MENTHOL DERIVATIVE WS-12 SELECTIVELY ACTIVATES TRANSIENT RECEPTOR POTENTIAL MELASTATIN-8 (TRPM8) ION CHANNELS

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ABSTRACT

Transient receptor potential melastatin-8 (TRPM8), a cationic ion channel is involved in detection of normal cooling-sensation in mammals. TRPM8 activation by cooling or chemical agonists have been shown to produce profound, mechanistically novel analgesia in chronic pain states such as neuropathic pain in rodents. Known TRPM8 agonists such as menthol and icilin have a relatively low potency and cross-activate nociceptors like TRPA1; thus bearing a limited therapeutic usefulness. For that reason, characterising ligands, which selectively activate TRPM8, presents a clinical need. Using *Xenopus laevis* oocytes as expression system, we evaluated WS-12, a menthol derivative, for its potential interaction with all six thermo-sensitive TRP ion channels. Oocytes were injected with cRNA of gene of interest and incubated for 3-5 days (at 16°C) before testing for functional characterisation of the recombinant ion channels. Oocytes were superfused with the test and standard substances respectively. Responses were measured by two-electrode voltage clamp technique and the amplitudes of evoked currents were compared with baseline values. WS-12 robustly activated TRPM8 in low micromolar concentrations (EC_{50} 12±5 µM) thereby displaying a higher potency and efficacy compared to menthol (EC_{50} 196±22 µM). Any of the other described thermo-sensitive TRP ion channels including TRPV1, TRPV2, TRPV3, TRPV4 and TRPA1 were not activated at a concentration (1 mM) optimally effective for TRPM8 responses; a characteristic which is in sharp contrast to menthol as it activates TRPA1 and TRPV3 in addition to TRPM8. Unlike icilin (~75% reduction; $p<0.001$, $n=6$), WS-12 does not induce tachyphylaxis (4±2.3% increase in responses; $p<0.08$, $n=6$) of TRPM8 mediated currents to repeated exposure of 1 mM doses. In addition, acidosis or variations in extracellular calcium have no influence on potency/efficacy of WS-12 for TRPM8. The selectivity profile of WS-12, its several-fold higher potency and around two-fold increase in efficacy compared to menthol warrants its potential utility for therapy in chronic neuropathic pain states and as a diagnostic probe in prostate cancer.

Keywords: TRPM8 specific ligand; WS-12; menthol; neuropathic analgesia; *Xenopus laevis* oocytes.

INTRODUCTION

The transient receptor potential (TRP) ion channel family comprises about 28 mammalian cation channels. These channels are involved in a wide range of physiological and pathophysiological processes including thermal sensation, pain, cell cycle regulation, fertilization and taste. Due to a relatively recent discovery, the knowledge about expression patterns and/or the diversity of gating stimuli as well as the pharmacology and function of this family of ion channels is far from comprehensive (Clapham *et al*., 2001; Clapham 2003; Montell 2005 and Nilius *et al*., 2007). Six members of the TRP super family including vanilloid receptors like TRPV1, TRPV2, TRPV3 and TRPV4 as well as TRPM8 and ankyrin-repeat-1 (TRPA1) are believed to function as molecular sensors and detectors of chemical and thermal stimuli on primary afferent nerve fibers of the somatosensory nervous system (Jordt *et al*., 2003; Dhaka *et al*., 2006; Bautista *et al*., 2006; Lumpkin & Caterina, 2007; Levine & Alessandri-Haber 2007).

Owing to their varied expression patterns and diverse functionalities these TRP ion channels are emerging as promising targets for the treatment of various pathological conditions like neuropathic pain (Walker *et al*., 2003; Levine & Alessandri-Haber, 2007), pruritus (Stander *et al*., 2004; Biro *et al*., 2007), inflammatory hyperalgesia (Szallasi & Appendino, 2004) and human skin cancers (Yamamura *et al.*, 2008). In recent years, however, several studies focused on TRPV1 and established a pivotal role of the channel in nociception. Desensitisation of TRPV1 is thought to underlie the analgesic effect of natural compounds like capsaicin (an active ingredient of hot chili), and new synthetic antagonists are being actively developed for better drugs (Gunthorpe *et al*., 2004 and Weil *et al*., 2005).

A striking concept for novel analgesics involves TRPM8 as an alternate pharmacophore target. While moderate cooling has a long history in pain relief, the mechanism underlying such analgesia has remained unknown. The action of menthol and similar coolant compounds on "thermoreceptors" provides the "cool" sensation via cold

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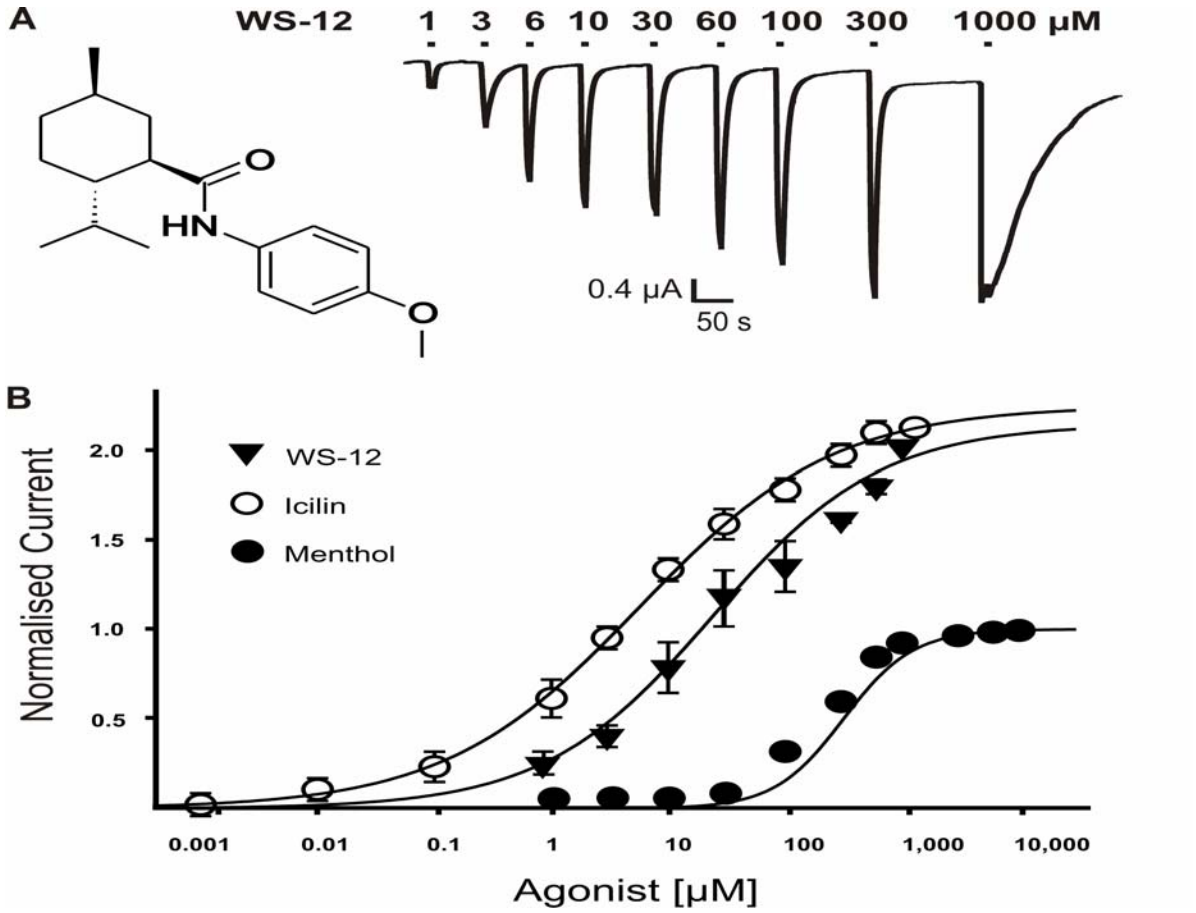

Fig. 1: Activation and dose-response relationship for WS-12. **A**: Chemical structure of WS-12 and a representative trace for TRPM8 activation by different concentrations of WS-12 depicting a dose-response relationship. **B**: Dose-response curve for icilin, menthol and WS-12 in 1.8 mM Ca^{2+}-containing solution. EC_{50} for all three compounds were structured from mean values of six independent experiments. Currents were normalized to the response to 10 mM Menthol. Note: - icilin does not activate TRPM8 in Ca^{2+}-free solutions so dose-response relationship cannot be established for comparison, in contrast, menthol and WS-12 do not require Ca^{2+} for inducing TRPM8 activation.

receptors. In the case of menthol and certain other coolant compounds one can also get a "hot" or stinging "pain" sensation. Menthol can act at high concentrations in much the same way as capsaicin to produce a hot sensation, but in this case, it stimulates the fibers that register both cold and warm temperatures. Recently, it was demonstrated that TRPM8, in particular, mediates analgesia caused by mild cooling and cooling agents such as menthol and icilin (Proudfoot *et al*., 2006). The inhibition of nociceptive input to the central nervous system was suggested as a possible underlying mechanism. TRPM8-expressing DRG neurons evoke release of glutamate at central synapses, which in turn inhibits the input of nociceptive afferents to dorsal horn neurons likely via activation of metabotropic glutamate receptors. Thus, the neural transmission through injury-activated or chronically active nociceptors will be attenuated (Proudfoot *et al*., 2006).

In this context, specific and potent TRPM8 agonists can play a very important role as potential therapeutics for the relief of traumatic or chronic neuropathic pain. Previously identified TRPM8 agonists such as menthol or icilin (Chuang *et al*., 2004; Peier *et al*., 2002) display either a relatively low affinity (Proudfoot *et al*., 2006) or cross activate other TRPs (Weil, 2005; Macpherson, 2006; Karashima, 2007), thus bearing limited therapeutic appliance and calling for new and improved TRPM8-targeting agents as potential analgesic drugs.

In the present work we present selectivity data about WS-12 {*N*- (4-methoxyphenyl)-p-menthone-3-carboxamide}, a menthol derivative also reported earlier (Beck *et al*., 2007: Bödding *et al*., 2007) to be the most potent agonist for TRPM8. We discovered WS-12 to be the most selective & efficacious TRPM8 agonist, which does not desensitize TRPM8 after repeated exposure and does not cross-activate any of the other six members of thermo-

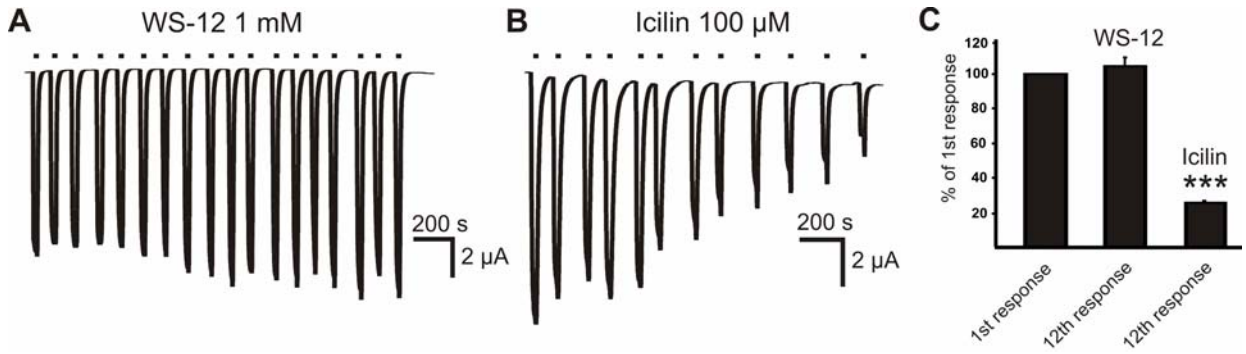

Fig. 2: Tachyphylaxis of TRPM8 mediated currents evoked by icilin versus WS-12. **A**: Representative trace showing no tachyphylaxis of TRPM8 mediated currents by repeated application of WS-12 (1 mM). **B**: Representative trace showing tachyphylaxis of TRPM8 responses to repeated application of 100-µM icilin. **C**: Comparative reduction in responses of TRPM8 at 12th application either to WS-12 or icilin. Response to WS-12 remained stable whereas that to icilin decreased ~ 74%.

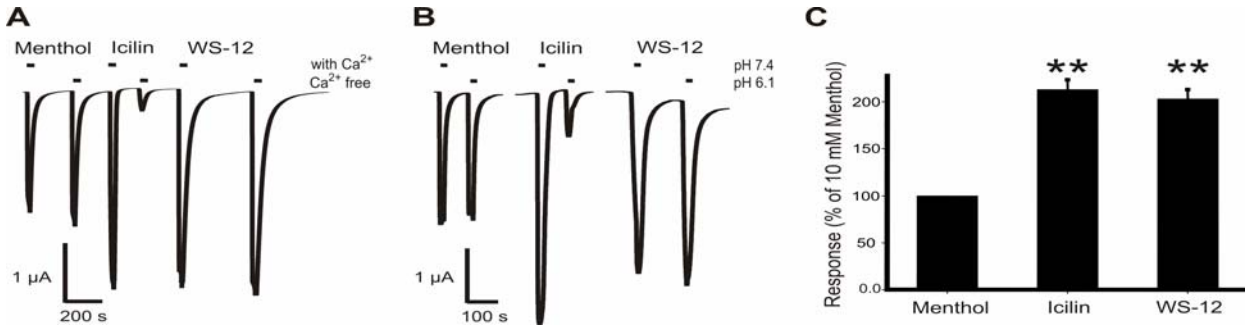

Fig. 3: Characterization of the mode of activation of TRPM8 by WS-12 in comparison to menthol and icilin. **A**: Activation of TRPM8 in the presence and absence of extracellular calcium. While menthol and its derivative WS-12 show similar amplitudes under both conditions, icilin evoked currents are strongly reduced. **B**: Activation of TRPM8 at different pH. Menthol and its derivative WS-12 activate similar currents at pH 6.1 and at pH 7.4, while icilin currents are reduced at an acidic pH. **C**: Comparative efficacy (means of maximum signal amplitudes) of WS-12 and icilin relative to menthol. Amplitudes of currents evoked by approximate saturating concentrations of WS-12 (1 mM) and icilin (100 µM) were normalized to 10 mM menthol responses.

TRPs subfamily as compared to menthol, which activates TRPV3 and TRPA1. In addition, the effects of WS-12 on TRPM8 are not affected either by pH variation (a hallmark of tissue acidosis and inflammation) or changes in extracellular Ca^{2+}, the conditions under which icilin loses its efficacy for TRPM8. Although the pharmacological efficacy of WS-12 is similar to that of icilin, it activates TRPM8 ion channels through a different mechanism.

MATERIALS AND METHODS

Expression vectors for thermo-TRP channels

Expression vectors for the TRP channels hTRPV1, rTRPV2, mTRPV3, rTRPV4, hTRPA1 and mTRPM8 (h, human, m, mouse, r, rat) were generous gifts from Dr David Julius (University of California, San Francisco), Dr M. Schaefer (Charite Berlin, Germany) and H.J-Behrendt. For the efficient expression in *Xenopus laevis* oocytes, cDNA inserts of hTRPV1, rTRPV2, mTRPV3, rTRPV4, hTRPA1 and mTRPM8 were subcloned by a PCR-based standard method into the oocyte expression vector pSGEM (Villmann *et al*., 1997).

Synthesis and injection of thermo-TRP cRNA

The generation of cRNA was performed by standard molecular-biology methods. In order to use plasmids containing cloned cDNA as a template for *in vitro* transcription, plasmids were linearized downstream of the end of the cDNA. Capped cRNAs were synthesized in the presence of capping analogue $m^7G(5')ppp(5')G$ using the AmpliCap-T7 MessageMaker Kit (Epicentre, Madison, WI). cRNA was ethanol-precipitated and re-dissolved in RNase-free water to give a final concentration of 1 µg/µl. Ovarian lobes were obtained from mature female *Xenopus laevis* frogs (anaesthetized by immersion in 0.15% 3-aminobenzoic acid ethyl ester and a partial ovarectomy was performed to isolate oocytes). The harvested ovarian

lobes were rinsed in Ca^{2+}-free modified Barth's solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 5 mM Tris-HCl, 0.82 mM $MgSO_4$, and 100 U/ml penicillin, 50 µg/ml streptomycin at pH 7.4). After treatment of the ovarian tissue with collagenase-Type I (4 mg/ml in Ca^{+2}-free Barth´s solution) for two hours at room temperature, the oocytes were treated with a calcium-gradient (increasing calcium concentrations) solution and incubated overnight in fresh Barth's solution (13°C). After 24 h, mature healthy and smart-looking oocytes (stage V to VI) were selected for cytoplasmic injection of cRNA (about 50 nano-liter per oocyte; approximate cRNA concentration 1 µg/µl) with a sharp pipette using a pulse-injector driven by air pressure (npi PDES 04T, Tamm, Germany). Afterwards, injected oocytes were placed in ND-96 solution (96 mM NaCl, 2 mM KCl, 0.8 mM $CaCl_2$, 1 mM MgCl, 5 mM HEPES, 10,000 units/ml penicillin G, 50µg/ml streptomycin sulphate and 25 µg/ml amphotericin B; pH 7.4) and incubated at 16°C. Oocytes were tested for functional expression of TRP channels encoded by specific cRNA messages after 3 to 5 days.

Electrophysiological recordings in oocytes

Two-electrode voltage-clamp recordings were performed to obtain current responses. Agonists and antagonists were diluted to the concentrations stated in the text in either calcium-containing (100 mM NaCl, 2.5 mM KCl, 1.8 mM $CaCl_2$, 200 µM flufenamic acid, 10 mM HEPES, pH 7.4) or calcium-free (100 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, 1.5 mM EGTA, 200 µM flufenamic acid and pH adjusted to 7.4) standard extracellular solutions. Agonists were applied by means of a multibarrel single tip superfusion device or by manual application. Application time was usually 10 sec. Electrodes were pulled from borosilicate glass using a Kopf vertical pipette puller (David Kopf Instruments, CA, USA). Electrodes were backfilled with 3M KCl. Membrane holding potential (V_h= -60 mV) was controlled and current signals were recorded with a two-electrode voltage-clamp amplifier (TURBO TEC-03, npi, Tamm, Germany) and the PCLAMP software (Axon Instruments, Sunnyvale, CA).

Statistics

All data were analysed for statistical significance by using student-t test (Microsoft Excel). To determine confidence intervals p values like $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***) were used to indicate various levels of statistical significance. Data are expressed as averaged mean ± S.E.M. of 6 means from independent experiments under similar experimental conditions unless otherwise stated.

Reagents

All cell culture reagents were obtained from Invitrogen (Karlsruhe, Germany). Chemicals for intracellular and extracellular solutions in voltage-clamp measurements were obtained from Sigma Aldrich. WS-12, a menthol derivative was kindly provided by Prof. Edward T. Wei from the University of California at Berkeley, USA.

RESULTS

Activation of TRPM8

The menthol-derivative WS-12 was tested for its capacity to activate TRPM8 channels expressed in *Xenopus* oocytes (Fig 1A). Application of WS-12 was found to robustly activate TRPM8 at a holding potential of V_h = -60 mV. We quantified the activation of TRPM8 by WS-12 with concentrations ranging from 1 to 1000 µM and determined an EC_{50} value of 12 ± 5 µM (Fig 1B). The potency of WS-12 is therefore several-fold higher as compared to menthol EC_{50} 196 ± 22 µM, a result similar to previous reports (Liu *et al*., 2006) in oocytes expression system and is roughly equal to that of the synthetic cooling agent icilin EC_{50} 7 ± 3 µM (Fig 1B).

Tachyphylaxis of TRPM8 mediated currents

Previous studies observed desensitization of TRPM8 channels during repeated exposure to icilin (Chuang *et al*., 2004; Andersson *et al*., 2004), which is commonly referred as tachyphylaxis. We asked whether tachyphylaxis would occur at some stage in consecutive exposure of WS-12. In contrast to icilin (74±2 % reduction in response; $p<0.001$, n=6) WS-12 evoked currents did not show any rundown or tachyphylaxis (4±2.3 % increase in response; is non-significant, $p<0.08$, n=6) during repeated exposures (Fig 2A, B & C), indicating a different mode of activation of the two compounds.

Mode of activation

A recent report on TRPM8 studying activation by menthol and icilin illustrated two different activation modes based on the changes in extracellular Ca^{2+} and pH (Chuang *et al*., 2004; Andersson *et al*., 2004). While the activation by menthol is independent of intra- and extracellular [Ca^{2+}] and pH (fig. 3A, B and C; changes in responses are ~10-12%, n=6), the structurally unrelated synthetic agonist icilin requires Ca^{2+} to fully activate TRPM8. Furthermore, it was described that TRPM8 activation by icilin is blocked by acidic pH. We asked whether extracellular Ca^{2+} is required for activation of TRPM8 channels by WS-12 and compared the results with those obtained for icilin. WS-12 fully activated TRPM8 channels in the absence of external Ca^{2+} and current amplitudes showed little variation in Ca^{2+}-containing versus Ca^{2+}-free solution (fig. 3A; 1-3 %, n=6). In contrast, currents elicited by icilin were reduced by a factor of 14 in Ca^{2+}-free solution (fig. 3A; 95±2 % decrease, $p<0.001$, n=6). Acidification of the applied solution to pH 6.1 did not affect currents elicited by WS-12 (14±3 % increase, n=6), but decreased icilin-evoked currents by ~ 80±3 % ($p<0.001$, n=6), a result which is

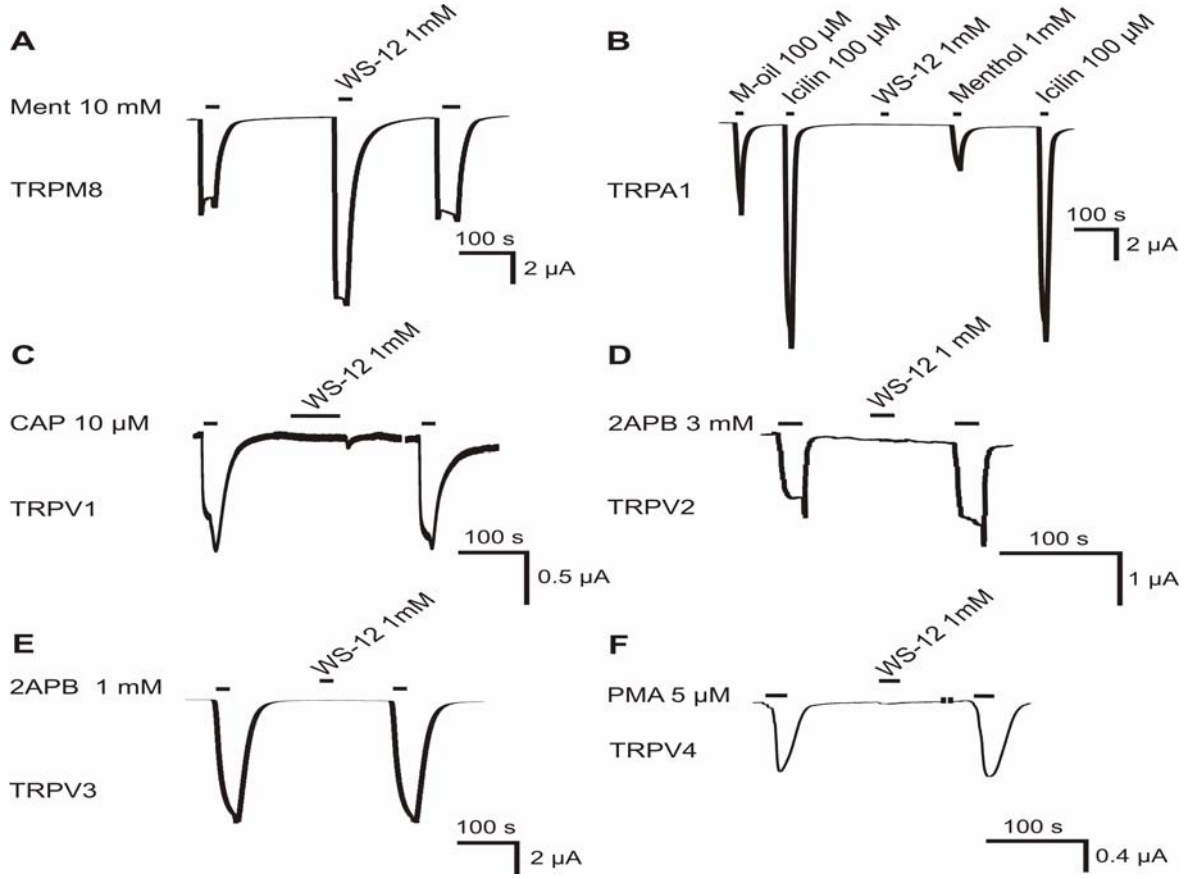

Fig. 4: Specificity of WS-12 as tested for various thermo-TRPs. Representative traces are shown for each thermo-TRP tested in oocytes expression system (n = 6). **A**: TRPM8 mediated currents evoked by WS-12 and menthol (Ment). **B**: TRPA1 mediated currents evoked by mustard oil (M-oil) while WS-12 did not elicit any significant currents. **C**: TRPV1 activation by capsaicin (CAP) while WS-12 did not elicit any significant currents. **D:** TRPV2 activation by 2-APB while WS-12 did not elicit any significant currents. **E**: TRPV3 activation by 2-APB while WS-12 did not elicit any significant currents. **F**: TRPV4 activation by PMA, while WS-12 did not elicit any significant currents.

consistent with previous reports (Chuang *et al*., 2004; Andersson *et al.,* 2004) on TRPM8 (fig. 3B).

In addition, we observed that a saturating concentration of icilin (100 µM) and of WS-12 (1 mM) evoked roughly two fold higher maximum amplitudes (213 ± 9 % and 203 ± 8 % respective increase) than 10 mM menthol taken as 100% (fig. 3C and 4A).

Selectivity of TRPM8 agonists

Since a significant overlap in the pharmacology of several TRP channels has been reported (Story *et al*., 2003; Behrendt *et al*., 2004; Weil *et al*., 2005; Macpherson *et al*., 2006) and specificity of a drug is highly relevant for clinical application, we next attempted to work-out any significant effects of WS-12 on other members of the thermo-TRPs subfamily of TRP ion channels including TRPA1 and TRPV1-4.

As shown in fig. 1A and 4A, WS-12 elicited a robust response in TRPM8 expressing oocytes. Although previous studies on TRPA1 revealed that both icilin and menthol activate the channel (Story *et al*., 2003; Karashima *et al*., 2007), WS-12 did not elicit any substantial currents in TRPA1 expressing *Xenopus* oocytes at 1 mM doses (fig. 4B). To confirm expression of TRPA1, we applied mustard oil (100 µM) and icilin (100 µM) as positive controls, since both of these substances are known TRPA1 agonists, and observed robust responses (fig. 4B).

We further tested the effects of WS-12 on TRPV1-4 expressed in *Xenopus* oocytes and compared the responses with known activators for these channels. Since TRPV1 is reported to play a role in nociception (Caterina *et al*., 1999; Dhaka *et al*., 2006; Levine and Allessandri-Haber, 2007) a lack of activation by WS-12 is a crucial advantage for any potential analgesic drug. As shown in

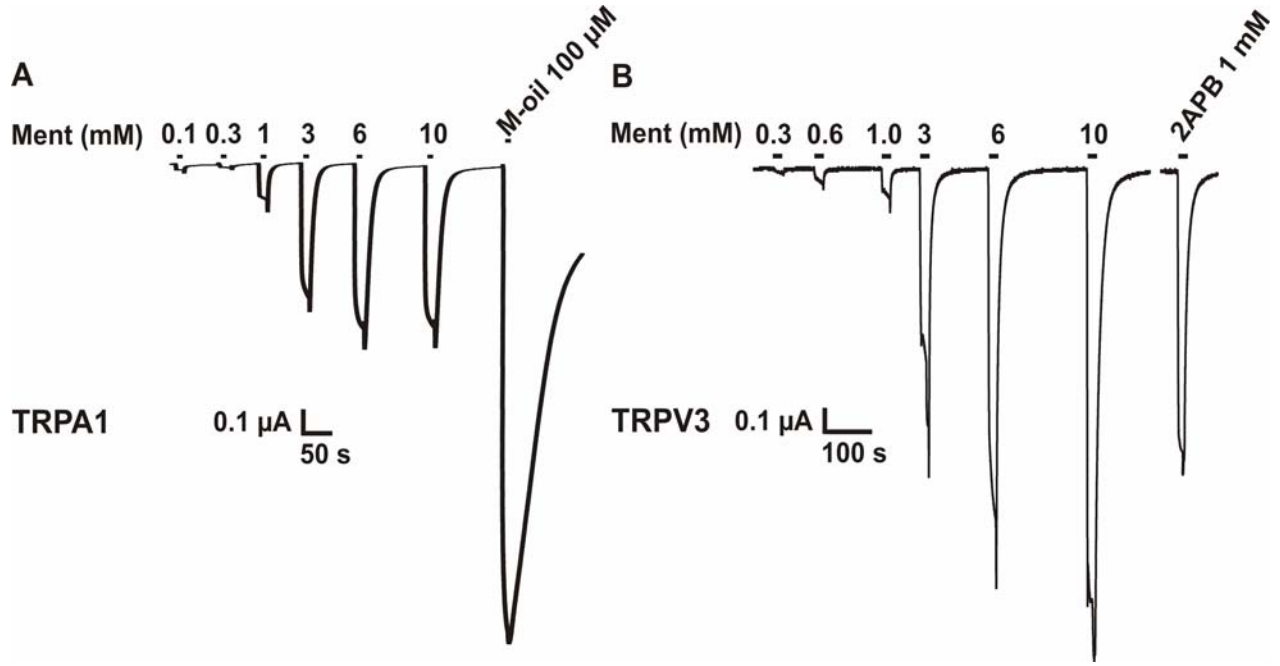

Fig. 5: Menthol activates TRPA1 and TRPV3 in a dose dependent manner. **A**: Representative trace showing activation of TRPA1 by menthol in a dose-dependent manner. **B**: Representative trace showing dose-dependent activation of TRPV3 by menthol. Experiments were conducted in Ca^{2+}-free background and at room temperature (~23°C) with oocytes clamped at –60 mV holding potentials. Ment means menthol and M-oil is mustard oil.

fig. 4C, WS-12 only elicited negligible currents at 0.1-1 mM in oocytes expressing TRPV1 (< 5% of the current evoked by 100 µM capsaicin). Moreover, we could not detect any substantial current induced by WS-12 (0.1-1 mM) in oocytes expressing TRPV2, V3 or V4, although the common agonists 2-APB and PMA robustly activated the channels (fig. 4 D-F).

Menthol activation of hTRPA1 and TRPV3

Menthol is reported to either block mouse TRPA1 (Macpherson, *et al*. 2006) or activate it at lower doses and quickly desensitises at higher doses (Karashima *et al*., 2007). The non-selectivity of menthol action in comparison to WS-12 is further investigated testing different concentration ranges of menthol (100 µM to 10 mM) on human TRPA1 (hTRPA1) and mTRPV3 expressing oocytes. Oocytes expressing hTRPA1 or mTRPV3 were superfused with different menthol concentrations and we observed significant activation at tested doses (figs. 5A and 5B).

DISCUSSION

Several members of the TRP super family have emerged as important targets for new drugs, especially analgesics, due to their expression in sensory neurons and their critical role in nociception. The most investigated ones are TRPV1 (a noxious heat sensor) and TRPA1 (a noxious cold-sensor), but other key members also include TRPV2, TRPV3 & TRPV4; since they have been shown to be involved in inflammatory and neuropathic pain in addition to their role as molecular thermo-sensors (Caterina *et al*., 1999; Jordt *et al*., 2003; Story et al., 2003; Dhaka *et al*., 2006; Hu *et al*., 2006; Alessandri-Haber *et al*., 2006; Lumpkin *et al*., 2007; Levine & Alessandri-Haber 2007). Cooling of injured areas by e.g. cold compresses / ice packs or application of menthol for pain relief has a long history in medicine. While the mechanism of these traditional treatments has long been obscure, the recent demonstration that TRPM8 activation acts through central inhibition of nociceptive input opened the door to a new generation of analgesics targeting TRPM8 (Proudfoot *et al*., 2006). While the mechanism has been shown in principle by using menthol and icilin, the limits of these drugs become obvious because of simultaneous proalgesic properties where the analgesic effect reverses to hyper sensitisation (Proudfoot *et al*., 2006). This is likely due to the activation of additional receptors like TRPA1, which responds to irritating compounds like garlic or mustard oil, and the sensations connected with TRPA1 activation are described as very unpleasant (Namer *et al*., 2005). In fact, menthol was recently shown to evoke pain in humans through activation and sensitisation of C-fibers (Wasner, 2004; Namer *et al*., 2005). Activation of either mouse TRPA1 by menthol (Karashima *et al*., 2007) or mouse TRPV3 (Macpherson *et al*, 2006; Vogt-Eisele *et al*., 2007) may account for this painful sensation. In our present investigation we studied human TRPA1, which showed consistent activation to various doses of menthol.

Previous investigations (Macpherson *et al*., 2006; Karashima *et al*., 2007), reported either block or desensitisations of TRPA1 after activation in different expression systems using a different construct (mouse cDNA). This difference in species or expression systems may account for the fact that we do not see either a block or desensitisation at higher doses. Additionally, icilin is recently reported to produce rapid and prolonged hyperthermia in rats when injected intramuscularly to produce cold analgesia (Ding *et al*., 2008); hence therapeutic utility of icilin to produce cold analgesia too becomes questionable.

We report here a discovery concerning WS-12, which is a carboxamide derivative of menthol, yet activates TRPM8 with an EC_{50} up to 20-fold lower than menthol. It additionally displays high selectivity for TRPM8 ion channels. The bulky methoxyphenyl group when replaced for hydroxyl group in menthol apparently conferred this selectivity. The same structural modification seems to play a critical role in improving the potency/efficacy of WS-12 for TRPM8. Icilin, which is a structurally unrelated ligand with similar modulating ability for TRPM8, but it, definitely activates the ion channel through a different mechanism. Icilin activation of TRPM8 depends on the presence of calcium and this activation is also affected by prevailing pH. Ca^{2+} has diverse effects on different TRP channels like activation of TRPA1 (Doerner *et al*., 2007; Zurborg *et al*., 2007), or inhibition of TRPV3 (Xiao *et al*., 2008). In such a background activation of TRPM8 by WS-12 through a Ca^{2+}-independent mode is advantageous and indicates direct action of the drug on the channel protein. Inflammatory conditions are associated with a drop in pH of the tissue. This may limit the efficacy of icilin in the inflammatory domain of affected tissue, but is unlikely to effect WS-12 since pH does not affect WS-12-induced TRPM8 responses. In addition WS-12 does not desensitize (no tachyphylaxis observed after repeated application) TRPM8 (Fig 2 A & C) under similar conditions, thereby allowing effective cooling over long durations and hence may produce better analgesic effects.

The pharmacological profiles of different TRPs overlap to a large and yet so far poorly characterized extent, making the development of specific drugs difficult. TRPM8 appears to have a unique role within thermo-TRPs, as it is not mediating the pain perception. TRPM8 does not colocalize with nociceptive markers (Kobayashi *et al*., 2005) and actually antagonizes nociceptive input (Proudfoot *et al*., 2006). Thus, targeting this channel with highly potent and selective agonists is a unique strategy for the development of novel analgesics. It is certain that structural derivatization of menthol truncates its interaction with other thermo-TRP ion channels while simultaneously improves its efficacy and affinity for TRPM8. The present data provides substantial evidence that WS-12 is highly selective at least within members of the thermo-TRPs. These characteristics render WS-12 a lead candidate for therapeutic application under conditions like neuropathic or chronic inflammatory pain, where traditionally available remedies do not work optimally. Previous studies reveal that a radio labelling of this compound does not affect its affinity for TRPM8 (Beck *et al*., 2007). Therefore, based on the selectivity profile, WS-12 may also present a diagnostic tool in prostate cancer where TRPM8 is reportedly over-expressed (Bidaux *et al*., 2007). However, currently no data are available providing substantial evidence whether TRPM8 over-expression accounts for prostate cancer or whether over-expression of TRPM8 is a consequence of a hyperactive prostate. Further studies are required to evaluate WS-12 as diagnostic tool in prostate cancer beside its possible appliance as an analgesic using *in vivo* animal models.

CONCLUSION

Since WS-12 does not activate any of the thermo-TRPs at optimally effective concentrations as opposed to its parent compound menthol, we believe that the development of this new class of selective TRPM8 activators provide an important lead for clinically valuable agents with potential applications in chronic sensitized pain states such as neuropathic or inflammatory pain, counter-irritant and as anti-itch agents. Additionally these ligands may be better diagnostic probes for prostate cancer. Part of the data was published in abstract form (Sherkheli *et al*., 2007; Sherkheli *et al.*, 2008).

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Abbreviations used

TRP (transient receptor potential); PMA (phorbol 12-myristate-13-acetate); 2APB (2-aminoethoxydiphenyl borate); HEK-293 (Human Embryonic Kidney-293). Menthol in this paper means (-)-Menthol which is {(1R,2S,5R)-2-isopropyl-5-methylcyclohexanol)}

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