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Preclinical Assessment of Novel Therapeutics on the Cough Reflex: Cannabinoid Agonists as Potential Antitussives

Maria G. Belvisi

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Abstract Cough, a reflex defense mechanism, is a common symptom of many airway inflammatory diseases. At present there are no satisfactory treatments for cough that have an acceptable side effect profile. Recent data have described the inhibitory effect of selective cannabinoid CB_2 receptor agonists on sensory nerve activity *in vitro* and the cough reflex in a guinea pig model. CB_2 receptor expression is limited in the central nervous system (CNS) and hence the development of selective agonists may provide a new therapeutic strategy for treatment of cough devoid of the CNS-mediated side effects that are normally associated with nonselective cannabinoid agonists.

Keywords Cannabinoids · Cough · Sensory nerves

Cough

Cough, a reflex defense mechanism, is a most common symptom of many inflammatory diseases of the airways such as asthma and chronic obstructive pulmonary disease (COPD), post viral infections, pulmonary fibrosis, and bronchiectasis [1]. Indeed, it is the first and most persistent symptom of diseases such as asthma and COPD. At present there are no satisfactory treatments for cough, as was outlined in a recent review [2] in which over-the-counter cough medicines were assessed. They concluded that the currently available medicines could not be recommended because there was no good evidence for their effectiveness. The identification of new therapeutic targets for the treatment of chronic cough therefore will be of immense therapeutic benefit and will greatly enhance the quality of life of patients.

M. G. Belvisi (✉)
Respiratory Pharmacology Group, National Heart & Lung Institute, NHLI, Faculty of Medicine, Imperial College London, Guy Scadding Building, Dovehouse Street, London SW3 6LY, UK
e-mail: m.belvisi@imperial.ac.uk

Animal Models

It is therefore essential to develop animal models of cough, models that reflect the disease in man. Therefore, a reliable, robust, and reproducible model of cough is essential to profile, and establish the efficacy of, novel antitussive therapies under development before testing in man. The chosen model should also allow the study of the physiology of cough and the mechanisms and mediators that lead to cough or the exacerbation of cough. Therefore, a requirement of the animal of choice for the model is that the physiology should resemble as closely as possible that of man, which in models used to study the cough reflex means not only the structure of the lungs, but the innervation of the trachea, bronchi, and intrapulmonary airways.

Guinea Pig Cough

In an attempt to accurately reflect the disease in man, several different species have been used to provide a variety of models of cough. Most preclinical studies of neural pathways involved in the cough reflex and the pharmacologic regulation of those pathways have been conducted in mice, rats, guinea pigs, rabbits, cats, and dogs [3], and more recently in conscious pigs [4]. However, the most useful and commonly used model for cough studies in recent years has been the conscious guinea pig [5, 6]. Much

information has now been gathered in this model regarding the pharmacologic modulation of the cough reflex. Various tussive stimuli have been examined with the most commonly used being inhaled citric acid or capsaicin. In these experiments cough can be detected by putting the guinea pig in a transparent Perspex chamber, exposing it to aerosols of tussive stimuli, and measuring changes in airflow, observing the characteristic posture of an animal about to cough, and recording the cough sound [7–10].

Tussive Agents

In addition to capsaicin and low pH solutions (commonly used to elicit cough experimentally both clinically and in animal models), a variety of proinflammatory mediators evoke cough, including bradykinin and prostaglandin E_2. However, it is not clear whether all tussive agents share a common mechanism of action with regards to their ability to stimulate sensory nerve activity. Capsaicin has been demonstrated to elicit cough in a TRPV1-dependent manner as determined using tool compounds known to inhibit this receptor such as capsazepine [10, 11]. However, preliminary evidence suggests that other agents may not elicit cough via the same mechanism [10]. Therefore, when investigating the action of novel antitussive agents in both clinical and preclinical models, it may be important to test agents against a number of tussive agents that could be acting via different mechanisms.

Disease Models

Models have also been configured to mimic the exaggerated cough seen in disease conditions, e.g., allergic models to mimic the cough in asthma [12, 13] and cigarette smoke exposure to mimic enhanced cough in COPD [14, 15]. Interestingly, in cigarette smoke models the response to certain tussive agents is increased, decreased, or not changed depending on the cough-provoking agents examined. For example, the numbers of cough produced by capsaicin and low pH solutions were increased and those provoked by hypertonic saline remained unchanged [15]. In conclusion, understanding the patterns of cough elicited by tussive agents and how these can be modulated in "disease" may lead to a greater understanding of the mechanisms surrounding cough and the development of novel antitussive agents.

Cannabinoids and Cough

Currently there is renewed interest in the therapeutic potential of cannabinoids, including the major active principal of marijuana, Δ^9-tetrahydrocannabinol (THC). Nonselective cannabinoids have been shown to have wide therapeutic applications for a number of important medical conditions, including pain, anxiety, glaucoma, nausea, emesis, muscle spasms, and wasting diseases. However, associated side effects such as sedation, cognitive dysfunction, tachycardia, and psychotropic effects have hampered the use of these compounds in treatment protocols [16]. Cannabinoids mediate their effects via at least two specific G-protein-coupled receptors, termed the CB_1 and CB_2 receptors [17, 18] (see Table 1 for a list regarding CB receptor tools). CB_1 receptors are distributed predominantly throughout the brain and spinal cord and are also expressed at low levels in several peripheral tissues. In contrast, CB_2 receptors are not commonly expressed in the central nervous system (CNS) [19–21] but primarily on immune tissues such as the spleen and tonsils and lymphocytes [21]. Studies suggest that cannabinoids have diverse effects on sensory nerve function. Activation of spinal CB_1 receptors inhibits nociceptive transmission [22], hyperalgesia, and neuropeptide release from central primary afferent fibers. More recently, CB_2 receptor activation has been demonstrated to be sufficient to inhibit acute nociception, inflammatory hyperalgesia, and the allodynia and hyperalgesia produced in models of neuropathic pain [23, 24].

Table 1 Commercially available cannabinoid ligands that can be used to investigate the role of the CB_1 and CB_2 receptors in biological processes

Nomenclature	CB_1	CB_2
Main transduction pathway	$G_{i/o}$	$G_{i/o}$
Selective agonists	O-1812	JWH 133
		L759633
		HU308
Selective antagonists	SR 141716A	SR144528
	LY 320135	AM630
Radioligands	[^{3}H]-CP55940	[^{3}H]-CP55940
	[^{3}H]-SR141716A	

There is still very little information about CB_2 receptors on peripheral sensory nerves and their involvement in tussive responses in the airways. We have previously demonstrated that the cannabinoid ligands and, in particular, the CB_2 receptor agonist JWH 133 inhibited capsaicin-induced guinea pig and human sensory nerve activation (Fig. 1) and citric acid–induced cough in conscious guinea pigs [25]. Although JWH 133 is reported to possess 200-fold selectivity for the CB_2 over the CB_1 receptor, it is not clear how selective this agonist is in *in vivo* situations. This is an important consideration given the reported antitussive activity of CB_1 receptor agonists probably due to their sedative activity [26, 27].

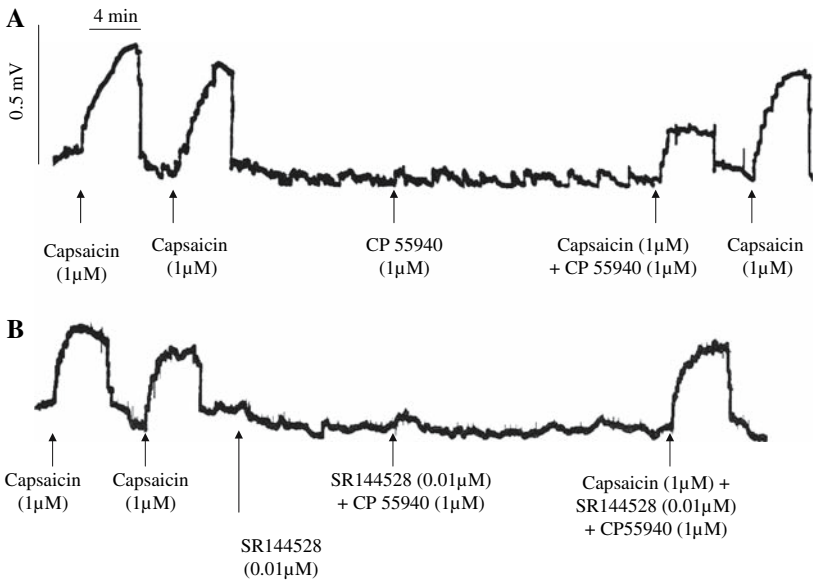

Fig. 1 Effect of cannabinoid ligands on nerve depolarizations of isolated guinea pig vagus. Traces showing (**A**) the inhibitory effect of the cannabinoid agonist CP 55,940 on nerve depolarizations induced by capsaicin, and (**B**) the blockade of this response by the CB_2 receptor antagonist SR144528 on guinea pig vagus nerve

Confirmation of the role of the CB_2 receptor as a target for antitussives has now been achieved by experiments demonstrating the sensitivity of this inhibitory response to blockade by a selective CB_2 receptor antagonist (unpublished data). These findings have important implications for the therapeutic potential of cannabinoids. There is limited CB_2 receptor expression in the CNS and hence the development of CB_2 receptor–selective agonists will provide a new therapeutic strategy for treatment of airway inflammatory diseases such as asthma and COPD that should be devoid of the CNS-mediated side effects that are normally associated with nonselective cannabinoid agonists.

Conclusions

Recent data has described an inhibitory effect of selective cannabinoid CB_2 receptor agonists on sensory nerve activity *in vitro* and the cough reflex in a guinea pig model. CB_2 receptor expression is limited in the CNS and hence the development of selective agonists may provide a new therapeutic strategy for treatment of cough devoid of the CNS-mediated side effects that are normally associated with nonselective cannabinoid agonists.

References

1. Widdicombe JG (1999) Advances in understanding and treatment of cough. Monaldi Arch Chest Dis 54:275–279
2. Shroeder K, Fahey T (2002) Systematic review of randomised controlled trials of over the counter cough medicines for acute cough in adults. BMJ 324:1–6
3. Karlsson JA, Fuller RW (1999) Pharmacological regulation of the cough reflex – from experimental models to antitussive effects in man. Pulm Pharmacol Ther 12:215–228
4. Moreaux B, Beerens D, Gustin P (1999) Development of a cough induction test in pigs: effects of SR 48968 and enalapril. J Vet Pharmacol Ther 22:387–389
5. Forsberg K, Karlsson JA, Theodorsen E, Lundberg JM, Persson CGA (1988) Cough and bronchoconstriction mediated by capsaicin-sensitive sensory neurons in guinea pigs. Pulm Pharmacol 1:33–39
6. Fox AJ, Barnes PJ, Urban L, Dray A (1993) An *in vivo* study of the properties of single vagal afferents innervating guinea pig airways. J Physiol 469:21–35
7. Fox AJ (1996) Modulation of cough and airway sensory fibres. Pulm Pharmacol 9:335–342
8. Fox AJ, Lalloo UG, Belvisi MG, Bernareggi M, Chung KF, Barnes PJ (1996) Bradykinin-evoked sensitization of airway sensory nerves: a mechanism for ACE-inhibitor cough. Nat Med 2:814–817
9. Fox AJ, Barnes PJ, Venkatesan P, Belvisi MG (1997) Activation of large conductance potassium channels inhibits the afferent and efferent function of airway sensory nerves in the guinea pig. J Clin Invest 99:513–519
10. Lalloo UG, Fox AJ, Belvisi M, Chung K, Barnes PJ (1995) Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs. J Appl Physiol 79:1082–1087
11. Trevisani M, Milan A, Gatti R, Zanasi A, Harrison S, Fontana G, Morice AH, Geppetti P (2004) Antitussive activity of iodo-resiniferatoxin in guinea pigs. Thorax 59(9):769–772
12. Liu Q, Fujimura M, Tachibana H, Myou S, Kasahara K, Yasui M (2001) Characterization of increased cough sensitivity after antigen challenge in guinea pigs. Clin Exp Allergy 31(3):474–484
13. Oribe Y, Fujimura M, Kita T, Katayama N, Nishitsuji M, Hara J, Myou S, Nakao S (2005) Attenuating effect of H+K+ATPase inhibitors on airway cough hypersensitivity induced by allergic airway inflammation in guinea-pigs. Clin Exp Allergy 35(3):262–267
14. Lewis CA, Ambrose C, Banner K, Battram C, Butler K, Giddings J, Mok J, Nasra J, Winny C, Poll C (2007) Animal models of cough: literature review and presentation of a novel cigarette smoke-enhanced cough model in the guinea-pig. Pulm Pharmacol Ther 20(4):325–333
15. Nasra J, Birrell MA, Poll C, Lewis CA, Belvisi MG (2007) Distinct patterns of response to tussive agents in a guinea-pig

model of cough: effect of cigarette smoke exposure. Am J Respir Crit Care Med 41:A667
16. Porter AC, Felder CC (2001) The endocannabinoid nervous system: unique opportunities for therapeutic intervention. Pharmacol Ther 90:45–60
17. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564
18. Munro S, Thomas KL, Abu-Shaar M (1993) Molecular characterisation of a peripheral receptor for cannabinoids. Nature 365:61–65
19. Buckley NE, McCoy KL, Mezey E, Bonner T, Zimmer A, Felder C, Glass M, Zimmer A (2000) Immunomodulation by cannabinoids is absent in mice deficient for the cannabinoiod CB_2 receptor. Eur J Parmacol 396:141–149
20. Griffin G, Fernando SR, Ross RA, McKay NG, Ashford MLJ, Shire D, Huffman JW, Yu S, Lainton JAH, Pertwee RG (1997) Evidence for the presence of CB_2 like cannabinoid receptors on peripheral nerve terminals. Eur J Pharmacol 339:53–61
21. Galiegue S, Mary S, Marchland J, Dussossoy D, Carriere D, Carayon P, Bouaboula M, Shire D, Le Fur G, Casellas P (1995) Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. Eur J Biochem 232:54–61
22. Richardson JD, Aanonsen L, Hargreaves KM (1998) Antihyperalgesic effects of spinal cannabinoids. Eur J Pharmacol 345:145–153
23. Clayton N, Marshall FH, Bountra C, O'Shaughnessy (2002) CB_1 and CB_2 cannabinoid receptors are implicated in inflammatory pain. Pain 96:253–260
24. Malan TP, Ibrahim MM, Vanderah TW, Makriyannis A, Porreca F (2002) Inhibition of pain responses by activation of CB_2 cannabinoid receptors. Chem Physics Lipids 121:191–200
25. Patel HJ, Birrell MA, Crispino N, Hele DJ, Venkatesan P, Barnes PJ, Yacoub MH, Belvisi MG (2003) Inhibition of guinea pig and human sensory nerve activity and the cough reflex in guinea pigs by cannabinoid (CB_2) receptor activation. Br J Pharmacol 140:261–268
26. Calignano A, Katona I, Desarnaud F, Giuffrida A, La Rana G, Mackie K, Freund TF, Piomelli D (2000) Bidirectional control of airway responsiveness by endogenous cannabinoids. Nature 408:96–101
27. Morita K, Kamei J (2003) Antitussive activity of WIN 55212-2, a cannabinoid receptor agonist. Eur J Pharmacol 427:269–272