

Theobromine inhibits sensory nerve activation and cough

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SPECIFIC AIMS

Cough is a condition that affects the vast majority of people at some point in their lives and is the most common complaint for which medical attention is sought. Currently, no effective treatment exists. The aim of this study was to investigate the utility of a novel antitussive called theobromine, a methylxanthine derivative present in cocoa and chocolate, on cough and airway sensory nerve function in humans.

PRINCIPAL FINDINGS

1. Theobromine as a potential antitussive

Several synthetic antitussives are characterized by the presence of a 1,2,4-oxadiazole ring in their chemical structure. With the renaissance of the methylxanthine theophylline to treat asthma in the 1970s, a series of novel compounds with an oxadiazolylalkyl substituent at the N7 atom on the basic xanthine skeleton was synthesized and investigated as potential antiasthmatic and antitussive agents.

With two of these compounds selected for preclinical testing, 3,7-dihydro-3-methyl-7-/(5-methyl-1,2,4-oxadiazol-3-yl)methyl/-1*H*-purine-2,6-dione, and 3,7-dihydro-1,3-dimethyl-7-/(5-methyl-1,2,4-oxadiazol-3-yl)methyl/-1*H*-purine-2,6-dione, there was an unexpected correlation between chemical structure and antitussive potency. When the N1 atom of the xanthine skeleton remained unsubstituted, the antitussive effect of these 1*H*-xanthine derivatives increased considerably compared with the corresponding N1-methyl analogs. The antitussive effect of 3,7-dihydro-3-methyl-7-/(5-methyl-1,2,4-oxadiazol-3-yl)methyl/-1*H*-purine-2,6-dione was about eightfold stronger than that of 3,7-dihydro-1,3-dimethyl-7-/(5-methyl-1,2,4-oxadiazol-3-yl)methyl/-1*H*-purine-2,6-dione. This correlation among the synthetic xanthine derivatives suggests that if the association also applies to the three

natural methylxanthine alkaloids, theobromine, theophylline and caffeine, then theobromine, which, in contrast to theophylline and caffeine, is unsubstituted at N1, should exhibit a therapeutically significant antitussive effect.

2. Theobromine inhibits citric acid-induced cough in the guinea pig model

We investigated the antitussive effect of theobromine on citric acid-induced cough in guinea pigs, using codeine hydrochloride as a positive control. Cough provocation in conscious guinea pigs is widely used to test the efficacy of new cough treatments. Theobromine and codeine showed a dose-dependent antitussive effect on citric acid-induced cough in guinea pigs compared with the vehicle-treated control groups. The antitussive effect of theobromine and codeine was similar, but significantly better than vehicle treatment using doses between 4–64 mg/kg and 8–64 mg/kg, respectively. Vehicle treatment itself caused some decrease in the numbers of citric acid-induced coughs, which were similar within the two treatment groups. Theobromine at a dose of 32 mg/kg produced a long-lasting antitussive effect that was statistically significant for up to 4 h after treatment.

3. Theobromine inhibits capsaicin-induced cough in man

We next examined whether theobromine could inhibit induced cough in human subjects. Capsaicin is a well-established, reproducible, sensory C-fiber stimulant in vitro, widely used as a tussive stimulant in clinical research to determine the cough threshold. In ten healthy volunteers, theobromine markedly increased

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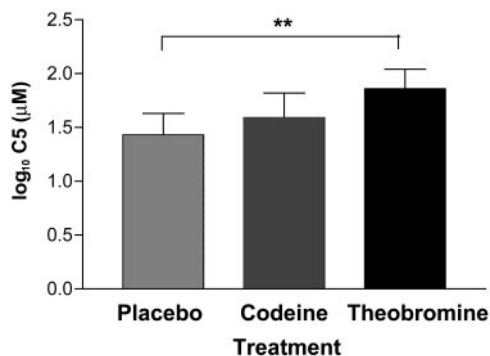


Figure 1. The effect of theobromine, codeine, and placebo on the capsaicin concentration required to induce five coughs (C5) in 10 healthy volunteers. Values shown represent mean \pm SE, where $**P < 0.01$.

the capsaicin concentration required to induce five coughs (C5, cough threshold) when compared with placebo ($P < 0.01$) and codeine phosphate ($P = 0.07$) (Fig. 1). The log C5 (\pm SD) was 1.43 ± 0.65 , 1.59 ± 0.74 , and 1.86 ± 0.58 for placebo, codeine, and theobromine, respectively. There was no significant difference between codeine and placebo ($P = 0.25$). No adverse effects, particularly cardiovascular or central nervous system, were observed.

4. Theobromine inhibits capsaicin-induced sensory nerve depolarization of guinea pig vagus and human vagus nerves in vitro

To ascertain whether the mechanism of action was peripheral or central, the effect of theobromine and codeine on capsaicin-induced guinea pig vagus nerve depolarization was investigated. Perfusion of guinea pig isolated vagus nerve preparations with theobromine (0.01–100 μ M) inhibited capsaicin-induced nerve depolarization in a concentration-dependent manner ($pD_2 = 5.2$). Maximum inhibition of $94.9 \pm 3.8\%$ was observed at the highest concentration used. Similarly, nerve preparations treated with codeine (0.01–100 μ M) showed markedly reduced sensory nerve depolarization induced by capsaicin in a concentration-dependent manner ($pD_2 = 5.6$); complete inhibition of induced nerve depolarization was achieved at 100 μ M.

We studied the effects of theobromine on depolarization of a human vagus nerve preparation by capsaicin. Human vagus preparations were used to confirm the observations seen with guinea pig vagus nerves to

provide the appropriate validation of this hypothesis in the relevant tissue in humans and confirm clinical relevance. Theobromine (100 μ M) inhibited capsaicin (10 μ M)-induced nerve depolarization of the human vagus by 66.7% (Fig. 2).

CONCLUSIONS AND SIGNIFICANCE

Cough is a protective, primitive reflex in healthy individuals, however, when cough serves no useful role, it is the most common respiratory complaint for which medical attention is sought. Persistent cough can be debilitating, socially distressing, and adversely impair quality of life. Cough leads patients to use over the counter remedies as first line treatments, where annual US sales exceed \$2 billion dollars. A recent meta-analysis, however, established that evidence regarding the effectiveness of such remedies was inconclusive.

Narcotic agents with a morphine skeleton, such as the opioids codeine and dextromethorphan, are the most widely used antitussives in cough remedies. However, they have unpredictable efficacy and undesirable central nervous and peripheral side effects that often lead to their discontinuation. The recent 2nd International Cough Symposium concluded that there is a great need for effective new cough treatments, and a better understanding of the complex genesis and pathophysiology of cough to guide the development of pharmacological approaches.

The cough reflex is initiated by stimulation of two different classes of sensory afferent fiber, namely the myelinated rapidly adapting receptors (RAR), and non-myelinated C-fibers with bronchial or pulmonary endings. Inappropriate activation of these nerves can occur in allergic diseases (e.g., asthma) and chronic obstructive pulmonary disease (COPD) and lead to many symptoms including coughing. Mechanisms involved in the abnormal functioning of airway nerves have not yet been described, but are thought to involve the release of inflammatory mediators (in asthma) and cigarette smoke (in COPD), which sensitize the nerve fibers leading to increased electrical activity of these fibers and an increase in the release of various neurotransmitters from the nerve endings (Fig. 3).

We hypothesized that agents that inhibit sensory nerve activity (i.e., nerve depolarization) will also inhibit the cough reflex. Although many compounds demonstrate promising characteristic antitussive effects

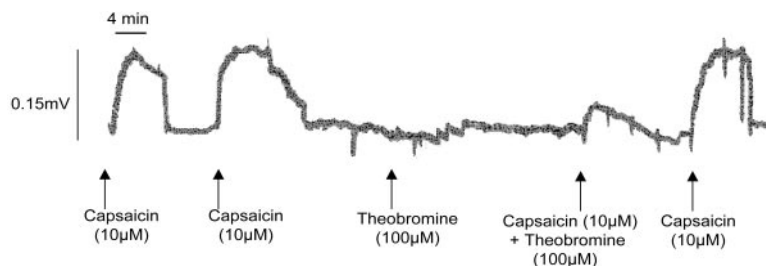


Figure 2. Theobromine mediated inhibition of capsaicin-induced nerve depolarization of isolated human vagus nerve. Tracing shows the inhibitory effect of theobromine on human vagus nerve depolarization induced by capsaicin.

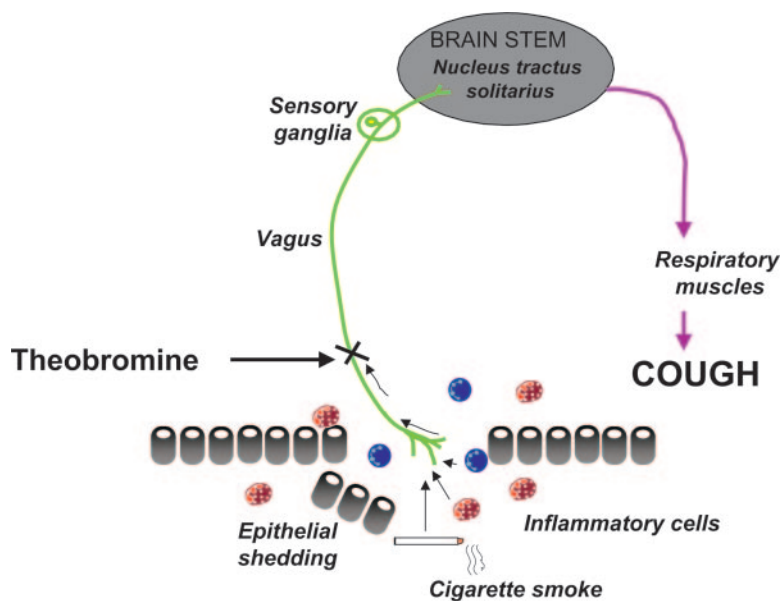


Figure 3. The cough reflex is initiated by stimulation of 2 different classes of sensory afferent fiber, namely the myelinated rapidly adapting receptors (RAR), and nonmyelinated C-fibers with bronchial or pulmonary endings. Inappropriate activation of these nerves can occur in allergic diseases (e.g., asthma) and chronic obstructive pulmonary disease (COPD) and lead to many symptoms including coughing. The mechanisms involved in the abnormal functioning of airway nerves have not yet been described. However, they are thought to involve the release of inflammatory mediators (in asthma) and cigarette smoke (in COPD) that sensitize the nerve fibers leading to increased electrical activity of these fibers and an increase in the release of various neurotransmitters from the nerve endings. Potential antitussives could act centrally or peripherally or at both sites. Theophylline inhibits vagal sensory nerve activation in vitro suggestive of a peripheral site of action for this novel antitussive therapy.

in animal models, few have shown any clinical benefit. Here we describe theophylline, a methylxanthine alkaloid derivative predominant in cocoa, as a novel and promising therapy for the treatment of cough. Recent studies have demonstrated a unique antitussive effect, unlike the other methylxanthines, in a series of pharmacological studies in the guinea pig cough model using a synthetic analog.

In these studies, we have demonstrated for the first time that theophylline, a methylxanthine derivative present in cocoa, effectively inhibited citric acid-induced cough in conscious guinea pigs in vivo, and using a randomized double-blind study we showed that theophylline inhibited capsaicin-induced cough in healthy human volunteers.

To investigate the mechanism of action we determined the effect of theophylline on the isolated guinea pig vagus nerve preparation, which is a validated in vitro model that allows evaluation of compounds on sensory nerve depolarization. Our data demonstrate that guinea pig and human vagus nerves respond similarly to theophylline inhibition of capsaicin-induced depolarization. The inhibitory effect of theophylline on the isolated nerve preparations from both species would suggest that this compound is inhibiting the cough reflex via a peripherally mediated mechanism (Fig. 3).

Taken together, the findings with theophylline in the guinea pig and humans cough models in vivo and in the guinea pig and human nerve preparations in vitro, are consistent with the hypothesis that the anti-

tussive action was due to direct inhibition by theophylline of capsaicin-induced sensory nerve activation rather than by a centrally mediated mechanism. The demonstration of inhibitory activity of theophylline on capsaicin-induced nerve depolarization using an isolated human vagus nerve preparation, provides in vitro proof of concept for this mechanism of action in humans.

In our human study, no cardiovascular or central nervous system adverse effects were observed, and these data support the use of theophylline as an effective antitussive agent in humans, with a safe therapeutic index. We compared theophylline to codeine phosphate, often used as a benchmark against which new cough treatments are compared, and although systemic opiates suppress capsaicin-induced cough, they are associated with many unacceptable adverse effects when used in effective antitussive doses.

Our studies have identified theophylline as a novel antitussive agent devoid of central side effects. The data show a significant antitussive effect of theophylline in healthy subjects when compared with placebo. Further studies are needed to see whether these effects can be extrapolated to patients with chronic persistent cough who have increased sensitivity to the tussive effect of inhaled irritant capsaicin, and where there is increased sensitivity of capsaicin-sensitive afferent nerves. Theophylline could form the basis for development of novel and safe antitussive agents for the treatment of a very common and troublesome symptom. FJ