

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

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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 CB₂ receptor agonists on sensory nerve activity *in vitro* and the cough reflex in a guinea pig model. CB₂ 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.

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

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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 E₂. 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

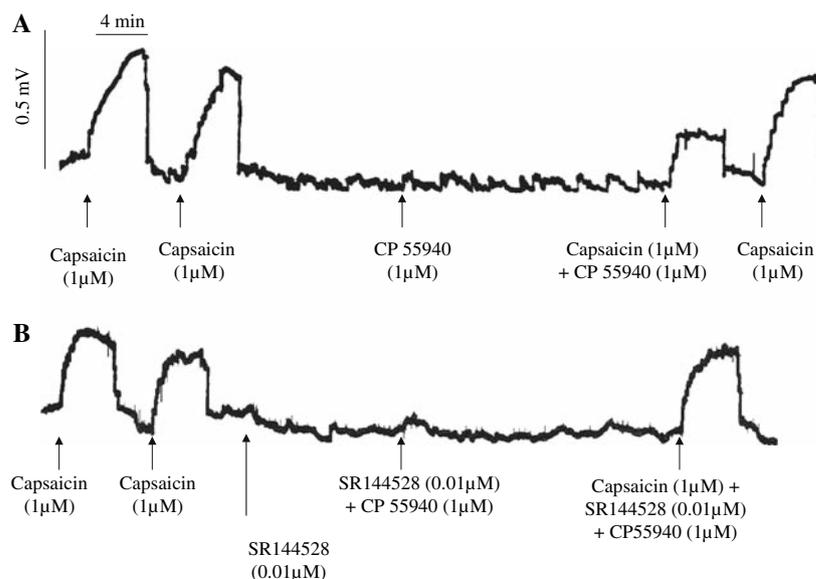
Table 1 Commercially available cannabinoid ligands that can be used to investigate the role of the CB₁ and CB₂ receptors in biological processes

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 LY 320135	SR144528 AM630
Radioligands	[³ H]-CP55940 [³ H]-SR141716A	[³ H]-CP55940

principal of marijuana, Δ⁹-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 CB₁ and CB₂ receptors [17, 18] (see Table 1 for a list regarding CB receptor tools). CB₁ receptors are distributed predominantly throughout the brain and spinal cord and are also expressed at low levels in several peripheral tissues. In contrast, CB₂ 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 CB₁ receptors inhibits nociceptive transmission [22], hyperalgesia, and neuropeptide release from central primary afferent fibers. More recently, CB₂ 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].

There is still very little information about CB₂ 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 CB₂ 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 CB₂ over the CB₁ 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 CB₁ 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 CB₂ receptor antagonist SR144528 on guinea pig vagus nerve



Confirmation of the role of the CB₂ receptor as a target for antitussives has now been achieved by experiments demonstrating the sensitivity of this inhibitory response to blockade by a selective CB₂ receptor antagonist (unpublished data). These findings have important implications for the therapeutic potential of cannabinoids. There is limited CB₂ receptor expression in the CNS and hence the development of CB₂ 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 CB₂ receptor agonists on sensory nerve activity *in vitro* and the cough reflex in a guinea pig model. CB₂ 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.

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