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Inhibition of guinea-pig and human sensory nerve activity and the cough reflex in guinea-pigs by cannabinoid (CB_2) receptor activation

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1 There is considerable interest in novel therapies for cough, since currently used agents such as codeine have limited beneficial value due to the associated side effects. Sensory nerves in the airways mediate the cough reflex *via* activation of C-fibres and RARs. Evidence suggests that cannabinoids may inhibit sensory nerve-mediated responses.

2 We have investigated the inhibitory actions of cannabinoids on sensory nerve depolarisation mediated by capsaicin, hypertonic saline and PGE_2 on isolated guinea-pig and human vagus nerve preparations, and the cough reflex in conscious guinea-pigs.

3 The non-selective cannabinoid (CB) receptor agonist, CP 55940, and the selective CB_2 agonist, JWH 133 inhibited sensory nerve depolarisations of the guinea-pig vagus nerve induced by hypertonic saline, capsaicin and PGE_2. These responses were abolished by the CB_2 receptor antagonist SR144528, and unaffected by the CB_1 antagonist SR141716A. Similarly, JWH 133 inhibited capsaicin-evoked nerve depolarisations in the human vagus nerve, and was prevented by SR144528.

4 Using a guinea-pig *in vivo* model of cough, JWH 133 (10 mg kg^{-1}, i.p., 20 min) significantly reduced citric acid-induced cough in conscious guinea pigs compared to those treated with the vehicle control.

5 These data show that activation of the CB_2 receptor subtype inhibits sensory nerve activation of guinea-pig and human vagus nerve, and the cough reflex in guinea-pigs, suggesting that the development of CB_2 agonists, devoid of CB_1-mediated central effects, will provide a new and safe antitussive treatment for chronic cough.
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Keywords: Cannabinoids; airway sensory nerves; cough; human; guinea-pig

Abbreviations: COPD, chronic obstructive pulmonary disease; KHS, Krebs–Henseleit solution; PGE_2, prostaglandin E_2; RARs, rapidly adapting receptors; THC, Δ^9-tetrahydrocannabinol; TRPV1, vanilloid receptor 1

Introduction

Cough is a dominant and persistent symptom of many inflammatory lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), viral infections, pulmonary fibrosis and bronchiectasis. Chronic cough can also be idiopathic in nature, where no obvious causal mechanism is evident. Cough is the most common complaint for which medical attention is sought, and although effective treatments for cough are not available, narcotic agents, such as the opioid codeine, are often used. However, such agents have only limited beneficial value due to the associated side effects such as constipation, nausea, vomiting and drowsiness. Therefore, the identification of novel therapies, devoid of central activity, for the treatment of chronic cough would be of significant therapeutic benefit and greatly enhance the quality of life of patients who suffer from this condition.

The cough reflex is predominantly under the control of two different classes of sensory afferent nerve fibres, namely the myelinated, rapidly adapting receptors (RARs or Aδ fibres), and nonmyelinated C-fibres with bronchial or pulmonary endings (Coleridge & Coleridge, 1984; Sant'Ambrogio, 1987; Lalloo *et al*., 1995), activation of which elicits cough *via* an afferent central reflex pathway. Evidence suggests that sensory nerve activity may be enhanced in inflammatory lung diseases such that the normally protective cough reflex becomes exacerbated and deleterious (Carr & Undem, 2001). Hence, theoretically, agents that inhibit sensory nerve activity (i.e. nerve depolarisations) will ultimately lead to a reduction in the cough reflex. In fact, this paradigm exists not only in relation to the cough response (Fox *et al*., 1997), but also with other sensory nerve-mediated responses such as vagally-induced plasma extravasation into the airways (Birrell *et al*., 2002). Moreover, such agents would act peripherally and therefore avoid the CNS side effects of centrally acting drugs such as opioids.

Agents such as hypertonic saline (Lalloo *et al*., 1995; Fox *et al*., 1996; 1997; Pedersen *et al*., 1998), capsaicin (Lalloo *et al*., 1995; Fox *et al*., 1996) and the endogenous prostanoid,

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prostaglandin E_2 (PGE_2; Roberts *et al*., 1985; Smith *et al*., 1998), are known sensory nerve stimulants. Furthermore, isolated guinea-pig and human vagus nerve preparations have been shown to elicit similar nerve depolarisation responses to these stimulants (Belvisi *et al*., 1998). Moreover, these agents, along with citric acid, are known tussigenic agents in human (Costello *et al*., 1985; Stone *et al*., 1992; Laude *et al*., 1993) and animal studies (Lalloo *et al*., 1995). Both *in vivo* cough studies (Lalloo *et al*., 1995) and *in vitro* single-fibre recordings of sensory nerves innervating the airways have shown that hypertonic saline excites RARs (conducting in the Aδ range) and C-fibres, and that citric acid and capsaicin excite C-fibres based on the sensitivity of these responses to the capsaicin receptor (vanilloid receptor 1 (TRPV1)) antagonist, capsazepine (Fox *et al*., 1993; 1995). Furthermore, i*n vivo* studies suggest that PGE_2 activates C-fibres (Coleridge *et al*., 1976) and RARs (Mohammed *et al*., 1993).

There is interest in the therapeutic potential of cannabinoids, including the major active principle of marijuana, Δ^9-tetrahydrocannabinol (THC). Non-selective cannabinoid receptor agonists have been shown to have therapeutic applications for a number of important medical conditions, including pain, anxiety, glaucoma, nausea, emesis, muscle spasm and wasting diseases (Porter & Felder, 2001). Although non-selective cannabinoids, such as anandamide, have been shown to suppress the cough reflex (Gordon *et al*., 1976; Calignano *et al*., 2000), the associated side effects such as sedation, cognitive dysfunction, tachycardia and psychotropic effects have hampered the use of such agonists for treatment purposes (Porter & Felder, 2001). Furthermore, appropriate validation of this hypothesis in the relevant human tissue (i.e. the vagal sensory nerves) or *in vivo* in man has not yet been provided.

Cannabinoids mediate their effects *via* CB_1 and CB_2 receptor subtypes (Matsuda *et al*., 1990; Munro *et al*., 1993). CB_1 receptors are predominantly distributed throughout the brain and spinal cord, and are also expressed at low levels in several peripheral tissues. In contrast, CB_2 receptors have not, to date, been found to be expressed in the CNS (Munro *et al*., 1993). In this study, we determined whether activation of specific cannabinoid receptor subtypes could inhibit sensory nerve activity (i.e. nerve depolarisations) in the airways and the cough reflex. Guinea-pig vagus preparations were used to characterise the cannabinoid receptor subtype involved, as pharmacological profiling *in vitro* is often more straightforward when drug action is not complicated by pharmacokinetic issues. Finally, human vagus preparations were used to confirm observations generated in the guinea-pig, to provide the appropriate validation of the target in man and confirm clinical relevance.

In these experiments, we have used the non-selective agonist CP 55940, the CB_2-selective agonist JWH 133, the CB_1 receptor antagonist SR141716A and the CB_2-receptor antagonist SR144528 as pharmacological tools with which to characterise the cannabinoid receptor subtype involved in this response. CP 55940 has essentially the same affinity for CB_1 and CB_2 receptors. The affinities for both receptors are in the nanomolar range, and this agonist exhibits relatively high efficacy at both these receptor types (Pertwee, 1999). JWH133 is the most selective CB_2 receptor agonist that is currently available commercially. Its binding affinities (K_i) for CB_2 and CB_1 receptors are 3.4 ± 1.0 and 677 ± 132 nM, respectively (Huffman *et al*., 1999). The diarylpyrazole SR141716A was developed by Sanofi, and is a highly potent and selective CB_1 receptor antagonist ($K_i = 5.9$ nM for CB_1 and $> 1\,\mu$M for CB_2; pA_2 for SR141716A at CB_1 receptors = 7.9; Rinaldi-Carmona *et al*., 1994). SR144528 is also a diarylpyrazole developed by Sanofi that binds with markedly higher affinity to CB_2 than CB_1 receptors ($K_i = 0.6$ nM for CB_2 and 437 nM for CB_1; pA_2 for SR144528 at $CB_2 = 6.3$; Rinaldi-Carmona *et al*., 1998).

Methods

Measurement of sensory nerve depolarisation in isolated vagus nerve preparations

Male Dunkin–Hartley guinea-pigs (300–350 g) were housed in a temperature-controlled (21°C) room with food and water freely available. Guinea-pigs were killed by cervical dislocation and the vagus nerves, caudal to the nodose ganglion, were carefully removed and placed in Krebs–Henseleit solution (KHS) of the following composition (mM): NaCl – 118; KCl – 5.9; $MgSO_4$ – 1.2; NaH_2PO_4 – 1.2; $CaCl_2$ – 2.5; glucose – 6.6; $NaHCO_3$ – 25.5, and bubbled with 95% O_2/5% CO_2. Human trachea, with branches of the cervical vagus still attached, was obtained from a donor patient (male, 45 years) for a heart or heart/lung transplant. Relevant approvals were obtained from the Royal Brompton and Harefield Trust Ethics Committee. Segments of human and guinea-pig vagus nerve (40–50 mm) were cleared of connective tissue, and carefully desheathed under a dissecting microscope. Throughout, care was taken to ensure that the nerve trunks remained in oxygenated KHS, and that they were not stretched or damaged in any way. The desheathed nerve trunk was mounted in a 'grease-gap' recording chamber as previously described (Rang & Ritchie, 1988; Birrell *et al*., 2002). Briefly, the nerve was drawn longitudinally through a narrow channel (2 mm diameter, 10 mm length) in a Perspex block. The centre of the channel was filled with petroleum jelly, injected through a side arm when the nerve was in place, onto the middle of the vagus, creating an area of high resistance, and electrically isolating the extracellular space between the two ends of the nerve. One end of the nerve emerged into a wider channel, and was constantly superfused with KHS at a flow rate of approximately 2 ml min^{-1}. The other nerve ending remained in a second, smaller chamber containing oxygenated KHS throughout the experiments. Ag/AgCl electrodes (Mere 2 Flexible reference electrodes, World Precision Instruments (WPI)), filled with KHS, made contact at either end of the nerve trunk and recorded DC potential *via* a DAM 50 differential amplifier (WPI). DC voltages were amplified $\times 10$, filtered at 1000 Hz, and sampled at 5 Hz. During each experiment, simultaneous recordings were made from two nerves. The temperature of the perfusate was maintained at 37°C by means of a water bath. The pen recorder was calibrated such that 1 mm was equivalent to 10 mV (incorporating the $\times 10$ amplification using a DAM 50 amplifier). The superfusing Krebs solution could be quickly changed by means of a tap, with little artefact, and the new solution reaching the vagus with a delay of approximately 10 s. Drugs were applied at known concentrations into the perfusing solution of the first channel only, and depolarising responses recorded onto a chart recorder (Lectromed Multi-Trace 2).

Sensory nerve activity, that is, nerve depolarisations, were induced by perfusion of the vagus nerve with pre-established (data not shown) submaximal concentrations of either hypertonic saline (2%), capsaicin (1 μM) or PGE_2 (1 μM). The stimulants were applied for a period of 4 min, after which the tissue was washed until the baseline response of the nerve was regained. After two reproducible responses to the nerve stimulants, the non-selective cannabinoid agonist CP 55940 or the CB_2 receptor agonist JWH 133 were added to the KHS, perfusing the nerves for 20 min prior to a subsequent administration of stimulant, while still in the presence of the agonist. In separate experiments, the CB_1 receptor antagonist SR141716A or the CB_2 receptor antagonist SR144528 were added 10 min prior to application of the agonist, and were also present for the duration of the experiment. Only one concentration of one agonist and/or antagonist was tested per vagus preparation. For each experimental condition using guinea-pig vagus preparations, $n=4$ determinations were performed. Due to the limited availability of human vagus nerve, only key experiments were performed.

Measurement of cough in conscious guinea-pigs

Male Dunkin–Hartley outbred guinea-pigs (300–350 g) were housed in a temperature-controlled (21°C) room with food and water freely available for at least 1 week before the commencement of experiments. The procedure for measuring cough in conscious guinea-pigs was as previously described (Lalloo *et al*., 1995). Cough sounds were amplified and recorded concurrently *via* a microphone placed inside the cough chamber, and recorded as spikes on a chart recorder. Solutions were delivered by aerosol *via* a nebuliser (De Vilbiss, Somerset, PA, U.S.A.). Coughs were counted by a trained observer and recognised from the characteristic opening of the mouth and posture of the animal, the sound produced, and the sound and airflow recordings. Using these criteria together, cough was easily distinguished from sneezes and augmented breaths. All animals were treated with terbutaline sulphate (0.05 mg kg^{-1}, i.p.) 10 min before the cough challenge, to minimise respiratory distress due to bronchoconstriction. JWH 133 (10 mg kg^{-1}, i.p., $n=8$) or vehicle (0.5% methyl cellulose with 0.2% Tween 80 in saline, i.p., $n=8$) was administered 20 min prior to exposure to the tussive agent citric acid (0.3 M) for 10 min, during which time the number of coughs were counted.

Materials

All Krebs compounds were obtained from BDH (Dorset, U.K.), and KHS was made fresh on a daily basis. SR141716A and SR144528 were kind gifts from Novartis Institute, London, U.K. Cannabinoid agonists were obtained from Tocris Cookson Ltd (Bristol, U.K.). All other chemicals were obtained from Sigma Aldrich. Stock concentrations of PGE_2 and CP 55940 were diluted in 100% ethanol and stock concentrations of capsaicin, SR141716A, SR144528 and JWH 133 were made in 100% DMSO. Further dilutions of all compounds were such that a final concentration of 0.1% of the diluent was always achieved. For the *in vivo* experiments, all drug solutions were freshly prepared on the day of each experiment. JWH 133 was suspended 0.5% methyl cellulose with 0.2% Tween 80 in saline (vehicle).

Statistical analysis

All the values in the figures and text are expressed as mean $\pm$ s.e.m. For the *in vitro* studies, two vagal preparations were obtained from each animal. Only one concentration of one agonist was tested per vagus nerve preparation, and experiments were randomised; hence, different concentrations of different drugs were tested on vagi from the same animal on the same day. Nerve depolarisation responses were measured in mm after 4 min from the time of stimulant addition, and then expressed as mV depolarisation. In experiments where tissues were treated with test compounds, responses were expressed as mV before (control response) and after drug additions, and then expressed as a percentage change from control. In these experiments, the data were subjected to a paired two-tailed *t*-test (significance is denoted by $P<0.05$*, $P<0.01$**, $P<0.001$***), since the response to a stimulant was measured before and after drug intervention within the same nerve. An unpaired *t*-test ($P<0.001$ denoted by ###) was used, where two different treatment groups were compared. For the *in vivo* cough experiments, the numbers of coughs during the 10 min exposure to citric acid were compared between treated and control groups using an unpaired *t*-test ($P<0.01$**). pD_2 values (−log of the EC_{50} defined as the concentration of drug required to elicit 50% of the maximum inhibition) and statistical significance were calculated using 'GraphPad Instat™' (© GraphPad software).

Results

In individual guinea-pig nerve preparations, control responses were obtained to hypertonic saline (2%), capsaicin (1 μM) or PGE_2 (1 μM). These stimuli elicited nerve depolarisations of 0.61 ± 0.04, 0.34 ± 0.03 and 0.18 ± 0.01 mV, respectively.

Perfusion of the vagus preparations with the non-selective cannabinoid agonist CP 55940 (0.03–3 μM) inhibited capsaicin-induced nerve depolarisation in a concentration-dependent manner (p$D_2=6.2$). CP 55940 (concentrations between 0.03 and 100 μM) also inhibited, in a concentration-dependent manner, depolarisation of the guinea-pig vagus elicited by PGE_2 and hypertonic saline (2%) (pD_2 values of 6.0 and 5.55, respectively). Complete inhibition was observed in each case (Figure 1a–c). Similarly, nerve preparations treated with the selective CB_2 receptor agonist JWH 133 (Huffman *et al*., 1999; Pertwee, 1999) (concentrations between 0.3 and 100 μM) markedly reduced sensory nerve depolarisation induced by capsaicin, PGE_2 and hypertonic saline (2%) in a concentration-dependent manner, with pD_2 values of 5.5, 5.4 and 5.1. Maximal inhibition was achieved at concentrations of 10 (97.5 ± 2.5%), 30 (100%) and 30 μM (91.6 ± 0.7%), respectively (Figure 1d–f).

The involvement of CB_2 receptors, and not CB_1 receptors, in mediating the inhibitory action of CP 55940 or JWH 133 was confirmed in experiments where the CB_1-selective antagonist SR141716A (0.01 μM; Rinaldi-Carmona *et al*., 1994) or the CB_2-selective antagonist SR144528 (0.01 μM; Rinaldi-Carmona *et al*., 1998) was perfused 10 min prior to application of a submaximal concentration of either CP 55940 or JWH 133. Submaximal concentrations of CP 55940 (1 μM) or JWH 133 (3 μM, where the stimulus was capsaicin, and 10 μM, where the

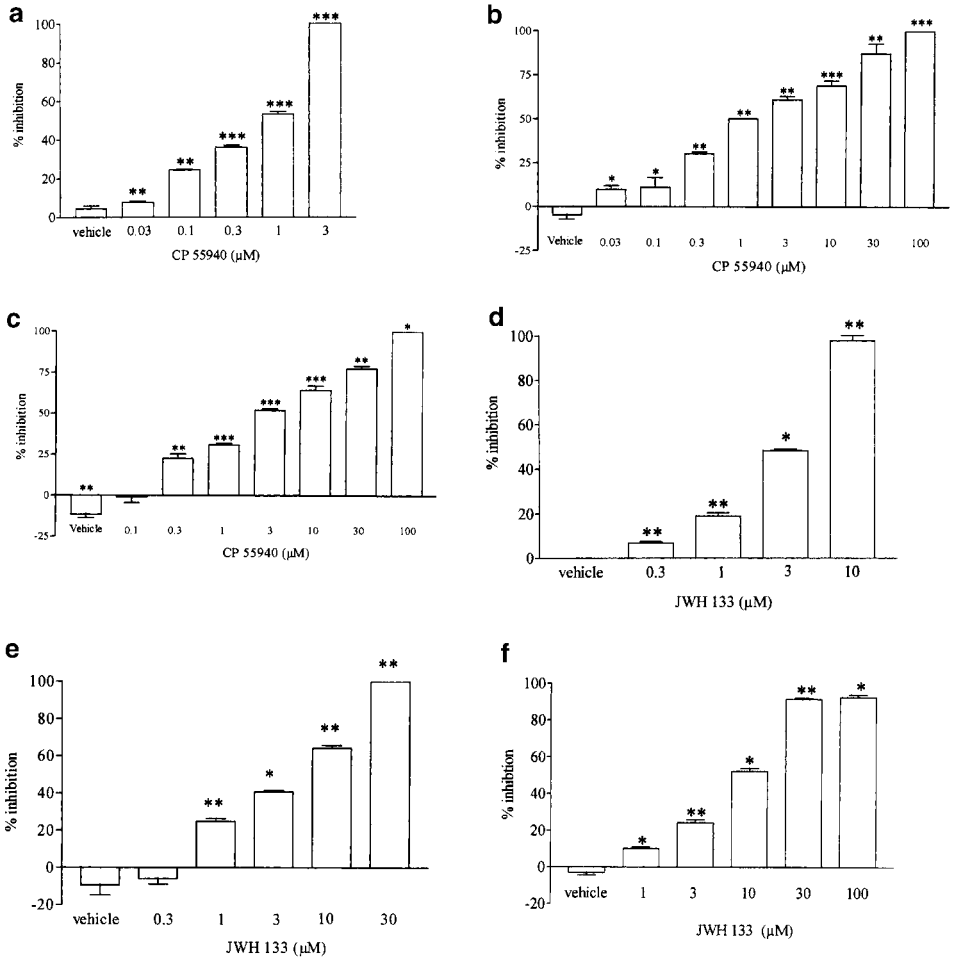

Figure 1 Inhibition of nerve depolarisation by cannabinoids. The nonselective cannabinoid agonist CP 55940 inhibits (a) capsaicin (1 μM), (b) PGE_2 (1 μM) and (c) hypertonic saline (2%)-induced depolarisation of the guinea-pig vagus nerve. Similarly, the CB_2-selective receptor agonist JWH 133 inhibits (d) capsaicin (1 μM), (e) PGE_2 (1 μM) and (f) hypertonic saline (2%)-induced depolarisation of the guinea-pig vagus nerve. Nerve depolarisation responses were expressed as absolute values in mV depolarisation before and after drug additions, and then expressed a percentage change. The data were subjected to a paired two-tailed *t*-test, since the response to a stimulant was measured before and after drug treatment within the same nerve. $*P<0.05$, $**P<0.01$ and $***P<0.001$ denote statistical significance. Values are presented as the mean $\pm$ s.e.m. percentage change of $n=4$ determinations. pD_2 values (−log of the EC_{50} defined as the concentration of drug required to elicit 50% of the maximum response) and statistical significance were calculated using 'GraphPad Instat™' (© GraphPad Software).

stimulus was hypertonic saline or PGE_2) were selected based on their respective concentration–response curves. CP 55940 (1 μM) inhibited capsaicin-induced nerve depolarisations of guinea-pig vagus nerve (before: 0.39 ± 0.04 mV; after: 0.21 ± 0.02 mV; $n=4$; $P<0.01$). This effect was completely blocked in the presence of SR144528 (before: 0.38 ± 0.05 mV; after: 0.53 ± 0.1 mV; $n=4$), and was unaffected by SR141716A (before: 0.5 ± 0.05 mV; after: 0.23 ± 0.03 mV; $n=4$; $P<0.01$). The vehicles for these agents (0.1% DMSO for SR141716A and SR144528 or 0.1% ethanol for CP 55940) had no significant effect on capsaicin-induced nerve depolarisations, either alone or in combination. Inhibitory responses induced by JWH 133 were also completely blocked by SR144528 and unaffected by SR141716A in all cases, regardless of the stimulus used (Figure 2). Experiments performed on the human vagus nerve confirm a similar inhibitory effect of JWH 133 (40% inhibition at 10 μM) against nerve depolarisations induced by capsaicin (1 μM), which was prevented in the presence of SR144528 (Figure 3).

Based on these observations, experiments were performed using an *in vivo* model of cough, in order to determine if the inhibitory actions of JWH 133 on sensory nerves *in vitro* are also seen *in vivo*. Indeed, administration of JWH 133 (10 mg kg^{-1}, i.p.) 20 min prior to exposure to the commonly used tussive agent citric acid (0.3 M, 10 min) significantly reduced cough in conscious guinea-pigs (0.94 ± 0.24 cough min^{-1}), compared to those treated with the vehicle control (2.05 ± 0.24 cough min^{-1}; Figure 4). No sedation was observed in the guinea-pigs treated with JWH 133.

Discussion

In this study, we have shown for the first time that activation of the CB_2 receptor subtype inhibits both guinea-pig and human airway sensory nerve activity and the cough reflex in guinea-pigs. The isolated vagus preparation was used to characterise the cannabinoid receptor subtype involved in this

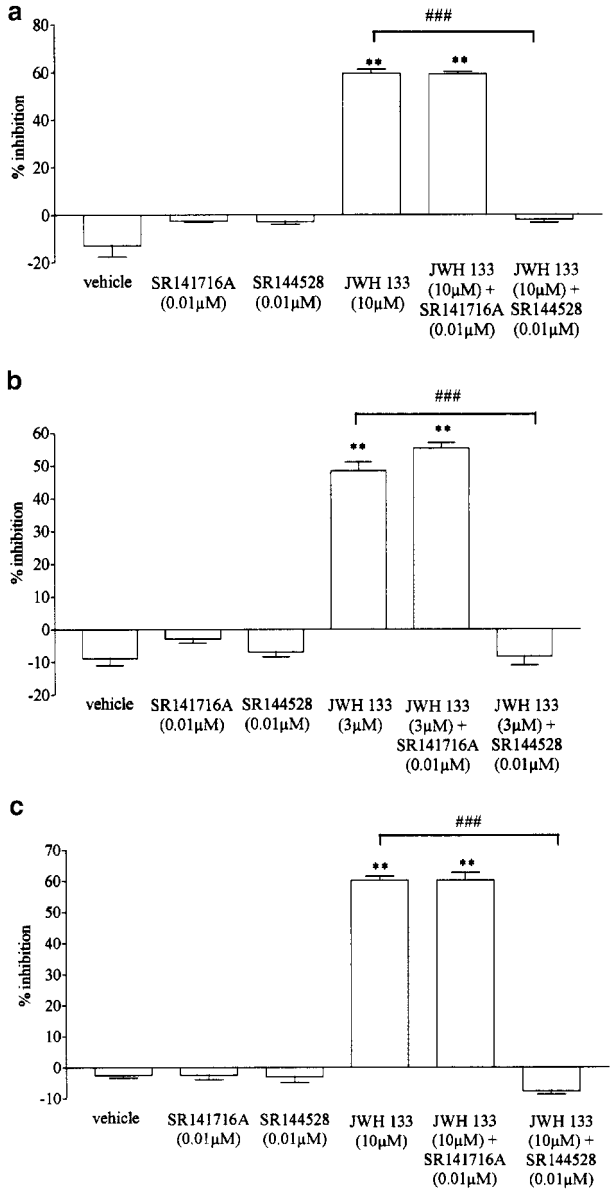

Figure 2 JWH 133-mediated inhibitory responses on nerve depolarisations are SR144528 sensitive. The inhibitory action of JWH 133 on guinea-pig vagus nerve depolarisations induced by (a) hypertonic saline (2%), (b) capsaicin (1 μM) and (c) PGE_2 (1 μM) is abolished in the presence of the CB_2 receptor antagonist SR144528, and is unaffected by the CB_1 receptor antagonist SR141716A. Nerve depolarisation responses were expressed as absolute values in mV depolarisation before and after drug additions, and then expressed a percentage change. The data were subjected to a paired two-tailed *t*-test, since the response to a stimulant was measured before and after drug treatment within the same nerve. $^{**}P<0.01$ denotes the statistical significance compared to control responses in the same tissue prior to drug treatment and $^{###}P<0.001$ denotes the statistical significance between two different treatment groups using an unpaired *t*-test. Values are presented as the mean $\pm$ s.e.m. percentage change of $n=4$ determinations.

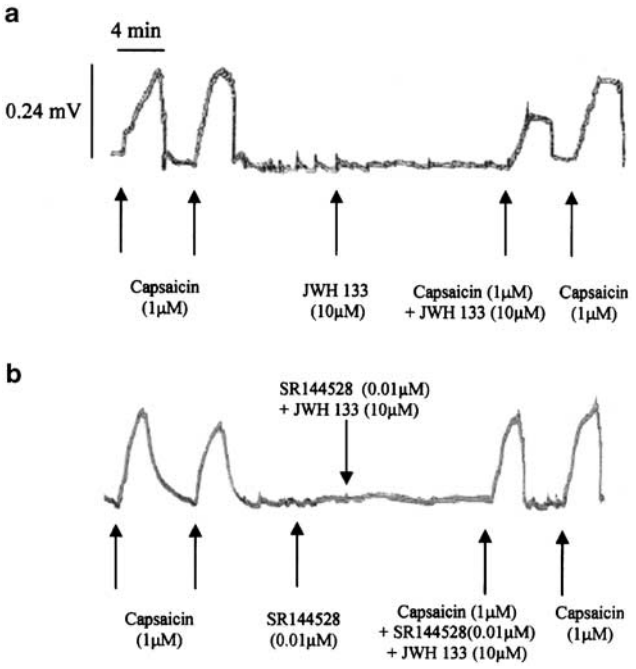

Figure 3 Effect of cannabinoid ligands on nerve depolarisations of isolated human vagus. Traces showing (a) the inhibitory effect of JWH 133 on nerve depolarisations induced by capsaicin, and (b) the blockade of this response by the CB_2 receptor antagonist SR144528 from human vagus nerve.

response, as pharmacological profiling *in vitro* is often more straightforward as pharmacokinetic issues do not complicate the interpretation of the drug action. However, although the isolated vagus preparation presents us with the ideal opportunity to conduct a comprehensive pharmacological assessment, data using this preparation should be interpreted with some caution since the pharmacological agents are applied to the axon of the isolated vagus nerve *in vitro*. Thus, the depolarisation signal recorded extracellularly represents a summation of the changes in membrane potential of all the axons *via* activation of receptors expressed in the neuronal membrane of the axon. Furthermore, the receptor expression and signal transduction mechanisms in the axon may not necessarily represent the behaviour of those elements in the peripheral endings.

Capsaicin, PGE_2 and hypertonic saline-induced nerve depolarisations of the guinea-pig vagus nerve were inhibited by the non-selective cannabinoid agonist CP 55940 in a concentration-dependent manner. Similarly, the CB_2-selective agonist JWH 133 (Huffman *et al*., 1999; Pertwee, 1999) reduced responses to hypertonic saline, capsaicin and PGE_2 in a concentration-dependent manner. Furthermore, the inhibitory responses induced by CP 55940 on depolarisation responses evoked by capsaicin were not affected when vagus preparations were pretreated with the selective CB_1 receptor antagonist SR141716A ($K_i=5.9$ nM for CB_1 and >1 μM for CB_2; Rinaldi-Carmona *et al*., 1994), but were completely abolished by the selective CB_2 receptor antagonist SR144528 ($K_i=0.6$ nM for CB_2 and 437 nM for CB_1; Rinaldi-Carmona *et al*., 1998). In addition, the inhibitory action of JWH 133 on nerve depolarisations induced by any of the stimuli was completely abolished by SR144528 and unaffected by SR141716A. Similarly, the inhibitory effect of JWH 133 on capsaicin-induced nerve depolarisation of the human vagus was abolished in the presence of SR144528. Antagonist affinity is the key factor when assessing receptor selectivity. The concentration of the antagonists used (0.01 μM) is similar to the

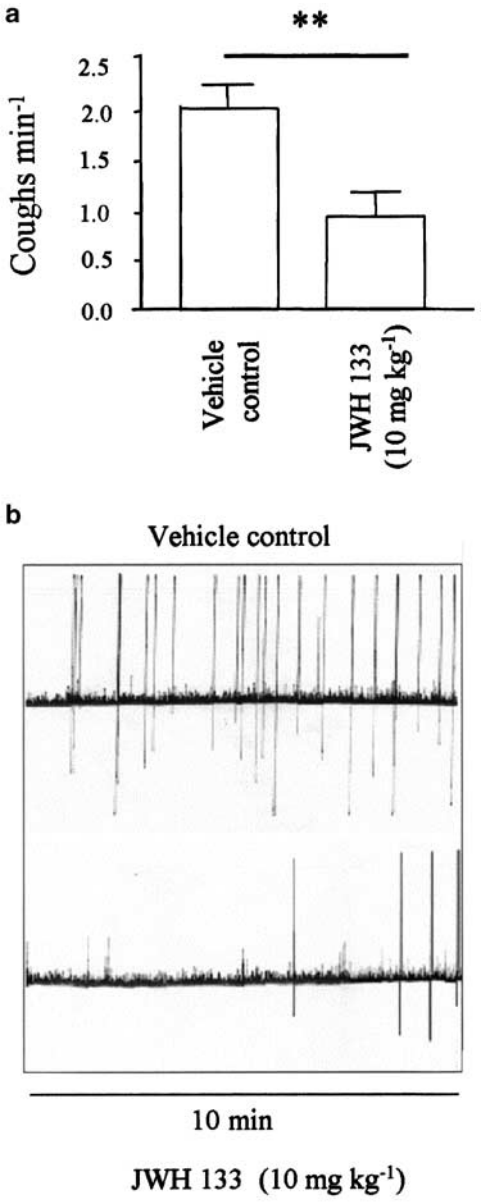

Figure 4 JWH 133 inhibits citric acid-induced cough in guinea-pigs. Histogram describing the mean data (a) and a representative trace (b) showing the effect of the CB_2-selective agonist JWH 133 ($10\,mg\,kg^{-1}$, i.p., 20 min, $n=8$) on citric acid (0.3 M, 10 min)-induced cough in conscious guinea-pigs compared to that of vehicle control-treated animals (0.5% methyl cellulose with 0.2% Tween 80 in saline, i.p., 20 min, $n=8$). The number of cough sounds detected by a microphone and recorded on a chart recorder (indicated by spikes) during the 10 min exposure period to citric acid (0.3 M) were compared between treated and control groups using an unpaired *t*-test ($^{**}P<0.01$).

pA_2 values for SR141716A at the CB_1 receptor ($pA_2=7.9$; Rinaldi-Carmona *et al*., 1994), and less than the pA_2 for SR144528 at the CB_2 receptor ($pA_2=6.3$; Rinaldi-Carmona *et al*., 1998). Hence, the data presented here clearly demonstrate that activation of CB_2 receptors mediates the inhibitory action of cannabinoids on sensory nerve depolarisation. This study is unique given the opportunity we have to validate the target, in this case the CB_2 receptor, in the relevant human tissue involved in evoking a tussive response, that is, human vagal sensory nerves.

We have previously demonstrated that agents shown to directly inhibit sensory nerve depolarisations *in vitro* also inhibit sensory nerve-mediated responses *in vivo*, such as plasma exudation (Birrell *et al*., 2002) and cough (Fox *et al*., 1997). Based on the observation that JWH 133 inhibits guinea-pig sensory nerve depolarisations induced by hypertonic saline, capsaicin and PGE_2, it is probable that JWH 133 inhibits the activity of both subpopulations of sensory nerves involved in the cough reflex (i.e. RARs and C-fibres). Indeed, the *in vivo* activity of JWH 133 was demonstrated by an inhibition of citric acid-induced cough in conscious guinea-pigs compared to those treated with vehicle control. No sedation was observed in guinea-pigs treated with the CB_2 agonist as measured by lack of activity, nonsupine position and responses to external environment, thus indicating that no central effects of this agent were observed, suggesting that this response is mediated *via* peripheral CB_2 receptor activation. Contrary to this, it has been suggested that the nonselective endocannabinoid anandamide suppresses cough in conscious guinea-pigs *via* activation of CB_1 receptors and not CB_2 (Calignano *et al*., 2000). However, such agents have sedative effects *via* activation of central CB_1 receptors and, therefore, the suppressive effect of anandamide on the cough reflex through sedation cannot be excluded (Lichtman *et al*., 1998; Manzanares *et al*., 1999). More recently, however, anandamide has been shown to increase the cough reflex *via* activation of TRPV1 receptors (Jia *et al*., 2002), and is consistent with data suggesting that anandamide (at high concentrations) activates rat and guinea-pig pulmonary vagal C fibres *via* TRPV1 receptor activation (Kagaya *et al*., 2002; Lin & Lee, 2002). The contrasting data from these studies may be due to the different experimental conditions employed, and in particular the doses of anandamide used, as this agent is considerably less potent than capsaicin at the TRPV1 receptor (Szallasi & Di Marzo, 2000; Ralevic *et al*., 2001).

The cough reflex is thought to be initiated *via* the activation of either RAR or C-fibre afferents. However, it has been suggested that effects such as bronchospasm, mucus secretion and plasma extravasation due to the release of neuropeptides following C-fibre activation may indirectly lead to RAR activation and the initiation of the cough reflex (Canning, 2002). Consistent with the notion of C-fibre-mediated RAR activation, capsaicin-induced cough can virtually be abolished by neurokinin receptor antagonists. Such effects of inhaled neurokinin antagonists (Girard *et al*., 1995; Xiang *et al*., 1998), and systemically administered, low CNS-penetrant compounds (Hay *et al*., 2002), in the guinea-pig cough model may argue for an indirect role of C-fibres in cough. Hence, it is possible that the inhibitory action of the CB_2 agonist on sensory nerve function may be due to a prejunctional effect on neurokinin release from airway C-fibres. However, contrary to this, Bolser *et al*. (1997) have demonstrated that neurokinin receptor antagonists inhibit cough in guinea-pigs and cats, solely *via* an effect on the central nervous system. On this basis, a central action of neurokinin receptor antagonists cannot be ruled out. Therefore, the suggestion that the CB_2 agonist directly inhibits airway C-fibres to inhibit cough cannot be excluded, and is consistent with the *in vitro* data presented on the isolated nerve preparation.

The present study indicates, with the use of pharmacological tools, the existence of neuronal CB_2 receptors in the airways. These data show, for the first time, that activation of the CB_2 receptor subtype inhibits both Aδ and C-fibre activation and the cough reflex in guinea-pigs. Moreover, the inhibitory action of the CB_2 receptor agonist and the prevention of this effect by the CB_2 receptor antagonist on human vagus nerve provides proof of concept for the mechanism in man, and is strong evidence to suggest that the development of CB_2 agonists, devoid of central effects, will provide a new and safe antitussive treatment for chronic cough.

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