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·Original Article·

Effects of a non-selective TRPC channel blocker, SKF-96365, on melittin-induced spontaneous persistent nociception and inflammatory pain hypersensitivity

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Abstract: Objective Melittin is the main peptide in bee venom and causes both persistent spontaneous nociception and pain hypersensitivity. Our recent studies indicated that both transient receptor potential (TRP) vanilloid receptor 1 (TRPV1) and canonical TRPs (TRPCs) are involved in mediating the melittin-induced activation of different subpopulations of primary nociceptive cells. Here, we further determined whether TRPC channels are involved in melittin-induced inflammatory nociceptive responses in behavioral assays. **Methods** The anti-nociceptive and anti-hyperalgesic effects of localized peripheral administration of three doses of the non-selective TRPC antagonist, SKF-96365 (1-{β-[3-(4-methoxyphenyl) propoxy]-4-methoxyphenyl}-1*H*-imidazole hydrochloride), were evaluated in melittin tests. Pain-related behaviors were rated by counting the number of paw flinches, and measuring paw withdrawal thermal latency (s) and paw withdrawl mechanical threshold (g), over a 1-h time-course. **Results** Localized peripheral SKF-96365 given before melittin prevented, and given after melittin significantly suppressed, the melittin-evoked persistent spontaneous nociception. Pre-blockade and post-suppression of activation of primary nociceptive activity resulted in decreased hypersensitivity to both thermal and mechanical stimuli applied to the primary injury site of the ipsilateral hindpaw, despite dose-effect differences between thermal and mechanical hyperalgesia. However, local administration of SKF-96365 into the contralateral hindpaw had no significant effect on any pain-associated behaviors. In addition, SKF-96365 had no effect on baseline threshold for either thermal or mechanical sensitivity under normal conditions. **Conclusion** Besides TRPV1, SKF-96365-sensitive TRPC channels might also be involved in the pathophysiological processing of melittin-induced inflammatory pain and hypersensitivity. Therapeutically, SKF-96365 is equally effective in preventing primary thermal and mechanical hyperalgesia as well as persistent spontaneous nociception. However, this drug is likely to be more effective in the relief of thermal hyperalgesia than mechanical hyperalgesia when applied 5 min after establishment of primary afferent activation.

Keywords: TRPC channels; melittin; persistent spontaneous nociception; primary thermal hyperalgesia; primary mechanical hyperalgesia

1 Introduction

Transient receptor potential (TRP) channels are a superfamily of non-selective cation-permeable channels that play important roles in somatosensation, including

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nociception[1-3]. Based on amino acid sequence homology, the mammalian TRP channel family can be divided into seven subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), TRPA (ankyrin) and TRPN (no mechanoreceptor potential C, or NOMPC)[2]. The TRPC subfamily members exhibit the highest homology to *Drosophila* TRPs and can be grouped into four subfamilies: TRPC1, TRPC2, TRPC3/6/7, and TRPC4/5, based on sequence homology and functional similarity[1,3,4]. TRPC channels are highly expressed in the central nervous system (CNS)[5,6] and rodent lumbar dorsal root ganglion (DRG)[7-9] where the mRNAs of TRPC1, TRPC3 and TRPC6 are most abundantly expressed in adults. Immunohistochemical staining also detects TRPC1, C3, C4 and C6 protein expression in rat lumbar DRG and in cultured DRG neurons[8], revealing the presence of TRPC channels in primary sensory neurons.

According to the mode of activation, TRPC channels are divided into receptor-operated and store-operated channels[10,11]. Both play important roles in brain development, including neural stem cell proliferation and differentiation, neuronal survival, axon pathfinding, neuronal morphogenesis, dendritic development, synaptogenesis, and growth cone development[12-14]. TRPC channels are also implicated in the onset or progression of many neurodegenerative diseases, such as Parkinson disease, Alzheimer disease and Huntington disease[12]. Yet no conclusion has been reached concerning the roles of TRPC channels in primary afferent nociception.

The melittin model, a well-established model of inflammatory pain[15,16], was used in this study. Melittin is a robust, basic 26 amino-acid polypeptide which is the active component of whole bee venom[17-19]. Our group has found that subcutaneous (s.c.) injection of melittin into rats causes not only long-term spontaneous pain but also strong mechanical and thermal hyperalgesia[20,21]. More recently, it was demonstrated that melittin activates primary nociceptive sensory neurons by activating TRPV1, via the phospholipase A2 (PLA2)-cyclooxygenases (COXs)/lipoxygenases (LOXs) cascade pathways, but not phospholipase C (PLC) signaling pathways[16]. Moreover, protein kinase A (PKA) was shown to be involved in the melittin-evoked activation of primary nociceptor cells, while PKC was not[16]. Interestingly, about 65% of these melittin-sensitive cells are IB4-positive DRG cells[16]. However, a previous study suggested that DRG cells co-expressing TRPC1 and IB4 or TRPC3 and IB4 do not contain TRPV1, while those with TRPC4 or TRPC6 do contain both TRPV1 and IB4[8], implying that some IB4-positive nociceptor cells sensitive to melittin do not contain TRPV1. In one of our more recent studies, we found that SKF-96365, a non-selective TRPC channel blocker, inhibits the melittin-evoked Ca^{2+} rise in 46.5% of primary sensory neurons[22]. Because SKF-96365-sensitive Ca^{2+} transients are not mediated by activation of TRPV1 channels[8], we proposed that the SKF-96365-blockable Ca^{2+} transients and currents evoked by melittin are not TRPV1-mediated. In the present study, we used the melittin test to determine whether SKF-96365 is effective in preventing and suppressing persistent spontaneous nociception, as well as primary heat and mechanical hyperalgesia.

2 Materials and methods

2.1 Animals Male albino Sprague-Dawley rats (weighing 180–220 g) were provided by the Laboratory Animal Facilities of both the Fourth Military Medical University and Capital Medical University. All experimental procedures were approved by Institutional Review Board of both universities and carried out in accordance with the guidelines for pain research in conscious animals. The animals were housed in plastic cages with food and water *ad libitum* under a 12 h:12 h light/dark cycle (with lights on from 08:00 to 20:00) at room temperature (22–26°C). Testing was done between 9:00 and 17:30. The rats were acclimated to test boxes for 5 days (>30 min per day) before the behavioral measurements. At the end of the experiments, all rats were sacrificed with an overdose of pentobarbital sodium (100 mg/kg, i.p.)

2.2 Drug administration and testing procedure A volume of 50 μL melittin solution (50 μg dissolved in 50 μL 0.9% sterile saline; Sigma, St. Louis, MO, USA) was used for all experiments. SKF-96365 (1-{β-[3-

(4-methoxyphenyl)propoxy]-4-methoxyphenyl}-1*H*-imidazole hydrochloride; Sigma) was dissolved in 0.9% sterile saline. To evaluate the effects of this inhibitor on the induction and maintenance of persistent spontaneous nociception, SKF-96365 (or saline as vehicle) was locally injected s.c. 5 min prior to or 5 min after s.c. melittin injection at the same site. For thermal and mechanical hyperalgesia, one of three doses of SKF-96365 (100, 10 or 1 μg in 20 μL saline, as indicated) was applied 5 min after s.c. melittin in the post-treatment paradigm, while one dose of SKF-96365 (100 μg in 20 μL) was administered 5 min before s.c. melittin in the pre-treatment paradigm. The paw withdrawal thermal latency (PWTL, s) and paw withdrawal mechanical threshold (PWMT, g) measurements were made 2 h after melittin injection. In addition, the baseline values of PWTL and PWMT in each group were measured prior to any treatment. Since there were no group differences in the baseline measurements for either PWTL or PWMT, the data were combined for further analysis. In general, the experimental order of these two measurements was randomized to rule out the effects of test order. The experimenters were blind to the treatment.

2.3 Quantitative analysis of spontaneous pain-related behaviors The paw flinch test was conducted based on our previously reports[20,21,23]. Briefly, a 30 × 30 × 30 cm^3 transparent Plexiglas test box with a transparent glass floor was placed onto a supporting frame 30 cm above the experimental table to allow the experimenters to observe the paws of the animals. The rat was placed into the test box for at least 30 min before administration of any chemical agents. After the acclimation period, s.c. injection of melittin was made into the center of the plantar surface of one hindpaw with minimal handling. The spontaneous nociceptive behaviour was determined by counting the number of paw flinches for 1 h following melittin injection.

2.4 Quantitative measurement of thermal pain sensitivity Thermal pain sensitivity was determined by measuring the PWTL in response to heat stimuli applied to the injected hindpaw. As described previously[20,21,23], the rat was placed on the surface of a 2-mm thick glass plate covered with a plastic chamber (20 × 20 × 25 cm^3), and heat stimuli were applied using a TC-1 radiant heat stimulator (RTY-3; Xi'an Bobang Technologies of Chemical Industry Co. Ltd., China). The radiant heat source was a high-intensity halogen lamp bulb (150 W) positioned under the glass floor directly beneath the target area on the hindpaw. Six stimuli were repeated for each site and the last five values were averaged as the mean PWTL. The inter-stimulus intervals were >10 min. The thermal latency was defined as the time from the onset of the heat stimulus to the occurrence of a hindpaw withdrawal reflex. A cutoff of 30 s was used to avoid excessive tissue injury.

2.5 Quantitative measurement of mechanical pain sensitivity Mechanical pain hypersensitivity was tested according to previous descriptions[20,21,23]. Briefly, the rat was placed onto a metal mesh floor covered with a plastic chamber, and von Frey filaments were applied from underneath the mesh to the testing site of the target hindpaw, in ascending bending forces of 2.0, 2.5, 3.0, 4.0, 5.0, 7.0, 10.0, 15.0, 20.0, 30.0, 35.0, 40.0, 50.0 and 60.0 g. Each von Frey filament was applied three times to the testing area. The bending force of the filament able to evoke an approximately 50% occurrence of the paw withdrawal reflex was identified as the PWMT. The stimulus was stopped if the threshold exceeded 60 g (cutoff value).

2.6 Statistical analysis All data are expressed as mean ± SEM. Two-independent-sample tests of non-parametric tests were used to analyze group differences in the mean time courses of spontaneous pain-related behaviors, as well as to compare differences in mean numbers of flinching reflexes during the 1-h period in the post- and pre-treatment paradigms. One-way ANOVA followed by *post hoc* Fisher's PLSD test was applied to the thermal or mechanical hyperalgesia measurements. A statistical difference was accepted as significant at $P < 0.05$.

3 Results

3.1 SKF-96365 reverses melittin-induced persistent spontaneous nociception We first determined whether s.c injection of SKF-96365 into the hindpaw affects the baseline value of PWTL and PWMT. Neither unilateral nor bilateral administration of 100 μg SKF-96365 affected the

thermal nociceptive latency (Fig. 1A) or the mechanical nociceptive threshold (Fig. 1B).

In accord with our previous reports[20,21], intraplantar s.c. injection of melittin solution immediately induced persistent spontaneous nociception as reflected by long-term spontaneous paw flinching or licking lasting for about 1 h (Fig. 2A, C). Ipsilateral post-treatment with 100 μg or 10 μg , but not 1 μg SKF-96365, resulted in significant attenuation of melittin-induced persistent paw flinches. The inhibitory action of SKF-96365 started 5 min after injection and lasted for nearly 20 min (Fig. 2A). The mean total numbers of paw flinches over the subsequent 55-min course following post-treatment with SKF-96365 are shown in Fig. 2B. The higher doses (100 μg and 10 μg) of SKF-96365 reduced the mean total number of paw flinches averaged for 1 h (P <0.01), while the low dose (1 μg) did not. The percentage inhibition of persistent spontaneous nociception by 10 μg SKF-96365 was 42.19% (n = 7, P <0.001) and by 100 μg was 44.37% (n = 6, P <0.001).

In the pre-treatment paradigm, this agent also remarkably suppressed the melittin-induced persistent nociceptive behavioral responses (Fig. 2C, D). Similarly, the duration of this inhibition was about 20 min (Fig. 2C). SKF-96365 treatment (100 μg in 20 μL) resulted in a 46.05% reduction of the mean total number of paw flinches (n = 5, P <0.001, Fig. 2D).

3.2 SKF-96365 reverses melittin-induced primary heat hyperalgesia To determine whether SKF-96365 reduces melittin-induced primary heat hyperalgesia, rats were subjected to radiant heat stimuli and the PWTL was recorded. Consistent with our previous results, s.c. melittin injection resulted in a significant reduction of thermal latency in the ipsilateral hindpaw (Fig. 3A, C). Post-administration of SKF-96365 at doses of 1, 10, and 100 μg increased the PWTL by 44.71% (n = 6, P <0.001), 46.60% (n = 5, P < 0.001) and 124.7% (n = 6, P <0.001) respectively (Fig. 3A).

Similarly, pre-treatment with 100 μg SKF-96365 inhibited the melittin-induced primary heat hyperalgesia (Fig. 3C). The decrease in thermal latency at the SKF-96365 injection site was almost abolished and the PWTL value was increased by 74.81% compared with the vehicle control (n = 5, P <0.001). This indicated that SKF-96365 reversed the melittin-induced thermal hyperalgesia at the primary injury site. However, SKF-96365 administration 5 min before or 5 min after melittin injection into the contralateral paw had little effect on melittin-induced reduction in PWTL (Fig. 3B, D).

3.3 SKF-96365 reduces melittin-induced primary mechanical hyperalgesia To determine whether SKF-96365 modulates melittin-induced mechanical hyperalgesia, von Frey monofilament tests were conducted. Melittin treatment induced a significant reduction in PWMT values, which was consistent with our previous results[20,21]. Post-

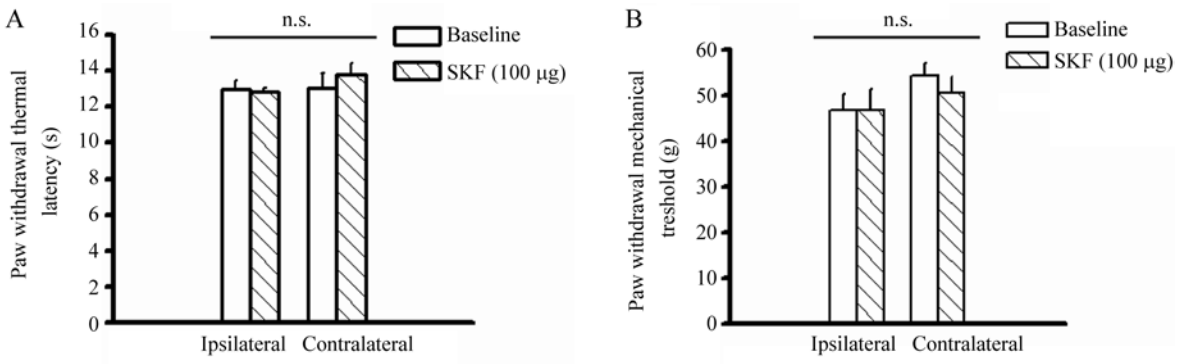

Fig. 1. SKF-96365 (SKF) did not affect the baseline value of thermal nociceptive latency and mechanical nociceptive threshold. SKF-96365 (100 μg in 20 μL) was injected s.c. into one hindpaw of the rats. Those receiving no treatment served as baseline controls. Two hours after SKF-96365 injection, paw withdrawal thermal latency (PWTL, s) and paw withdrawal mechanical threshold (PWMT, g) were recorded. SKF-96365 injection into one or both hindpaws did not affect the baseline PWTL (A) or the baseline PWMT value (B). Data are presented as mean ± SEM (n = 5 for each group). n.s., not significant.

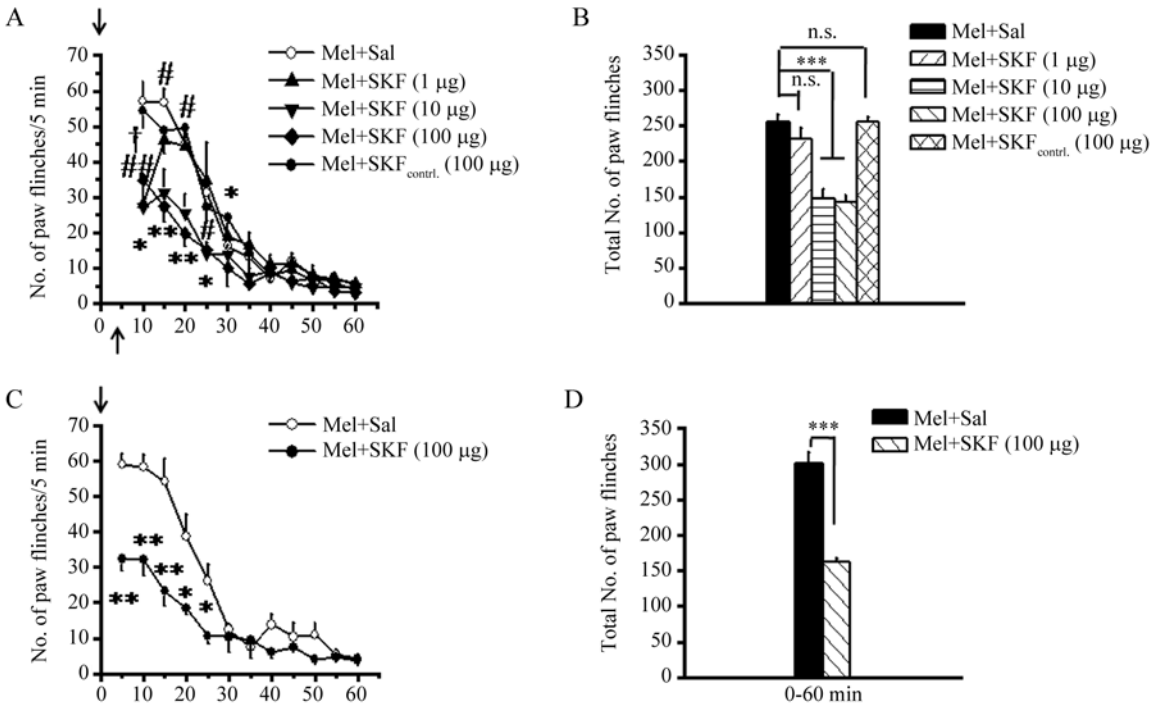

Fig. 2. SKF-96365 (SKF) both prevented and inhibited the maintenance of persistent spontaneous flinching reflexes induced by s.c. melittin (Mel). SKF-96365 was injected s.c. 5 min after or 5 min before melittin in the post- or pre-treatment paradigm. The spontaneous paw flinches were recorded for 1 h immediately after injection. The mean time courses recorded at each 5-min time block are shown in A and C, and the mean total numbers of paw flinches averaged from the 1 h period are shown in B and D. Post-treatment with SKF-96365 significantly inhibited (A, B), while pre-treatment with SKF-96365 profoundly reduced (C, D) melittin-induced persistent spontaneous flinching reflexes. Contrl., control group receiving SKF-96365 administration on the contralateral hindpaw. The reverse arrows at the ordinate indicate the starting point for melittin administration and the upright arrow at 5 min in A denotes the time of SKF-96365 injection for the post-treatment paradigm. Mean ± SEM (n = 5–8 for each group). *P < 0.05, **P <0.01, ***P <0.001, 100 μg SKF-96365 *vs* saline (Sal); $^{\#}P$ <0.05, $^{\#\#}P$ <0.01, 10 μg SKF-96365 *vs* saline; $^{\dagger}P$ <0.05, 1 μg SKF-96365 *vs* saline (two-independent-sample tests of non-parametric tests for the post-treatment paradigm and Student's *t*-test for the pre-treatment paradigm). n.s., not significant.

administration of 1 μg or 10 μg SKF-96365 only slightly increased the PWMT values in the melittin-treated hindpaw (Fig. 4A), but a higher dose (100 μg in 20 μL) partially blocked the generation of mechanical hyperalgesia, increasing the mechanical threshold (i.e., PWMT) by 80.87% (n = 6, P <0.01) compared with the vehicle control group (Fig. 4A). Pre-administration of SKF-96365 (100 μg in 20 μL) at the melittin injection site increased the mechanical threshold by 190.9% (n = 7, P <0.001), suggesting that pre-treatment also partially blocked the development of primary mechanical hyperalgesia (Fig. 4C). So, both post- and pre-treatment partially reversed the established mechanical hyperalgesia tested 2 h after intraplantar melittin injection, whereas neither post- nor pre-treatment with SKF-96365 on the contralateral hindpaw had any significant influence on PWMT values (Fig. 4B, D).

4 Discussion

4.1 Possible roles of TRPC channels in the mediation of melittin-evoked activation of primary sensory neurons In the present study, we demonstrated that s.c. injection of SKF-96365 did not affect the baseline thermal and mechanical sensitivity under normal conditions, but was effective in preventing the occurrence of both persistent spontaneous nociception and thermal/mechanical hyperalgesia in the primary injury site after melittin injection. These anti-nociceptive and anti-hyperalgesic actions of the drug were clearly local rather than systemic because local

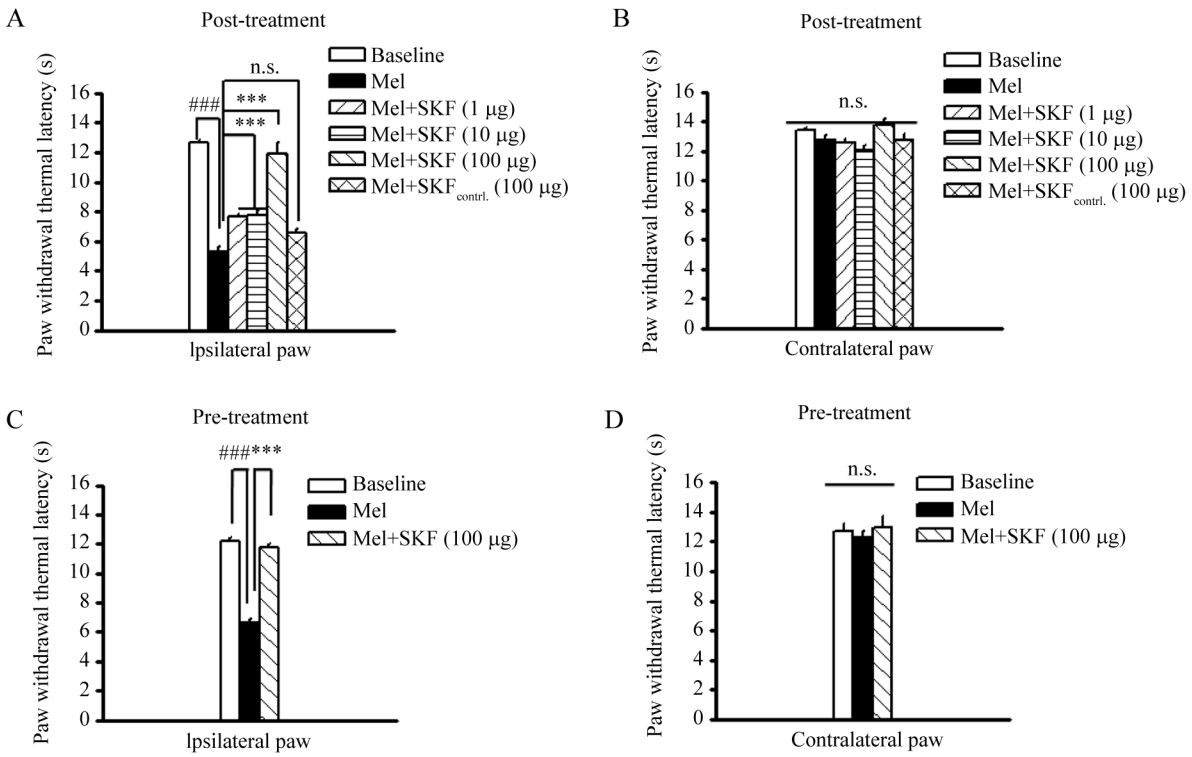

Fig. 3. SKF-96365 (SKF) reverses melittin-induced heat hyperalgesia. SKF-96365 was injected s.c. 5 min after or 5 min before melittin (Mel) in the post- or pre-treatment paradigm. Two hours after melittin injection, the paw withdrawal thermal latency (PWTL, s) was measured. A: Post-injection of SKF-96365 to the melittin site significantly reversed melittin-induced heat hyperalgesia on the ipsilateral side, but injection of SKF-96365 into the contralateral paw showed no significant effect on PWTL. B: The same post-treatment with SKF-96365 to the melittin site had no significant effect on PWTL of the contralateral side. C: Pre-treatment with SKF-96365 on the melittin-injected paw significantly reversed the melittin-induced heat hyperalgesia of the ipsilateral paw. D: The same pre-treatment with SKF-96365 at the melittin site had no significant effect on PWTL of the contralateral paw. Baseline, averaged values of PWTL obtained prior to any treatment. Contrl., control group receiving SKF-96365 on the contralateral hindpaw. Values are mean ± SEM (*n* = 5–6 for each group). ******P* <0.001; ###*P* <0.001 (one-way ANOVA with Fisher's PLSD *post hoc* test for the post-treatment paradigm and Student's *t*-test for the pre-treatment paradigm). n.s., not significant.

administration of SKF-96365 into the contralateral hindpaw had no effect on any of the pain-associated behaviors. SKF-96365 was primarily identified as an inhibitor of voltage-gated and receptor-mediated Ca^{2+} entry[24]. However, it was also shown to be a potent blocker of voltage-gated Ca^{2+} channels (VGCCs) and TRP channels[25,26]. Both TRPC3-[27] and TRPC6-mediated Ca^{2+} entry can be blocked by SKF-96365[28-30]. Moreover, SKF-96365 is believed to be a non-selective blocker of TRPC3/6/7 channels, leading to its wide use as a tool-drug for TRPC channels[3,31]. As for the functional roles of TRPC channels in primary sensory neurons, it has been demonstrated that TRPC3 mRNA is expressed in 35% of DRG neurons that are almost exclusively small, IB4-positive non-peptidergic nociceptor cells that are largely TRPV1-negative[7]. Also, melittin, on one hand, can excite primary nociceptive sensory neurons by activating TRPV1 via PLA2-COXs/LOXs cascade pathways (but not PLC signaling pathways)[16], while on the other hand, it can activate another subpopulation of DRG cells that is sensitive to SKF-96365[22], suggesting the involvement of TRPC and VGCCs. However, VGCCs are not likely to be involved in the melittin-evoked activation of nociceptor cells because the clamp voltage for inward current recordings was set at -70 mV under which VGCCs cannot be activated at all[16,22]. It is also of interest that 65% of the melittin-sensitive DRG cells are IB4-positive[16]. However, DRG cells that co-express TRPC1 and IB4 or TRPC3 and IB4 were shown to be TRPV1-negative[8],

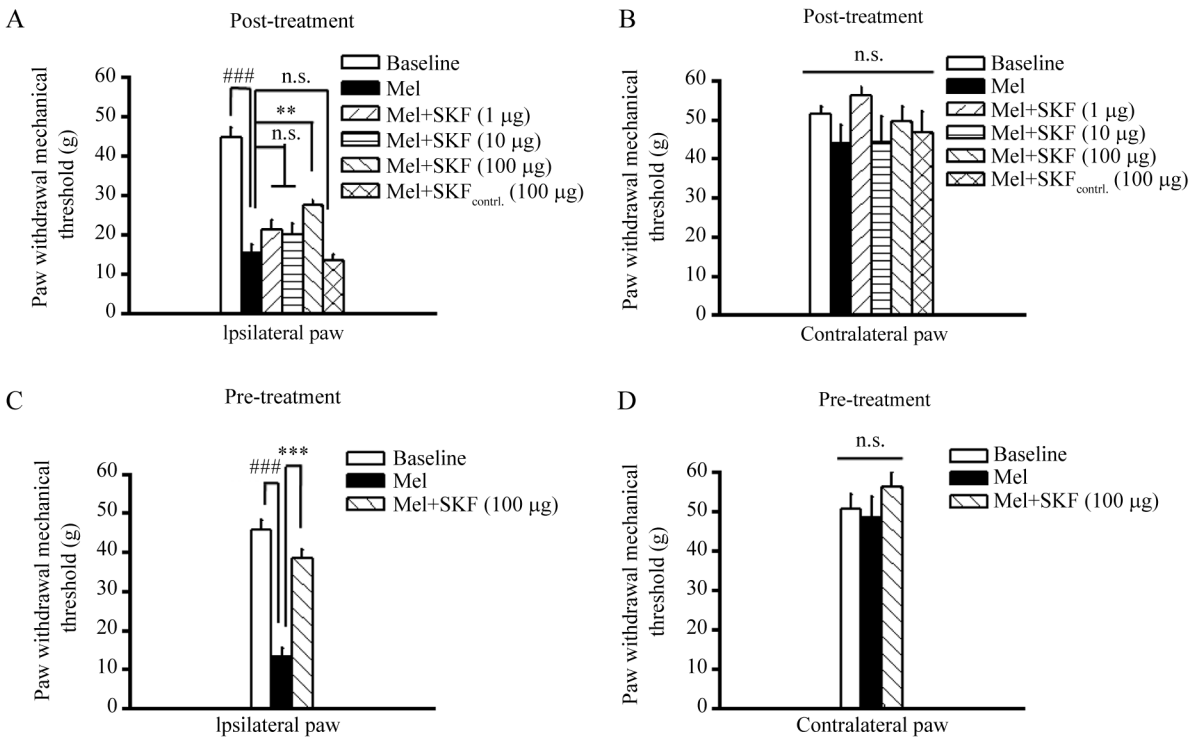

Fig. 4. SKF-96365 (SKF) reverses melittin-induced mechanical hyperalgesia. SKF-96365 was injected s.c. 5 min after or 5 min before melittin in the post- or pre-treatment paradigm. Two hours after melittin injection, the paw withdrawal mechanical threshold (PWMT, g) was measured and recorded. A: Post-treatment with 100 μg SKF significantly reversed melittin-induced mechanical hyperalgesia, but injection of SKF to the contralateral paw had no significant effects on PWMT. B: The same post-treatment with SKF-96365 to the melittin site had no significant effect on PWMT of the contralateral side. C: Pre-treatment with SKF at the melittin site greatly reversed melittin-induced mechanical hyperalgesia of the ipsilateral paw. D: The same pre-treatment with SKF-96365 to the melittin site had no significant effect on PWMT of the contralateral paw. Baseline, averaged values of PWMT obtained prior to any treatment. Contrl., control group receiving SKF-96365 in the contralateral hindpaw. Values are mean ± SEM (n = 5–6 for each group). $^{**}P$ <0.01, $^{***}P$ <0.001 SKF-96365 *vs* vehicle; $^{###}P$ <0.001 melittin *vs* baseline (one-way ANOVA with Fisher's PLSD *post hoc* test for the post-treatment paradigm and Student's *t*-test for the pre-treatment paradigm). n.s., not significant.

implying that some IB4-positive non-peptidergic nociceptor cells that are sensitive to melittin do not contain TRPV1. This is similar to the pharmacological results demonstrating that SKF-96365-sensitive Ca^{2+} transients are not mediated by the activation of TRPV1 channels[8]. We thus propose that the SKF-96365-blockable Ca^{2+} transients and currents evoked by melittin are not mediated by TRPV1 but more likely by TRPC channels.

4.2 Possible roles of TRPC channels in mediation of melittin-evoked persistent spontaneous nociception As shown in our previous behavioral and electrophysiological studies[15,20,21,32-34], melittin-evoked persistent spontaneous paw flinches are driven by persistent spike discharges of spinal dorsal horn pain-related neurons that are dependent upon the long-term activation of nerve terminals of primary nociceptive neurons in the primary injury site. As proposed above, melittin, which can directly activate PLA2[15,19], recruits the production of both COXs and LOXs, leading to the opening of TRPV1 channels in a subpopulation of nociceptor cells[16]. Meanwhile, melittin, which can insert itself into the lipid membrane, causes pore formation that leads to the release of ATP and the activation of P2X channels and P2Y G protein-coupled receptors (GPCRs)[32]. Activation of GPCRs by melittin results in the production of diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3). DAG is an endogenous activator of TRPC3/6/7 channels that are sensitive to SKF-96365[31,36-37], and this may serve as another route for melittin to activate the subpopulation

of nociceptor cells containing IB4, but not TRPV1. Whether the melittin-evoked Ca^{2+} rise and inward current in DRG cells that are SKF-96365-sensitive but capsazepine-insensitive, are mediated by the PLC pathway, is not clear and requires further studies. Taken together, the melittin-evoked persistent spontaneous nociception may be initiated by activation of both TRPV1 and TRPCs channels.

4.3 Possible roles of TRPC channels in mediation of melittin-evoked primary thermal and mechanical hyperalgesia As for the melittin-induced primary thermal hyperalgesia, it is clear that TRPV1, a thermal nociceptor, participates in this process[16,20]. Moreover, ATP receptors (P2X and P2Y) and mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 MAPK, are also involved in the sensitization of thermal nociceptors[32-35]. Based on the present results, SKF-96365-sensitive TRPCs are also likely to be involved in the development of primary thermal hyperalgesia.

Unlike primary thermal hyperalgesia, the melittin-induced primary mechanical hyperalgesia is not mediated by the activation and sensitization of TRPV1[16,20] or activation of the MAPKs (ERK, JNK, and p38 MAPK)[33-35]. Surprisingly, only ATP receptors (P2X and P2Y) are involved in this mechanical hyperalgesia[32]. It has been reported that activation of the $P2Y_2$ receptor, via G protein and stimulation of PLC, induces TRPC3/6/7 channel opening in rat cardiomyocytes[38]. As a new target, TRPCs might be important in the induction of primary mechanical hyperalgesia based on the following evidence: (1) TRPC6 is directly activated by stretch in non-neuronal cells[39-41]; and (2) TRPC1 and TRPC6 channels cooperate with TRPV4 to mediate mechanical hyperalgesia and nociceptor sensitization under inflammatory conditions[42]. Accordingly, all these findings add strength to the pivotal involvement of SKF-96365-sensitive TRPC channels in the primary mechanical hyperalgesia induced by melittin.

5 Conclusion

Taken together with our previous results, besides TRPV1, SKF-96365-sensitive TRPC channels might also be involved in the pathophysiological processing of melittin-induced inflammatory pain and hypersensitivity. As for therapeutic uses, SKF-96365 is equally effective in preventing the occurrence of primary thermal and mechanical hyperalgesia as well as persistent spontaneous nociception. However, this drug is likely to be more effective in the relief of thermal hyperalgesia than mechanical hyperalgesia when applied 5 min after the establishment of primary afferent activation.

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