provided by the Animal Facility of Dalian Medical University. The rats were housed in standard transparent plastic cages under a 12 h/12 h light-dark cycle regime and were offered free access to food and water. The animal ethics committee of the Dalian Medical University approved all the study

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protocols and it was by the declaration of the national institutes of health guide for the care and use of laboratory animals.

2.2. Cell culture

Human embryonic kidney 293 (HEK 293) cells were purchased from the American Type Culture Collection (Beijing Zhongyuan limited, China). Using short tandem repeat (STR) analysis, the HEK293 cells were authenticated by Beijing Micro read Genetics (Beijing, China) before purchase. The HEK293 cells were routinely maintained in DMEM/F12 (Gibco, USA) medium, supplemented with 10 % fetal bovine serum (FBS) (Gibco, USA), penicillin 100 units/ml, and streptomycin 100 µg/ml (TransGen Biotech, China). The cells were maintained in an incubator at 37 °C humidified air with 5 % CO₂ atmospheric condition. The HEK293 cells were stably transfected with Nav1.7, KCNQ2/3, or TRPV1. The transfected cells were subcultured in a 35-mm Petri dish. Cell densities at plating were 800–1000 cells/ml and placed in a 37 °C/5 % CO₂ incubator for at least 24 h before being used for the experiments.

2.3. Drugs and reagents

Preservative-free 2 % lidocaine (31802232) was purchased from Tianjin Shuicheng Pharmaceutical limited by share Ltd, China, while Penicillin and streptomycin were purchased from TransGen Biotech, China. DMEM/F12, DMEM, horse serum, and fetal bovine serum were purchased from Gibco. DMSO, Retigabine, NaCl, CsF, CsCl, MgCl₂, CaCl₂, KCl, Glucose, HEPES, EGTA, and KF were purchased from Sigma-Aldrich, whereas Capsaicin was purchased from MedchemExpress, China. Stock solutions of lidocaine or Retigabine were diluted or dissolved in DMSO to prepare a 100 mM storage solution, and an extracellular gradient dilution solution was used to obtain the detection concentration for the experiment. A stock solution of capsaicin was also dissolved in DMSO to prepare a 300 µM storage solution and extracellular gradient dilution was used to obtain the detection concentration for the experiment.

2.4. Preparation of AGAP

The recombinant AGAP (rAGAP) was provided by Shenyang Pharmaceutical University School of Life Science and Bio-pharmaceutics (Shenyang, China). The AGAP was obtained as described previously [8]. The expression and purification of recombinant AGAP were primarily performed according to the Novagen pET system manual.

Different concentrations of AGAP (12.5, 25, 50, 75, 100 µg/kg) were injected intrathecally to determine the dose-response of AGAP. Base on the dose-response, we then selected different concentrations to further investigate the analgesic effect of AGAP along with lidocaine.

2.5. Cell viability assay

As previously described [9], to determine the cytotoxicity effect of AGAP and lidocaine, we seeded HEK293 cells in 96-well plates at a density of 1×10^4 cells per well. The cells were then incubated at 37 °C overnight. AGAP 10 µg/ml, lidocaine 100 µgM or AGAP and lidocaine combined were then used to treat cells and incubated in a humidified atmosphere of 5% CO₂ at 37 °C for 24, 48, 72 or 96 h. MTT stock solution (5 mg/mL) 20 µL was added to each well and the cells were incubated for an additional 4 h at 37 °C. The MTT solution was then discarded, and 100 µL of dimethyl sulfoxide (DMSO) solution was added into each well and incubated for 30 min in the dark to dissolve the insoluble formazan crystals. Optical density (OD) was measured at a test wavelength of 590 nm and a reference wavelength of 650 nm using an enzyme-linked immunosorbent assay (ELISA) multi-well plate reader (BioTek Instruments, Winooski, USA). To calculate percentage cell viability, OD values were used in the formula [9, 10]:

$$\% \text{ of cell viability} = \frac{\text{OD value of experimental sample (treated cells)}}{\text{OD value of experimental control (untreated cells)}} \times 100 \quad (1)$$

2.6. Chronic constrictive injury model (CCI)

The chronic constrictive injury (CCI) model was developed following the method of Bennett and Xie [11]. The individual rats were anaesthetized with sodium pentobarbital (20 mg/kg, intraperitoneal injection) and a small incision made at the mid-thigh level to expose the left sciatic nerve. Unilateral tight ligation of one-third of the sciatic nerve was induced with a single ligature (5-0 silk thread). The incision made was closed in layers and the surgical wound was treated with Vetericyn Plus antimicrobial hydrogel once daily.

2.7. Drugs application

The doses of the drugs were determined based on the results of previous studies and our preliminary experiments (AGAP [9, 12], and lidocaine as per manufacture protocols). An equal volume of AGAP (25, 50, 100 µg/kg) and preservative-free lidocaine 0.005 mg/ml were prepared using 0.9 % saline. The pH of lidocaine solution was adjusted to 6.8 with NaOH to avoid possible variations of pH with different drug concentrations that could alter local pharmacokinetics. We confirmed the presence of mechanical allodynia in rats on the second day after CCI. Rats were then randomly assigned to one of six groups using a computer-generated random number table. The group allocation was concealed in sealed opaque envelopes which were opened just before the administration of the drugs. Group A (n = 12) received intrathecal injection of saline as the negative control; Group B (n = 12) received intrathecal injection of lidocaine 0.005 mg/ml; Group C (n = 12) received intrathecal injection of AGAP 100 µg/kg; Group D (n = 12) received intrathecal injection of lidocaine 0.005 mg/ml + AGAP 25 µg/kg; Group E (n = 12) received intrathecal injection of lidocaine 0.005 mg/ml + AGAP 50 µg/kg; and Group F (n = 12) received intrathecal injection of lidocaine 0.005 mg/ml + AGAP 100 µg/kg. An independent researcher specialized in regional anaesthesia was assigned to carry out the intrathecal injections of the drugs. The method used to inject the drugs into the subarachnoid space was the same as previously described [13, 14].

A 28-gauge short bevel needle attached to a 25 µl Hamilton micro-syringe was inserted between the lumbar 5 and lumbar 6 vertebrates of conscious rats. A sudden slight flick of the tail indicated entry into the subarachnoid space. A volume of 15 µl of drug solution or 0.9 % saline was injected into the subarachnoid space for a period of 30 s. Motor function was assessed through observation of placing or stepping reflexes 5 min before assessing mechanical allodynia. Rats with complete motor blocks were excluded from the analysis. Three independent researchers specialized in pain assessment and were blinded to the administration of the drugs were assigned to monitor mechanical allodynia in the rats.

2.8. Measurement of mechanical allodynia

Mechanical allodynia was assessed using von Frey filaments (North Coast Medical, Inc., San Jose, CA) starting from 2 g and ending with 0.16 g or 15 g filaments as the cut-off values using the “up-and-down” method. The paw withdrawal threshold (PWT) was determined by consecutively increasing and decreasing the magnitude of the stimulus (“up-and-down” method). Before testing, an equal volume (15 µl) of drug solution or 0.9 % saline was injected into the subarachnoid space. The individual rats were then placed in a plastic box (20 × 25 × 15 cm) on a metal mesh floor and allowed for 20 min of adaptation. The filaments were then presented, in ascending order of strength (0.16, 0.4, 0.6, 1, 1.4, 2, 4, 6, 8, and 15 g), and held for 6–8 s perpendicular to the plantar surface with sufficient

force to cause slight bending against the paw. Paw flinching or quick withdrawal was considered a positive response to pain. A successful block was defined as a sensory block in all regions assessed within 30 min of local anaesthetic injections. The block performance time (time elapsed from intrathecal injection to the end of local anaesthetic injection into the subarachnoid space) and the number of time attempts were recorded. The paw withdrawal threshold was measured in the rats using the left ligated paw. The paw withdrawal threshold was assessed and recorded every 30 min until the complete resolution of anaesthesia. Complications, including vascular puncture, were noted.

Duration of analgesia was defined as the time interval between the administration of drug solutions and the complete resolution of anaesthesia. Motor block (time since the administration of the block until the absence of movement in the left hindlimb). Rats that exhibited motor blocks were excluded from this study. At the end of the study, rats were euthanized and blood serum, excised spinal cord and left hind sciatic nerve were collected and snap-frozen in liquid nitrogen for further investigations.

2.9. Electrophysiological recordings

The electrophysiological recordings were performed by Henan Scope Research Institute of Electrophysiology, China. Each experiment was performed with one 35 mm Petri dish of cells. Cells were washed with extracellular fluid twice and placed on an inverted microscope stage. The whole-cell patch-clamp technique was used for Nav1.7 and TRPV1 recording and the whole-cell drilling patch-clamp technique was used for KCNQ 2/3 current recording. Amphotericin B was used for the patch-clamp technique. Amphotericin B powder, 3 mg was first dissolved with 50 μ l of DMSO using sonication to give a 60 mg/ml stock solution and then, 8 μ l of the 60 mg/ml stock amphotericin B was dissolved in DMSO using the ultrasonic and oscillating dissolution to give a final concentration of 0.25 mg/ml. The final concentration of amphotericin B for the patch-clamp experiments was stored on ice in amber light-resistant microtubes to avoid light. The whole-cell patch-clamp experiments were performed at room temperature. The tip resistance of borosilicate glass microelectrode was 1–2 M Ω (Nav1.7) or 2–4 M Ω (KCNQ2/3 and TRPV1).

2.10. TRPV1 channel recording scheme

After the whole-cell recording mode, the membrane potential was clamped at 0 mV, and the cell was stimulated with -100 mV polarization voltage every 2 or 5 s, sustained for 80 ms, and back slope depolarization to 100 mV for 320 ms. The recorded current was used for the calculation of the TRPV1 current. The calculated current was compared statistically at \pm 100 mV. The TRPV1 current was then maintained for 80 ms at 100 mV voltage. The TRPV1 current was stabilized in extracellular fluid for at least 2 min before the drug was added. When the amplitude of TRPV1 current changed less than 5 %, it was considered that the drug action was stable after perfusion. The concentration of the compound was terminated when the current does not reach a steady state within 5 min.

2.11. KCNQ2/3 channel recording scheme

After the whole-cell recording mode, the membrane potential was clamped at -80 mV. The cell was stimulated with -20 mV depolarization voltage every 5 s, and hyperpolarized to -20 mV for 300 ms after 700 ms. The recorded current at -80 mV was used as the baseline for calculating the KCNQ2/3 current. The KCNQ2/3 current was recorded stably in extracellular fluid for at least 2 min before the addition of the compound. When the amplitude of KCNQ2/3 current changed less than 5 %, it was considered that the drug action reached a steady state after perfusion. When the current does not reach a steady state within 5 min, the concentration of the compound was terminated.

2.12. Nav1.7 channel recording scheme

After the formation of the whole-cell configuration, the cell was clamped at -130 mV and depolarized to -10 mV for 15 mini seconds (ms), then recovered to -130 mV. The clamp voltage of 20–30 % inactivation was selected for recording. The current was recorded in extracellular fluid for at least 2 min. When the amplitude of Nav1.7 changed less than 5 %, it was considered that the drug action was stable after perfusion.

2.13. Statistical analysis

Statistical analyses were carried out using GraphPad Prism v 5.01 (GraphPad Software, La Jolla, CA, US). The nonparametric method of Dixon as previously described [15] was used to analyse the data by the von Frey filaments. The electrophysiology raw data was recorded using Clampex 10.7 and stored as *.abf files on the SGs Computer Network. Data acquisition and analysis were performed using pCLAMP 10.7 software (Axon Instruments). The inhibition rates of TRPV1, KCNQ2/3 and Nav 1.7 currents measured were calculated using the formula [16, 17, 18, 19]:

$$\% \text{ Inhibition rate} = \{1 - (\text{Current Amplitude} / \text{Control Current Amplitude})\} \times 100 \quad (2)$$

All values are depicted as a mean \pm SD and considered significant if $P < 0.05$.

3. Results

3.1. Analgesic effects of intrathecal injection of AGAP in rat

To investigate the effects of AGAP along with lidocaine after intrathecal injection, we first examined the cytotoxic effect and the dose-response of AGAP and lidocaine in vitro and in vivo. The viability of HEK 293 cells from 94.6 % decreased to 77.2% at concentrations of 10 μ g of AGAP for 96 h. Similarly, at a concentration of 100 μ gM of lidocaine, the viability of HEK 293 cells from 80.9 % decreased to 75.0 % for 96 h. The dose-response of AGAP in rats using different concentrations (12.25, 25, 50, 75 100 μ g/ml) showed an increase PWTs in a dose-dependent manner (Figure 1 A, B, C).

We then injected lidocaine 0.005 mg/ml, AGAP 100 μ g/kg, lidocaine 0.005 mg/ml and AGAP 25, 50 or 100 μ g/kg combined or 0.9 % saline into the subarachnoid space to investigate the analgesic effects. The von Frey filaments were used to assess mechanical allodynia in rats every 30 min until complete resolution of anaesthesia (PWT returned to baseline). The time interval between the intrathecal injection of drug solutions and the complete resolution of anaesthesia was recorded as the duration of analgesia. The data indicated that the first 30 min of drug injections, rats from the lidocaine group exhibited an increased PWT of 11.1 g which gradually decreased to 1.3 g PWT at 240 min, whereas the rats from the AGAP 100 μ g/kg group exhibited PWT of 1.85 g at the first 30 min and decreased to 1.2 g PWT at 210 min. The results showed a significantly increased duration of analgesia in rats of the lidocaine group compared with rats of the AGAP 100 μ g/kg group ($P < 0.001$) (Figure 2A). The rats from the lidocaine and AGAP 25 μ g/kg combined group exhibited a 12.65 g increase in PWT at the first 30 min of drug administration and decreased to 1.2 g PWT at 270 min compared with rats from the lidocaine or AGAP 100 μ g/kg alone groups. The data indicated a significantly increased duration of analgesia in rats from the lidocaine 0.005 mg/ml and AGAP 25 μ g/kg combined group compared with rats from the lidocaine 0.005 mg/ml or AGAP 100 μ g/kg alone groups ($P < 0.001$) (Figure 2B). Rats from the lidocaine 0.005 mg/ml and AGAP 50 μ g/kg combined group showed a similar increase in PWT, 13.75 g after the first 30 min of drugs injections, which gradually decreased to 1.4 g PWT at 360 min when compared with rats from the lidocaine 0.005 mg/ml or AGAP 100 μ g/kg alone group (Figure 2C). Similarly, at the first 30 min of drugs injections, rats from the lidocaine

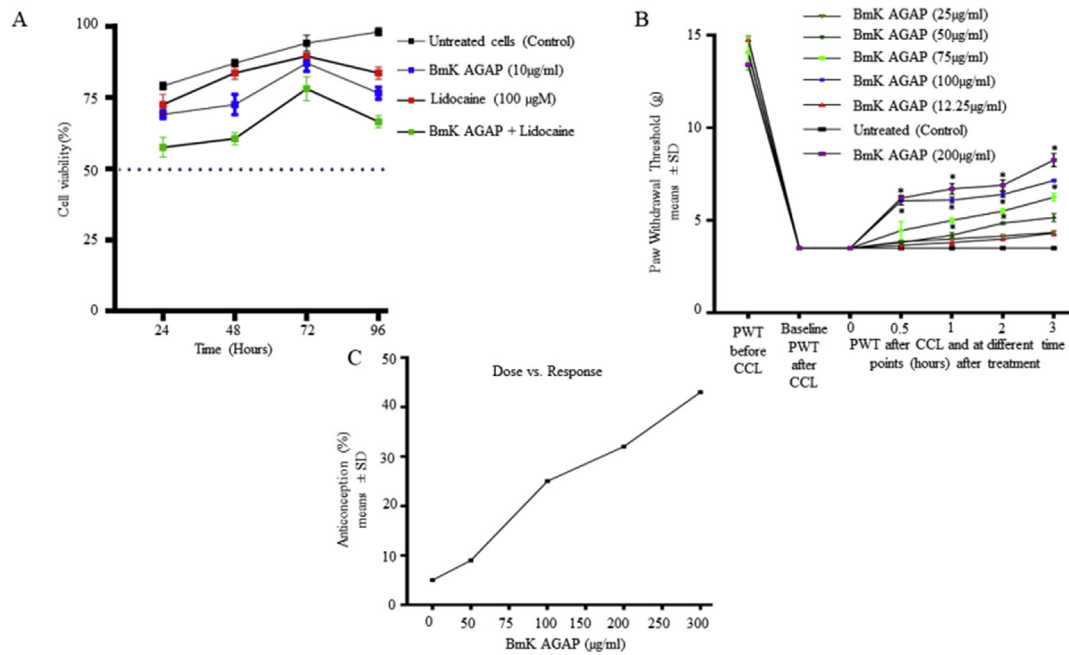


Figure 1. Cytotoxic effects of BmK AGAP and lidocaine. A. Percentage cell viability for the indicated time, B, C, dose-response of AGAP.

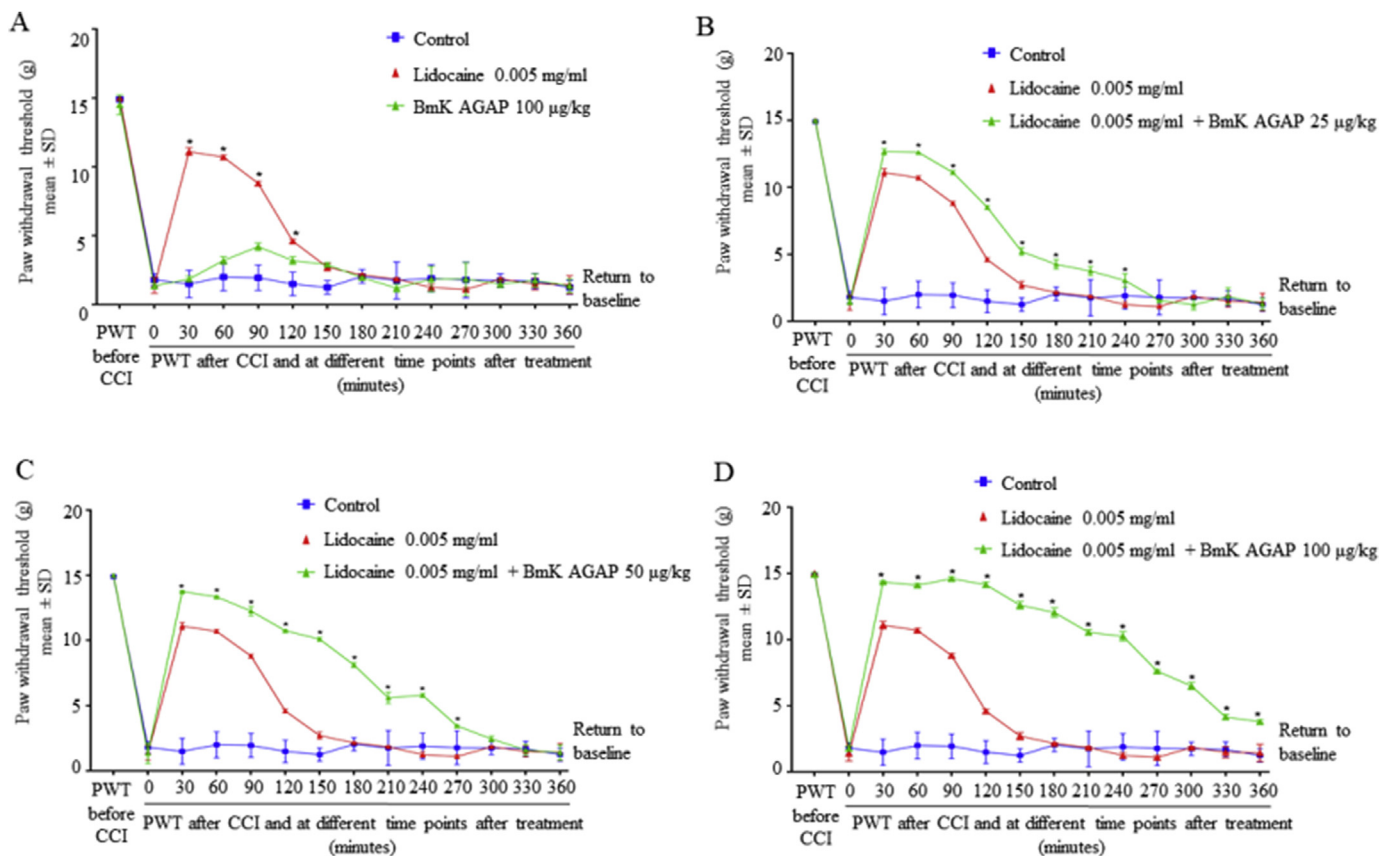


Figure 2. Intrathecal injection of BmK AGAP with lidocaine causes dose-dependent increase duration of analgesia. A, B, C, and D. between-group comparisons from 60-360 min found multiple significant differences at separate time points. PWT = paw withdrawal threshold. * Indicates statistical significance, $P < 0.05$.

0.005 mg/ml and AGAP 100 µg/kg combined group exhibited a similar significantly increase of 14.35 g PWT which gradually decreased to 3.8 g PWT at 360 min compared with rats from the lidocaine 0.005 mg/ml or AGAP 100 µg/kg alone group (Figure 2D). The data showed a

significantly increased PWT and duration of analgesia in rats that were supplemented with AGAP 25, 50, or 100 µg/kg ($P < 0.001$; $P < 0.01$; $P < 0.001$ respectively) when compared with rats from the lidocaine 0.005 mg/ml or AGAP 100 µg/kg alone group. It was observed that rats

that were supplemented with AGAP exhibited dose-dependent increases in PWT and duration of analgesia.

3.2. Potent modulation of TRPV1 channels by AGAP

TRPV1 is a nonselective cation channel that may be activated by a wide variety of exogenous and endogenous physical and chemical stimuli. The activation of TRPV1 leads to a painful, burning sensation [20]. To determine the mechanism by which AGAP potentiates the analgesic effect of lidocaine, we further tested to investigate whether AGAP affects capsaicin-activated TRPV1. The finding of this study showed that AGAP 10 $\mu\text{g/ml}$ significantly inhibited Capsaicin activated TRPV1 current between $47.73 \pm 2.71\%$ (-100 mV) and $72.74 \pm 2.01\%$ ($+100$ mV) (Figure 3 A). The I-V curves recording of the Capsaicin-activated TRPV1 channels before and after the treatment also showed that AGAP 10 $\mu\text{g/ml}$ have an inhibitory effect on TRPV1 current (Figure 3 B).

3.3. AGAP and KCNQ2/3 channels

Voltage-gated Kv7 (KCNQ) channels are voltage-dependent potassium channels that are activated at resting membrane potentials and consequently provide a potent brake on neuronal excitability [21]. To determine the mechanism by which AGAP potentiates the analgesic effect of lidocaine during intrathecal injections, we further tested to determine whether AGAP have any effect on KCNQ2/3 currents. The whole-cell configuration was stable for 5 min and then tested at three different voltages; -40 mV, 0 mV, and 40 mV. The data showed that AGAP 10 $\mu\text{g/ml}$ significantly changed the current compared with control (Figure 4 A). I-V curves were also recorded before and after AGAP treatment. The data showed that AGAP 10 $\mu\text{g/ml}$ significantly inhibited KCNQ2/3 currents (Figure 4 B).

3.4. AGAP and voltage-gated sodium channels

Sodium channels are essential membrane proteins that generate and transfer action potentials in axons, dendrites, and muscle tissue, design and filter synaptic inputs, and initiate and sustain membrane potential oscillation [22]. Sodium channels contain the presenting site for local anaesthetics. Sodium channels share structural features with other voltage-gated ion channels such as voltage-gated calcium and potassium channels [23]. To determine the mechanism by which AGAP exhibits and potentiates the analgesic effect of lidocaine, we first tested the effect of AGAP on voltage-gated sodium channel Nav 1.7. The HEK293 cells were stably transfected with Nav1.7.

The whole-cell aspiration patch-clamp technique showed that AGAP at a concentration of 1 $\mu\text{g/ml}$ or 10 $\mu\text{g/ml}$ had a weak inhibitory effect on the Nav1.7 current, $14.96 \pm 2.89\%$ and $18.23 \pm 2.12\%$ respectively (Figure 5 A), while the lidocaine at 100 μM significantly inhibited the

Nav1.7 current by $49.34 \pm 3.70\%$. The results of the combined treatment showed a similar inhibitory effect on Nav1.7 current (Figure 5 B). The time constant τ was obtained by fitting the deactivation section of the current when depolarizing to -10 mV, and it was found that 10 $\mu\text{g/ml}$ AGAP significantly slow down the deactivation (Figure 5 C). Figure 5D displays the activation of the square wave, the activation of the representative map, and the fitting curve before and after the administration of drugs. The data showed that the curve moved slightly to the left by AGAP. Next, we determined the inactivation square wave, the inactivation representative map before and after administration of drugs, and the fitted inactivation curve. The data showed that the curve slightly shifted to the left after the administration of the drugs (Figure 5 E).

4. Discussion

Experimental and clinical data indicate that noxious stimuli may sensitize structures of central neural involved in the perception of pain. Clinically, most of the local anaesthetics used are comparatively hydrophobic molecules that diffuse into or through the cell membrane to gain access to their blocking site. Local anaesthetics are multipurpose drugs that have been applied for infiltration, nerve block, peripheral or neuraxial anaesthesia and intravenously. The nerve-blocking potency of local anaesthetics increases with increasing molecular weight and increasing lipid solubility. The effectiveness of a given local anaesthetic is influenced by the dose, site of administration, additive, and temperature [24]. The functional effects of local anaesthetics arise from their dealings with specific ionic channels, receptors, and perhaps, from their general effects on biological membranes. Local anaesthetics bind to the sodium channel and inhibit sodium ion permeability that underlines the action potentials in nerves [25]. Local anaesthetics blockade of voltage-gated sodium channels (VGSCs) prevent the generation of action potentials at the nerve endings and also blocks action potential conduction along axons during infiltration for peripheral blocks [26]. Despite the existence of many local anaesthetics, there is therapeutic and scientific interest in the prolonged duration of local anaesthetics that can be established by a single injection.

Studies have shown that AGAP is a sodium channel-specific neurotoxin. It binds to voltage-independently at site-3 of sodium channels and inactivates the activated sodium channels to mediate analgesic activity [12]. Ruan *et al.*, in 2018 reported that intrathecal injection of the Chinese scorpion neurotoxin AGAP inhibits neuropathic and inflammation-associated pain through a MARK-mediated mechanism [14]. This study was designed to test the hypothesis that AGAP may inhibit TRPV1 and KCNQ2/3 and may potentiate the analgesic effect of lidocaine. From the data presented, the following essential observations emerged; AGAP inhibited TRPV1 and KCNQ2/3 currents; AGAP exhibited a synergistic effect with lidocaine and weak inhibitory effect on voltage-gated sodium channel Nav 1.7. These emerging pieces of

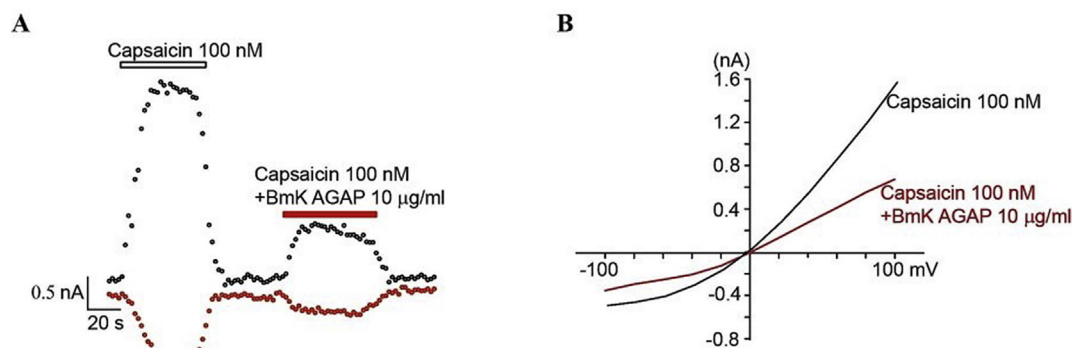


Figure 3. BmK AGAP inhibits Capsaicin-activated TRPV1 current. A. Paracellular administration of 100 nM Capsaicin activates TRPV1 channels and exhibits outwardly rectified features. Capsaicin 100 nM + BmK AGAP 10 $\mu\text{g/ml}$ significantly reduced the current was. B. I-V curves of Capsaicin activated TRPV1 channels before and after administration of BmK AGAP 10 $\mu\text{g/ml}$. Data were represented as mean \pm SD.

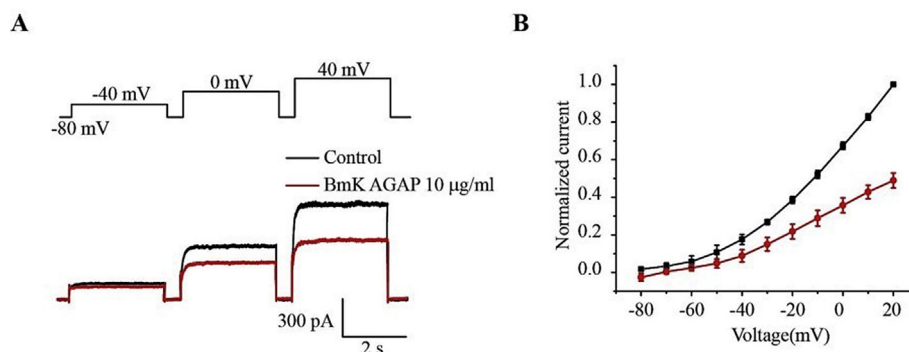


Figure 4. BmK AGAP inhibits KCNQ2/3 current. **A.** Stimulus curve and stabilization of the channel current before and after drug administrations. **B.** Shows the I–V curves of KCNQ2/3 channels before and after the administration of BmK AGAP 10 µg/ml. The data was statistically significant at * indicates $P < 0.05$; ** indicates $P < 0.01$; and *** indicates $P < 0.001$ as compared with control. Data were represented as mean \pm SD.

evidence suggested that AGAP may target TRPV1 and KCNQ2/3 to exhibit its analgesic effects.

TRPV1 has strongly been associated with nociception and pain, as well as thermal nociception and inflammatory hyperalgesia and allodynia, and neuropathic pain [27, 28, 29, 30, 31]. KCNQ2/3 channels expressed in dorsal root ganglion neurons are significant in pain transmission [32]. Study evidence shows that injection of QX-314 (lidocaine derivative) together with capsaicin into rat hind paws produces a long-lasting mechanical and thermal nociceptive threshold. Also, regional injection of QX-314 and capsaicin near the sciatic nerve produces a long-lasting decrease in pain sensitivity [33]. Monteiro de Araujo et al, reviewed existing literature on the role of pharmacological interventions on TRPA1 to reduce pain in numerous pain conditions and concluded that TRPA1 may be a therapeutic target for nociceptive pain [34]. Interestingly, while AGAP has been shown to directly block voltage-gated sodium channels, its effects on TRPV1 and KCNQ2/3 are yet to be known. The present study investigated the effect of AGAP on TRPV1 and KCNQ2/3 by electrophysiology. Our data revealed that AGAP has inhibitory effects on TRPV1 and KCNQ2/3 currents. Also, the findings of this study showed a long-lasting increase in nociceptive pain threshold by AGAP and lidocaine combined. These emerging pieces of evidence suggested that TRPV1 and KCNQ2/3 may be the main targets by AGAP to exhibit analgesic effects. We also observed that AGAP had a synergistic effect with lidocaine. Therefore, AGAP and local anaesthetics may be a novel therapeutic approach for optimising perioperative analgesia. A study by electrophysiology demonstrated that the expression of TRPV1 caused a 7.5-mV depolarizing shift in the voltage dependence of KCNQ2/3 activation. Activation of TRPV1 results in attenuation of KCNQ2/3 and reduction of KCNQ2/3-mediated current amplitude. Also, activation of TRPV1 suppresses the activity of the KCNQ2/3 channel. Hence, a negative regulatory mechanism mediated by KCNQ2/3 channels may be another way in which TRPV1 can facilitate its role in pain and establish a hyperexcitable state. Inhibitory effects of TRPV1 on KCNQ2/3 channels may reduce activation thresholds of nociceptor to other stimuli [35]. In this current study, we observed that AGAP exhibited significant inhibitory effects on TRPV1 and KCNQ2/3 and long-lasting analgesia when administered along with lidocaine. However, it remains to be shown the synergistic effects of AGAP with lidocaine on TRPV1 and KCNQ2/3. Determining the TRPV1 and KCNQ2/3 responses to different concentrations of AGAP and lidocaine combined may provide insights into a better understanding of the mechanism.

Voltage-gated sodium channels, such as Nav 1.6, Nav 1.7, Nav 1.8, and Nav 1.9 are implicated in pain signalling pathways [36, 37]. Electrophysiology by patch-clamp technique has demonstrated that AGAP inhibits Nav 1.8 current which decreases membrane excitability and blocks potential action propagation in nociception sensory neurons [11]. Voltage-gated sodium channel, Nav 1.9 is also found to play a role in AGAP analgesic activity. AGAP is reported to inhibit Nav 1.9 in

small-diameter dorsal root ganglion neurons and reduces the current which subsequently leads to loss of depolarisation and causes hyperpolarisation shift in the resting membrane potential. In so doing, it increases the threshold for initiating an action potential. AGAP inhibits Nav 1.9 current to attenuate inflammatory pain [11]. The genetic links of voltage-gated sodium channel Nav 1.7 to pain and Nav 1.8 as a result of its sensory neuron specificity have been focused on in particular as essential in the pathophysiology of pain [38]. In this present study, we demonstrated the effect of AGAP on voltage-gated sodium channel Nav 1.7. Contrarily to the findings of other studies, we observed that AGAP had a weak inhibitory effect on the voltage-gated sodium channel Nav 1.7. Hence, Nav 1.7 may not be the main target by AGAP to effect analgesic activity. However, our study is not without limitations. Further studies are needed on the mechanism of AGAP and lidocaine on TRPV1, KCNQ2/3 and Voltage-gated sodium channels.

5. Conclusion

The findings of this study demonstrated that AGAP inhibits TRPV1 and KCNQ2/3 currents and intrathecal injection of AGAP with lidocaine produces long-lasting analgesia. AGAP may have a synergistic effect with local anaesthetics which demonstrates a potential therapeutic approach for optimizing post-operative analgesia. However, a further detailed investigation is needed in the mechanisms by which the AGAP mediate lidocaine activities.

Declarations

Author contribution statement

Sylvanus Kampo: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

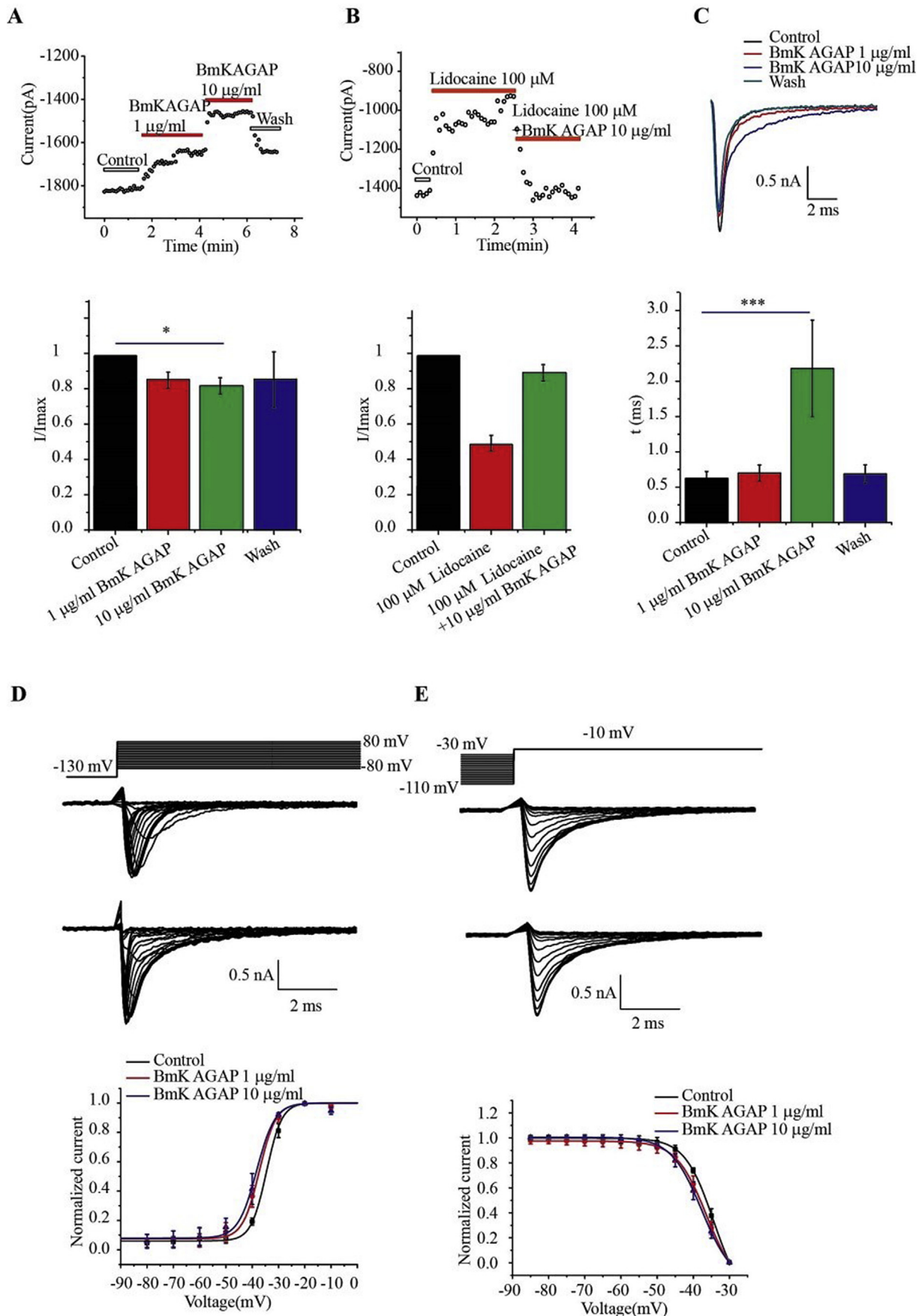
Yong Cui: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Jiachuan Yu; Thomas Winsum Anabah; Aglais Arredondo Falagán; Marcel Tunkumngen Bayor: Contributed to analyzing and interpreting the data; contributed reagents, materials, analysis tools or data.

Qing-Ping Wen: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Figure 5. BmK AGAP slightly inhibits Nav1.7 channel peak current and slow down activation. A. Paracellular perfusion administration of BmK AGAP 1 or 10 µg/ml. B. The whole-cell aspiration patch-clamp technique showed that BmK AGAP at a concentration of 1 µg/ml or 10 µg/ml had a weak inhibitory effect on the Nav1.7 current. C. The time constant τ was obtained by fitting the deactivation section of the current when depolarizing to -10 mV. D. Displays the activation of the square wave, the activation of the representative map and the fitting curve before and after the administration of drugs. E. The inactivation square wave, the inactivation representative map before and after administration of drugs and the fitted inactivation curve. The data was statistically significant at * indicates $P < 0.05$; ** indicates $P < 0.01$; and *** indicates $P < 0.001$ as compared with control. Data were represented as mean \pm SD.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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