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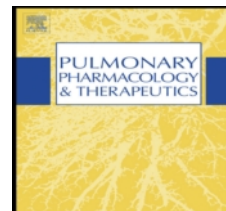
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TRPV1 receptors in sensitisation of cough and pain reflexes

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TRPV1, pain, cough, sensory nerves, hyperalgesia, airway hyperresponsiveness

Abstract

Preclinical studies suggest that the vanilloid receptor (TRPV1) is an important component of several disease areas such as pain (inflammatory, visceral, cancer and neuropathic), airway disease (including chronic cough), inflammatory bowel disease (IBD), interstitial cystitis, urinary incontinence, pancreatitis and migraine. TRPV1 is a member of a distinct subgroup of the transient receptor potential (TRP) family of ion channels. The neuronally expressed TRPV1 is a non-selective, Ca^{2+} -preferring, cation channel. In addition to capsaicin, this channel is activated by a number of different stimuli including heat, acid, certain arachidonic acid derivatives and direct phosphorylation via protein kinase C (PKC). Moreover, there is also evidence that various inflammatory mediators such as adenosine triphosphate (ATP), bradykinin, nerve growth factor (NGF) or prostaglandin E_2 (PGE_2) may indirectly lead to activation of the TRPV1 channel via activation of their respective receptors. There is strong experimental evidence that the combination of direct and indirect mechanisms finely tune the TRPV1 activity. Each of the different known modes of direct TRPV1 activation (protons, heat and vanilloids) is capable of sensitising the channel to other agonists. Similarly, inflammatory mediators from the external milieu found in disease conditions can indirectly sensitise the receptor. It is this sensitisation of the TRPV1 receptor in inflammatory disease that could hold the key and contribute to the transduction of noxious signalling for normally innocuous stimuli, i.e. either hyperalgesia in the case of chronic pain or airway hyperresponsiveness/hypertussive responses in patients with chronic cough. It seems reasonable to suggest that the various mechanisms for sensitisation provide a scenario for TRPV1 to be tonically active and this activity may contribute to the underlying pathology – providing an important convergence point of multiple pain producing stimuli in the somatosensory system and multiple cough-evoking irritants in the airways. The complex mechanisms and pathways that contribute to the pathophysiology of chronic pain and chronic cough have made it difficult for clinicians to treat patients with current therapies. There is an increasing amount of evidence supporting the hypothesis that the expression, activation and modulation of TRPV1 in sensory neurones appears to be an integral component of pain and cough pathways, although the precise contribution of TRPV1 to human disease has yet to be determined. So the question remains open as to whether TRPV1 therapeutics will be efficacious and safe in man and represent a much needed novel pain and cough therapeutic.

1. Introduction

The vanilloid 1 (TRPV1 or VR1) receptor is a member of a subgroup/superfamily of transient receptor potential (TRP) ion channels which subserve a whole host of cellular roles including many features of sensory transduction [1]. The neuronally expressed TRPV1 is a non-selective, Ca^{2+} -preferring, cation channel. The TRPV1 channel is activated by a diverse range of chemical ligands such as capsaicin (the 'hot' component of chilli peppers) and other vanilloids (resiniferatoxin and the cannabinoid, anandamide), as well as acid (protons, H^+), physical stimuli such as heat, certain arachidonic acid derivatives and direct phosphorylation via protein kinase C (PKC) [2-5]. In addition, TRPV1 is also activated (directly and indirectly) by a variety of mediators thought to contribute to neuroinflammation [6-7]. Moreover, various endogenous mediators such as bradykinin, substance P, glutamate, prostaglandins, hydroperoxy fatty acids, and adenosine triphosphate (ATP) sensitise TRPV1 [7].

It is this sensitisation of the TRPV1 receptor in inflammatory diseases that could hold the key and contribute to the transduction of noxious signalling for normally innocuous stimuli, i.e. either hyperalgesia in the case of chronic pain or airway hyperresponsiveness/hypertussive responses in patients with chronic cough. It seems reasonable to suggest that the various mechanisms for sensitisation provide a scenario for TRPV1 to be tonically active and this activity may contribute to the underlying pathology – providing an important convergence point of multiple pain-producing stimuli in the somatosensory system and multiple cough-evoking irritants in the airways. Whilst there is evidence that this is indeed the case in pain producing pathways, there is no direct evidence that there is tonic activity of TRPV1 in cough receptors. However, as discussed in section 4 of this manuscript, it seems likely that TRPV1 plays a critical role in the sensory regulation and/or sensitisation of the cough reflex in animals and humans and in so doing may be tonically active in airway sensory nerves. This review examines the various mechanisms that can activate and sensitise TRPV1 receptors and the evidence that suggests that these receptors are important in pain and cough pathways. A decade after the first reported cloning of the receptor [2], TRPV1 research has moved into another era. The availability of novel, potent and selective TRPV1 antagonists, together with a greater understanding of

TRPV1 physiology and pharmacology, should enable crucial questions with respect to the potential therapeutic benefits of targeting this mechanism in disease. To date the foremost application of TRPV1 receptor antagonists/agonists has been, understandably, in pain. However, the potential therapeutic benefit of targeting TRPV1 for a wide range of other disease areas including asthma and cough [8] has recently been highlighted.

2. Activation and sensitisation of TRPV1 receptors

Sequence analysis of the cloned capsaicin receptor VR1 revealed that it belongs to the TRP superfamily, characterised by having six transmembrane domains, and having a pore region between the fifth and sixth transmembrane domains [2]. Once activated by vanilloid molecules the channel allows the influx of the cations Ca^{2+} and Na^{+} . TRPV1 mRNA is highly expressed in a subset of primary sensory neurones with A δ - and C-fibres that respond to chemical, mechanical and thermal stimuli and, therefore, they are classified as polymodal nociceptors (Fig.1). Recent studies have demonstrated that several endogenous chemical substances can activate TRPV1 in various tissues. The most prominent feature of TRPV1 is its responsiveness to physicochemical agents/noxious stimuli, such as temperature and protons. TRPV1 can be activated by acidic solutions with a pH of 5-6, which can be produced in tissues during pathological conditions with inflammation [9]. TRPV1 is a thermosensor on afferent nerves, activated by temperatures between 42 and 53°C [2], which coincides with the threshold temperature of thermal pain perception [9]. The effect of temperature on airway afferent nerves has not been as widely studied as have the cutaneous temperature sensors. However, it is probably unlikely that these temperatures are achieved in the lower airways, even in the inflamed lung. Whilst it is known that noxious cold air can induce cough, which may implicate TRPM8 receptors, there appears to be little or no evidence to show whether hot air can cause or sensitise the cough reflex. Recently, several members of the TRP family, including TRPV1 and TRPV4, have been implicated in sensory nerve mechanotransduction [10]. Nonetheless, the molecular basis of mechanical transduction in the sensory terminals of the airways is little understood, but it would be fascinating to determine if TRPV1 receptors in airway sensory nerves can respond to mechanical stimuli that can cause cough. Additional stimuli of TRPV1 include elevated concentrations of the

endocannabinoid, anandamide [11], the lipoxygenase metabolites of arachidonic acid, Leukotriene B₄ (LTB₄), 12-(S)- and 15-(S)-hydroperoxyeicosatetraenoic acid (12S- and 15S-HPETE) [12], which can also sensitise TRPV1 receptors. Recently, N-arachidonoyl-dopamine (NADA) has been recognised as a TRPV1 stimulant, apparently more potent than anandamide [13-14].

It is well known that bradykinin activates sensory neurones; however, the mechanism by which this occurs is not well understood, although possible sensitisation pathways have been suggested. Bradykinin releases diacylglycerol (DAG), inositol-(1,4,5)-triphosphate (IP₃) and arachidonic acid from sensory neurones [9]. Thus, it is likely that arachidonic acid, generated by bradykinin, would in turn activate phospholipase A₂ (PLA₂) and result in the production of lipoxygenase products from arachidonic acid. A PLA₂-lipoxygenase-TRPV1 pathway for excitation of sensory neurones by bradykinin is therefore possible (Fig. 1).

It is well documented that a number of endogenous inflammatory mediators can modulate the sensitivity of TRPV1 during tissue inflammation. The exact mechanisms underlying the sensitisation of TRPV1 are not yet fully understood, but several signal transduction pathways are known to be involved (Fig.1). TRPV1 has several consensus phosphorylation sites that can be phosphorylated by protein kinases A, C, and G (PKA, C and G) and tyrosine kinase (Trk) [15-18], which ultimately results in sensitisation of TRPV1 receptors. PKC activation increases neuronal current responses to noxious heat and the activation of PKC by phorbol esters enhances the responses of TRPV1 to capsaicin, anandamide, acid and heat [17-19]. Thus, for example, bradykinin which, as already mentioned, could indirectly activate TRPV1 receptors via the production of arachidonic acid metabolites, could also sensitise the TRPV1 receptor by an indirect action on PKC also via the production of lipoxygenase products such as 15 (S)-HPETE, which in turn activate PKC. Furthermore, bradykinin, via activation of the B₂ receptor (Fig.1), is known to stimulate phospholipase C (PLC) and increase the production of DAG, which in turn activates PKC [20]. Prostaglandin E₂ (PGE₂) also sensitises sensory neurones via an effect on TRPV1 receptors (Fig. 1). Evidence suggests that PGE₂ activates the G_s protein-coupled EP₂ prostanoid receptor present on the membranes of these neurones, which upon activation increases the enzyme activity of adenylyl cyclase. The resulting rise in

cAMP may then stimulate PKA, which in turn increases the phosphorylation of TRPV1 and enhances its excitability [16, 21]. A further example is nerve growth factor (NGF): administration of NGF in somatic tissues induces a long-lasting increase in the sensitivity of TRPV1 receptors. This effect is believed to be mediated through the G-protein –coupled TrkA receptors, which in turn activates mitogen-activated protein kinase and the PLC signalling pathway, resulting in potentiation of the TRPV1 channel [20, 22].

Another pathway for sensitising TRPV1 involves the ‘disinhibition’ of the receptor. PLC cleaves phosphatidylinositol-(4,5)-biphosphate (PIP₂) to yield IP₃ and DAG. PIP₂ constitutively inhibits TRPV1, such that removal of PIP₂ from TRPV1 results in disinhibition of the receptor. When PLC is activated by bradykinin or NGF, PLC sequesters PIP₂ which release TRPV1 from the constitutive inhibition (Fig. 1) [9].

3. TRPV1 receptors in pain

Depending on its origin, pain can be classified as follows: pain caused by the activation of nociceptive receptors and transmitted over intact neuronal pathways is termed nociceptive pain; pain caused by damage to neural structures that disrupts the ability of the sensory nerves to transmit correct information to the brain is termed neuropathic pain; finally pain with no clear physiological origin can be termed psychological pain.

Growing evidence suggests several members of the TRP superfamily are involved in the detection of acute noxious, mechanical and chemical as well as in neuropathic pain [23]. The first evidence for the involvement of TRP channels in the pain pathway came with the cloning of the vanilloid receptor TRPV1, which is arguably the most extensively studied of the entire TRP superfamily [24]. The appropriate expression of the receptor in target tissues and the unmistakable pungency of capsaicin and many other agonists at the vanilloid receptor clearly define TRPV1 as a key transducer in the pain pathway and as an important integrator of responses to inflammatory mediators. Moreover, sensitisation of TRPV1 receptors during chronic pain is believed to contribute to the transduction of noxious signalling for normally

innocuous stimuli. Furthermore, TRPV1 has a unique expression profile in peripheral nociceptors and the ability to show polymodal activation.

Thus the expression of TRPV1 in the dorsal root ganglion (DRG) and nodose ganglion neurones, particularly in association with nociceptive afferent fibres, together with its activation by heat ($>43^{\circ}\text{C}$), acid and pungent vanilloid compounds strongly indicate that TRPV1 plays an important role in the detection and integration of noxious stimuli [2,3]. In gene-based disruption experiments, analysis of TRPV1 gene-knockout mice revealed that the channel contributes to the detection of acute painful chemical and thermal stimuli [25, 26]. In particular, *trpv1*^(-/-) mice showed reduced responses to noxious heat stimuli and complete indifference to pungent vanilloids.

In addition to their normal role as detectors of harmful stimuli, several pathological conditions lead to changes in the expression level and/or sensitivity of “pain” TRP channels. This can lead to exaggerated pain, when the experienced pain overestimates the harmfulness of the stimulus, or chronic pain, when the pain persists after the noxious stimulus has terminated. Many pathological conditions are characterised by hyperaesthesia, i.e. enhanced sensitivity to sensory stimuli. With respect to pain a distinction can be made between allodynia, when pain is experienced in response to non-noxious stimuli, and hyperalgesia, when exaggerated pain is experienced in response to noxious stimuli.

Mechanisms leading to allodynia and hyperalgesia are well described for TRPV1. The *trpv1*^(-/-) mice are not only less sensitive to acute painful thermal stimuli and chemical stimuli, but are also unable to develop inflammatory thermal hyperalgesia [25, 26]. Several mechanisms have been elucidated that contribute to the increased sensitivity of TRPV1 during inflammation and have been discussed earlier in this review (Fig. 1).

The well-established role of TRPV1 in the pain pathway, has given rise to the development of TRPV1-selective antagonists as new therapeutic targets for the treatment of clinical pain [27]. Recently, SB-705498 was reported as a potent

selective TRPV1 antagonist with good oral bioavailability and effectiveness in reducing hyperalgesia and allodynia in animal models [28, 29, 30]. Furthermore, encouraging pharmacodynamic effects, including an effect on heat pain threshold and a reduction in UV burn-induced flare in the skin, indicating on target activity of SB-705498 and activity versus inflammatory hyperalgesia, have been reported in Phase 1 healthy volunteer studies. This demonstrates that this compound is pharmacologically-active in humans at the dose tested and provides further confidence in the progression and design of clinical trials to assess the efficacy of TRPV1 antagonists in patients [31]. Similarly, AMG8562, a novel, second generation TRPV1 antagonist was shown to cause effective anti-nociceptive effects in several models of inflammatory and surgical pain [32]. Importantly, this compound did not cause hyperthermia (increase in body temperature), an effect that has been observed previously with other TRPV1 antagonists in animal and human studies [33, 34]. These examples illustrate the potential of TRPV1 antagonists in the treatment of varied forms of pain in humans and with the development of even more selective agents further understanding of the role of TRPV1 in pain is within reach.

4. TRPV1 receptors in cough

Cough is arguably the most common symptom associated with pulmonary diseases, such as asthma, bronchitis, chronic obstructive pulmonary disease (COPD) and the common cold. Chronic cough is a symptomatic manifestation of airway hyperresponsiveness. Receptors present on airway sensory nerve endings and in cell bodies of C-fibres and A δ -fibres are drug targets for chronic cough. Increasing evidence has suggested a significant role of TRPV1 in the genesis of cough. Firstly, good evidence suggests that airway sensory nerves expressing TRPV1 receptors are involved in eliciting cough reflexes [21,35] and that TRPV1 plays a critical role in the sensory regulation and/or sensitisation of the cough reflex in animals [35-37]. In animals and in humans, experimental cough can be induced by inhalation of citric acid, capsaicin and anandamide, all of which are potent TRPV1 activators [38-43]. Secondly, a number of endogenous inflammatory mediators that are known to upregulate TRPV1 sensitivity, such as PGE₂, bradykinin, and histamine can also enhance the cough sensitivity in experimental animals, as well as in humans [44-46]. Indeed, PGE₂, at low doses markedly enhances the excitability of vagal pulmonary C-

fibres in anaesthetised rats [47]. In cultured nodose and jugular pulmonary neurones, PGE₂ markedly increased the whole cell current density and number of action potentials evoked by capsaicin which suggest a sensitising effect of PGE₂ on TRPV1 receptors [16]. Likewise, it has recently been reported that activation of protease activated receptor-2 (PAR-2) up-regulates the excitability of isolated rat pulmonary chemosensitive neurones [48] and also increases TRPV1-mediated cough in guinea-pigs via activation of PKC and PKA signal transduction pathways [49]. Thus, PGE₂ and PAR-2, released during airway inflammation, may cause airway hyperresponsiveness and exaggerated cough reflexes via sensitisation of TRPV1 receptors in a similar manner to that described for the generation of thermal hyperalgesia in inflammatory pain. Thirdly, in humans, TRPV1 is upregulated in patients with chronic cough [50,51] and a significant correlation between the cough sensitivity to capsaicin inhalation challenge and the density of TRPV1-expressing nerves in the mucosa of patients with chronic cough was also observed. [50]. Moreover, capsaicin-evoked cough responses are increased in patients with inflammatory lung diseases such as asthma, bronchitis, COPD and upper respiratory tract infection, which could be as result of TRPV1 sensitisation.

Clearly, there is a growing body of evidence linking an important role of TRPV1 in airway inflammation, airway hyperresponsiveness and cough. Thus, as in the pain arena, this potential role of TRPV1 in the cough pathway, has given rise to studies examining the pharmacological antitussive activity of TRPV1 antagonists. Several studies have used the TRPV1 antagonist capsazepine and iodo-resiniferatoxin (iodo-RTX). These studies demonstrated antitussive activity, but the agents were not fully efficacious [40, 52]. Unfortunately, capsazepine and iodo-RTX are not particularly good pharmacological tools with limited selectivity and potency [53]. More encouragingly, preclinical studies have demonstrated clear antitussive efficacy with the more potent and more selective TRPV1 antagonists, BCTC and JNJ17203212 in a number of rodent models including capsaicin- and citric acid-evoked cough in guinea-pigs [53, 54]. Furthermore, the TRPV1 antagonist BCTC was also shown to possess antitussive activity in an antigen-evoked cough model in guinea-pigs [54] and also attenuated the hyperresponsiveness to capsaicin-evoked cough that develops following airway inflammation induced by the noxious gas sulphur dioxide [55]. Together, these findings provide convincing evidence to suggest that an increase in

expression and/or sensitivity of TRPV1 in the airway sensory nerves may be involved in the development of chronic cough. Unquestionably, the emergent evidence implicating a fundamental role for TRPV1 in airway inflammation means that TRPV1 antagonists may have important benefit for the treatment of patients suffering from chronic cough, asthma, COPD and allergic rhinitis.

5. Current status of novel TRPV1 antagonists for drug development

To date the foremost application of TRPV1 receptor antagonists/agonists has been, understandably, in pain. Several synthetic antagonists of the TRPV1 channel are being developed and are currently under investigation, focused primarily for use in pain, in particular dental pain and migraine. However, authoritative information regarding the exact progress of these molecules through pre-clinical and early clinical development is often difficult to acquire. A number of pre-clinical, Phase I and Phase II clinical studies/trials are currently in progress emanating from various different pharmaceutical companies and collaborations (Fig. 2). As discussed previously, encouraging pharmacodynamic effects have been obtained with SB-705498, demonstrating that this agent is pharmacologically active in humans [31]. Likewise, Merck-Neurogen and Glenmark have also recently announced completion of successful Phase I clinical trials with MK-2295(NGD-8243) and GRC6211, respectively, and are now in the process of assessing proof-of-concept studies in dental pain [56]. Unfortunately, Amgen recently announced that their molecule AMG517 caused marked hyperthermia in humans and stated that this would prevent it from further development [34]. Interestingly, hyperthermia has not been highlighted as a major issue in the other Phase I studies completed so far. Notwithstanding, Amgen have another, second-generation, TRPV1 antagonist (AMG8562) in pre-clinical development, which does not cause hyperthermia, but retains pharmacological efficacy in on-target (agonist) challenge models and rodent pain models. There are many other companies operational in this area (Fig. 2), [57,58] and no doubt further clinical trials will soon be underway. Indeed, Evotec AG very recently announced the initiation of a Phase I clinical trial of a TRPV1 antagonist under partnership with Pfizer Inc. To date the emphasis for TRPV1 antagonists from a clinical development viewpoint has been on pain, however, there is increasing preclinical and clinical

evidence which suggests that TRPV1 antagonists may have potential for the treatment of cough as well as a variety of other human disorders.

6. Conclusions

Preclinical studies suggest that the TRPV1 receptor is an important component of several disease areas such as pain (inflammatory, visceral, cancer and neuropathic) and airway disease (including chronic cough). In addition to capsaicin, this channel can be activated directly, indirectly and also sensitised by a number of different stimuli and mechanisms including heat, acid, and various inflammatory mediators. There is strong experimental evidence that the combination of direct, indirect and sensitising mechanisms finely tune the TRPV1 activity. It is this sensitisation of the TRPV1 receptor in inflammatory disease that could hold the key and contribute to the transduction of noxious signalling for normally innocuous stimuli, i.e., either hyperalgesia in the case of chronic pain or airway hyperresponsiveness/hypertussive responses in patients with chronic cough. It seems reasonable to suggest that the various mechanisms for sensitisation provide a scenario for TRPV1 to be tonically active and this activity may contribute to the underlying pathology – providing an important convergence point of multiple pain producing stimuli in the somatosensory system and multiple cough-evoking irritants in the airways. There is an increasing amount of evidence supporting the hypothesis that the expression, activation and modulation of TRPV1 in sensory neurones appears to be an integral component of pain and cough pathways, although the precise contribution of TRPV1 to human disease has yet to be determined. So the question remains open as to whether TRPV1 therapeutics will be efficacious and safe in man and represent a much needed novel pain and cough therapeutic. The availability of novel, potent and selective TRPV1 antagonists, together with a greater understanding of TRPV1 physiology and pharmacology, should enable crucial questions with respect to the potential therapeutic benefits of targeting this mechanism in disease.

References

- [1] Gunthorpe MJ, Benham CD, Randall, A, Davis JB. The diversity in the vanilloid (TRPV) receptor family of ion channels. *Trends Pharmacol Sci* 2002; 23:183-191.

- [2] Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat activated ion channel in the pain pathway. *Nature* 1997; 389: 816-824
- [3] Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D. The cloned capsaicin receptor integrates multiple pain producing stimuli. *Neuron* 1998; 21: 532-543.
- [4] Van Der Stelt M, Di Marzo V. Endovanilloids. Putative endogenous ligands of transient receptor potential vanilloid 1 channels. *Eur J Biochem* 2004; 271: 1827-1834.
- [5] Numazaki M, Tominaga T, Toyooka H, Tominaga M. Direct phosphorylation of capsaicin receptor VR1 by protein Kinase Cepsilon and identification of two target serine series. *J Bio Chem* 2002; 277: 13375-13378.
- [6] Tominaga M, Tominaga T. Structure and function of TRPV1. *Pflugers Arch* 2005; 451: 143-150.
- [7] Szallasi A, Cortright DN, Blum CA, Eid SR. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof of concept. *Nat Rev Drug Discov* 2007; 6: 357-372.
- [8] Jia Y, Lee LY. Role of TRPV receptors in respiratory diseases. *Biochimica et Biophysica Acta* 1772 2007; 915-927.
- [9] Hwang SW, Oh U. Hot channels in airways: pharmacology of the vanilloid receptor. *Curr Opin Pharmacol* 2002; 3: 235-242.
- [10] Lin SY, Corey DP. TRP channels in mechanosensation. *Curr Opin Neurobiol* 2005; 15: 350-357.

[11] Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Marzo V, Julius D, Hogestatt ED. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 1999; 400: 452-457.

[12] Hwang SW, Cho H, Kwak L, Lee SY, Kang CJ, Jung J, Cho S, Min KH, Suh YG, Kim D, Oh U. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci USA* 2000; 97: 6155-6160.

[13] Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci USA* 2002; 99: 8400-8405.

[14] Harrison S, De Petrocellis L, Trevisani M, Benvenuti F, Bifulco M, Geppetti P, Di Marzo V. Capsaicin-like effects of N-arachidonoyl-dopamine in the isolated guinea-pig bronchi and urinary bladder. *Eur J Pharmacol* 2003; 475: 107-114.

[15] Gu Q, Kwong K, Lee LY. Ca^{2+} transient evoked by chemical stimulation is enhanced by PGE_2 in vagal sensory neurones: role of cAMP/PKA signalling pathway. *J Neurophysiol* 2003; 89: 1985-1993.

[16] Kwong K, Lee LY. PGE_2 sensitises cultured pulmonary vagal sensory neurones to chemical and electrical stimuli

[17] Premkumar LS, Ahern GP. Induction of vanilloid receptor channel activity by protein kinase C. *Nature* 2000; 408: 985-990.

[18] Vellani V, Mapplebeck S, Moriondo A, Davis JB, McNaughton PA. Protein Kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. *J Physiol* 2001; 534: 813-825.

- [19] Tominaga M, Wada M, Masu M. Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. *Proc Natl Acad Sci USA* 2001; 98: 6951-6956.
- [20] Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P₂-mediated inhibition. *Nature* 2001; 411: 957-962.
- [21] Lee LY, Kwong K, Lin YS, Gu Q. Hypersensitivity of bronchopulmonary C-fibres induced by airway mucosal inflammation: cellular mechanisms. *Pulm Pharmacol Therap* 2002; 15: 199-204.
- [22] Ganju P, O'Bryan JP, Der C, Winter J, James IF. Differential regulation of SHC proteins by nerve growth factor in sensory neurones PC12 cells. *Eur J Neurosci* 1998; 10: 1995-2008.
- [23] Nilius B, Owsianik G, Voets T, Peters JA. Transient receptor potential cation channels in disease. *Physiol Rev* 2006; 87: 165-217.
- [24] Caterina MJ, Julius D. The vanilloid receptor: a molecular gateway to the pain pathway. *Annu Rev Neurosci* 2001; 24: 487-517.
- [25] Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Peterson-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 2000; 288: 306-313.
- [26] Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harris MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature* 2000; 405: 183-187.
- [27] Kraus JE, Chernard BL, Cortright DN. Transient receptor potential ion channels as targets for the discovery of pain therapeutics. *Curr Opin Invest Drugs* 2005; 6: 48-57.

- [28] Rami HK, Thompson M, Stemp G, Fell S, Jerman JC, Stevens AJ, Smart D, Sargent B, Sanderson D, Randall AD, Gunthorpe MJ, Davis JB. Discovery of SB-705498: a potent, selective and orally bioavailable TRPV1 antagonist suitable for clinical development. *Bioorg Med Chem Lett* 2006; 16: 3287-3291.
- [29] Rami HK, Thompson M, Wyman P, Jerman JC, Egerton J, Brough S, Stevens AJ, Randall AD, Smart D, Gunthorpe MJ, Davis JB. Discovery of small molecule antagonists of TRPV1. *Bioorg Med Chem Lett* 2004; 14: 3631-3634.
- [30] Gunthorpe MJ, Hannan SL, Smart D, Jerman JC, Arpino S, Smith GD, Brough S, Wright J, Egerton J, Lappin SC, Holland VA, Winborn K, Thompson M, Rami HK, Randall A, Davis JB. Characterisation of SB-705498, a potent and selective vanilloid receptor-1 (VR1/TRPV1) antagonist that inhibits the capsaicin-, acid-, and heat-induced activation of the receptor. *J Pharmacol Exp Ther* 2007; 321: 1183-1192.
- [31] Chizh BA, O'Donnell MB, Napolitano A, Wang J, Brooke AC, Aylott MC, Bullman JN, Gray EJ, Lai RY, Williams PM, Appleby JM. The effects of the TRPV1 antagonist SB-705498 on TRPV1 receptor-mediated activity and inflammatory hyperalgesia in humans. *Pain* 2007; 132: 132-141
- [32] Lehto SG, Tamir R, Deng H, Klionsky L, Kuang R, Le A, Lee D, Louise J-C, Magal E, Manning BH, Rubino J, Surapaneni S, Tamayo N, Wang T, Wang J, Wang J, Wang W, Youngblood B, Zhang M, Zhu D, Norman MH, Gavva NR. Antihyperalgesic effects of (R,E)-N-(2-Hydroxy-2,3-dihydro-1H-inden-4-yl)-3-(2-(piperidin-1-yl)-4-(Trifluoromethyl)phenyl)-acrylamide (AMG8562), a novel transient receptor potential vanilloid type 1 modulator that does not cause hyperthermia in rats. *J Pharmacol Exp Ther* 2008; 326: 218-229.
- [33] Gavva NR, Bannon AW, Surapaneni S, Hovland DNJr, Lehto SG, Gore A, Juan T, Deng H, Han B, Klionsky L, Kuang R, Le A, Tamir R, Wang J, Youngblood B, Zhu D, Norman MH, Magal E, James J. S. Treanor JJS, Louis J-C. The vanilloid receptor TRPV1 is tonically activated *in vivo* and involved in body temperature regulation. *J Neurosci* 2007; 27: 3366-3374.

[34] Gavva NR, Treanor JJS, Garami A, Fang L, Surapaneni S, Akrami A, Alvarez F, Bak A, Darling M, Gore A, Jang GR, Kesslak JP, Ni L, Norman MH, Palluconi G, Rose MJ, Salfi M, Tan E, Romanovsky AA, Banfield C, Davar G. Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. *Pain* 2008; 136: 202-210.

[35] Canning BJ, Mori N, Mazzone SB. Vagal afferent nerves regulating the cough reflex. *Respir Physiol Neurobiol* 2006; 152: 223-242.

[36] Mazzone SB. Sensory regulation of the cough reflex. *Pulm Pharmacol Therap* 2004; 17: 361-368

[37] Kollarik M, Udem BJ. Sensory transduction in cough-associated nerves. *Respir Physiol Neurobiol* 2006; 152: 243-254.

[38] Karlsson J-A, Fuller RW. Pharmacological regulation of the cough reflex – from experimental models to antitussive effects in Man. *Pulm Pharmacol Therap* 1999; 12: 215-228.

[39] Higenbottam T. Chronic cough and the cough reflex in common lung diseases. *Pulm Pharmacol Therap* 2002; 15: 241-247

[40] LallooUG, Fox AJ, Belvisi MG, Chung KF, Barnes PJ. Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea-pigs. *J Appl Physiol* 1995; 79: 1082-1087.

[41] Winning AJ, Hamilton RD, Shea SA, Guz A. Respiratory and cardiovascular effects of central and peripheral intravenous injections of capsaicin in man: evidence for pulmonary chemosensitivity. *Clin Sci* 1986; 71: 519-526.

[42] Ricciardolo FLM. Mechanism of citric acid-induced bronchoconstriction. *Am J Med* 2001; 111: 18S-24S.

[43] Jia Y, McLeod RL, Wand X, Parra LE, Egan RW, Hey JA. Anandamide induces cough in conscious guinea-pigs through VR1 receptors. *Br J Pharmacol* 2002; 137: 831-836.

[44] Choudry NB, Fuller RW, Pride NB. Sensitivity of the human cough reflex: effect of inflammatory mediators prostaglandin E₂, bradykinin and histamine. *Am Rev Respir Dis* 1989; 140: 137-141.

[45] Fox AJ, Lalloo UG, Belvisi MG, Bernareggi M, Chung KF, Barnes PJ. Bradykinin-evoked sensitisation of airway sensory nerves: a mechanism for ACE-inhibitor cough. *Nat Med* 1996: 814-817.

[46] Polosa R, Holgate ST. Comparative airway response to inhaled bradykinin, kallidin, and [des-Arg⁹] bradykinin in normal and asthmatic subjects. *Am Rev Respir Dis* 1990; 142: 1367-1371.

[47] Ho CY, Gu Q, Hong JL, Lee LY. Prostaglandin E₂ enhances chemical and mechanical sensitivities of pulmonary C fibres in the rat. *Am J Respir Crit Care Med* 2000; 162: 528-533

[48] Gu Q, Lee LY. Characterisation of acid signalling in rat vagal pulmonary sensory neurones. *Am J Physiol* 2006; 291: L58-L65

[49] Gatti R, Andre E, Amadesi S, Dinh QT, Fischer A, Bunnett NW, Harrison S, Geppetti P, Trevisani M. Protease-activated receptor-2 activation exaggerates TRPV1-mediated cough in guinea-pigs. *J Appl Physiol* 2006; 101: 506-511.

[50] Groneberg DA, Niimi A, Dinh QT, Cosio B, Hew M, Fischer A, Chung KF. Increased expression of transient receptor potential vallinoid-1 in airway nerves of chronic cough. *Am J Respir Crit Care Med* 2004; 170: 1276-1280.

[51] Mitchell JE, Campbell AP, New NE, Sadofsky LR, Kastelik JA, Mulrennan SA, Compton SJ, Morice AH. Expression and characterisation of the intracellular

vanilloid receptor (TRPV1) in bronchi from patients with chronic cough. *Exp Lung Res* 2005; 31: 295-306.

[52] Trevisani M, Milan A, Gatti R, Zanasi A, Harrison S, Fontana G, Morice AH, Geppetti P. Antitussive activity of iodo-resiniferatoxin in guinea-pigs. *Thorax* 2004; 59: 769-772.

[53] Bhattacharya A, Scott BP, Nasser N, Ao H, Maher MP, Dubin AE, Swanson DM, Shankley NP, Wickenden AD, Chaplan SR. Pharmacology and antitussive efficacy of 4-(3-Trifluoromethyl-pyridin-2-yl)-piperazine-1-carboxylic acid (5-Trifluoromethyl-pyridin-2-yl)-amide (JNJ17203212), a transient receptor potential vanilloid 1 antagonist in guinea-pigs. *J Pharmacol Exp Therap* 2007; 323: 665-674.

[54] McLeod RL, Fernandez X, Correll CC, Phelps TP, Jia Y, Wang X, Hey JA. TRPV1 antagonists attenuate antigen-provoked cough in ovalbumin sensitised guinea-pigs. *Cough* 2006; 2: 10

[55] McLeod RL, Jia Y, McHugh NA, Fernandez X, Mingo GG, Wang X, Parra LE, Chen J, Brown D, Bolser DC, Kreutner W, Hey JA. Sulfur-dioxide exposure increases TRPV1-mediated responses in nodose ganglia cells and augments cough in guinea-pigs. *Pulm Pharmacol Therap* 2006; 20: 750-757.

[56] Gunthorpe MJ, Szallasi A. Peripheral TRPV1 receptors as targets for drug development: new molecules and mechanisms. *Curr Pharm Des* 2008; 14: 32-41.

[57] Rami HK, Gunthorpe MJ. The therapeutic potential of TRPV1 (VR1) antagonists: Clinical answers await. *Drug Discov Today Therap Strategy* 2004; 1 97-104.

[58] Szallasi A, Cortright DN, Blum CA, Eid S. 10 years on from channel cloning to antagonist proof-of-concept. *Nat Rev Drug Disc* 2007; 6: 357-371.

Figure Captions

Fig. 1. Diversity of mechanisms either directly, indirectly or sensitising TRPV1 receptors in the terminals of primary sensory neurones. For explanation see text.

Fig. 2. Current status of novel TRPV1 antagonists for drug development.

ACCEPTED MANUSCRIPT

