

Adaptation of cough reflex with different types of stimulation

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ABSTRACT: Inhalation cough challenge has become an accepted method of investigating antitussive agents. It is, therefore, important to examine the degree of tachyphylaxis seen with repeated cough challenge. In addition, different types of challenge may reveal important differences in the neuronal pathways involved in the cough reflex. Citric acid, distilled water and capsaicin were examined to determine adaptation of the cough response during acute and long-term inhalation studies in healthy subjects.

To study acute tachyphylaxis two separate one minute continuous inhalation challenges (n=13) were performed. Long-term tachyphylaxis (n=10) was examined using citric acid and capsaicin inhalation at 10 min intervals for 40 min, and at 4 and 6 h. Cross-tachyphylaxis to citric acid and capsaicin was examined in a separate randomized crossover study (n=10).

Highly significant adaptation occurred between the first and last 10 s of the one minute challenge with citric acid (90-100%) and distilled water (74-84%), but was less pronounced with capsaicin (37-49%, at 2 μ M). Cough during the whole of the second test was significantly reduced for citric acid (50%). During long-term challenge cough was attenuated over 40 min both with citric acid (100 mM, $p<0.05$; 300 mM, $p<0.001$; 1 M, $p<0.001$) and with capsaicin (3 μ M, $p<0.01$; 10, 30, 100 μ M, $p<0.001$ each). With higher doses, tachyphylaxis was still present at 180 min with both citric acid (300 mM, $p<0.05$) and capsaicin (100 μ M, $p<0.008$). Total cough response was diminished by 24% when citric acid was inhaled after capsaicin ($p<0.05$), and 33% when capsaicin followed citric acid ($p<0.02$).

Citric acid and distilled water have a different pattern of adaptive response to capsaicin and may, thus, stimulate different receptor populations with separate afferent neuronal pathways.

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The defensive cough reflex may be provoked by numerous physical and chemical stimuli. These different types of tussive challenge produce differing responses in the respiratory tract. The cough produced in response to inhalation of citric acid and distilled water has long been used as a method of cough challenge [1] and is thought, in animals, to be produced by rapidly adapting receptors [2-4]. In man, tachyphylaxis to challenge with citric acid is recognized [5] but has not been extensively investigated. In contrast, cough produced by the pungent extract of peppers, capsaicin, is claimed to be consistent [6] but may be modulated by drugs and disease processes [6-9].

We have examined the effect of repeated cough challenge in a series of studies to determine the duration of tachyphylaxis with different tussive agents and to explore differences in the neuronal pathways involved in the cough reflex.

Patients and Methods

Three separate studies were performed. Acute tachyphylaxis was investigated by continuous inhalation of the tussive agent during one minute from a one litre deadspace. The effect of repeated separate one second inhalations of citric acid and capsaicin was examined over six hours to determine whether long-term tachyphylaxis occurred. Finally, the possibility of cross-tachyphylaxis with different agents was determined in a randomized, crossover study. All subjects gave informed written consent, and protocols were approved by the local hospital Ethics Committee.

Acute tachyphylaxis

Thirteen healthy subjects (7 men, 6 women; mean age 26 yrs, range 21-35 yrs), who had not previously

undergone cough challenge, were studied in the following order. Aerosols of distilled water and citric acid (0.68% in 0.79% saline) were delivered by a De Vilbiss 65 ultrasonic nebulizer at maximum output (6 ml·min⁻¹). This was followed by incremental doses of capsaicin (0.5–10 µmol·l⁻¹ in normal saline) administered *via* a jet nebulizer (System 22, Medic Aid Ltd, UK) run on oxygen at a flow rate of 7 l·min⁻¹. Two challenges for each agent were performed on separate days at least one week apart, at the same time of day in order to avoid possible diurnal changes. Subjects inspired normally with tidal respiration for one minute from a one litre deadspace containing the aerosol. There was a five minute interval between different challenges, and also between incremental doses of capsaicin. The cough response was recorded by means of a pneumotachograph attached to the expiratory port of the mouthpiece. Individual coughs were identified by large excursions on the pneumotachograph trace, which were consistently observed in association with the acoustic characteristics of cough.

Data were analysed by comparison of 10 s intervals during each one minute challenge. Statistical analysis was performed using non-parametric methods. The Page test for ordered alternatives was used to test whether the cough responses were ordered in a specific sequence according to time. For this analysis the Friedman two-way analysis of variance by ranks is too general. To ensure proper use of the Page test the order of the sequence of cough responses with time were specified *a priori* and so one-tailed values for this specific hypothesis are cited for the test [10]. The Wilcoxon signed ranks test was used to compare paired responses within individuals for tests performed one week apart.

Long-term tachyphylaxis

Repeated cough challenge with citric acid and capsaicin was investigated in 10 healthy subjects (6 men, 4 women; mean age 24 yrs, range 21–32 yrs), who had previously undergone cough challenge. Challenges were performed in a randomized order and commenced at 10 a.m. on the study day. Either log incremental doses of citric acid (10, 30, 100, 300 mM and 1 M) or capsaicin (1, 3, 10, 30 and 100 µmol) were delivered by a compressed air driven nebulizer controlled by a breath activated dosimeter (Mefar, Brescia, Italy). Four single inhalations of each dose were administered in succession and the dosimeter was preset to limit the delivery time to 1 s, with a 10 s interval between each of the four inhalations. The mean nebulizer output was 0.125 ml per inhalation, and thus the dose delivered ranged from 0.125–12.5 nmol and 1.25–125 µmol for capsaicin and citric acid, respectively. The cough response in the 10 s following each inhalation was recorded. The challenge was repeated at 10 min intervals for the first 40 min and then at 240 and 360 min. Measurement of ventilatory

function, including forced expiratory volume in one second (FEV₁), forced vital capacity (FVC) and peak expiratory flow rate (PEFR) was performed at baseline and after each challenge using a Vitalograph-compact (Vitalograph Ltd, UK).

The cough responses were analysed using the non-parametric Page test for ordered alternatives to compare the cough responses over the first 40 min. Wilcoxon ranked sum test was used to determine the reproducibility of repeated challenge at 240 and 360 min.

Cross-tachyphylaxis

Ten healthy subjects (6 men, 4 women, mean age 34 yrs, range 22–51 yrs), who had previously undergone cough challenge, were randomized to a balanced, single-blind, two-period, crossover study using the same cough challenge methodology as in the long-term tachyphylaxis study. Subjects were randomized to receive at first challenge either citric acid or capsaicin, followed by the other tussive agent. A week later, challenges were repeated in the reverse order. Statistical analysis was by the two-period cross-over method of HILLS and ARMITAGE [11].

Measurement of ventilatory function including FEV₁, FVC and PEFR was performed at baseline and after each complete challenge, using a Vitalograph-compact (Vitalograph Ltd, UK.).

Results

Acute tachyphylaxis

Citric acid. The results for the mean response (n=13) in each 10 s interval within the one minute citric acid challenge on both test days are shown in figure 1. On the first test day the cough response to citric acid diminished progressively throughout the 60 s, the number of coughs elicited in the first 10 s (mean 4.9 coughs, range 0–11) being tenfold higher than for the last 10 s of the challenge (mean 0.5 coughs, range 0–2). The cough response showed a progressive decrease, which was very highly significant (Page test $zL=3.97$, $p<0.0001$).

On the second test day, one week after the first, the corresponding mean values for the first and last 10 s intervals were 3.1 coughs (range 0–7) and 0 coughs (range 0), respectively (fig. 1). A difference in the cough response was again very highly significant (Page test $zL=3.97$, $p<0.0001$). The response to citric acid challenge was consistently greater on the first test day than for the second challenge. This difference in response within individuals for both test days was found to be significant (Wilcoxon signed ranked test, $p<0.02$ two-tailed).

Distilled water. As with citric acid the cough response to distilled water (n=13) (fig. 1) diminished

progressively throughout the 60 s, the number of coughs elicited in the first 10 s (mean 3.1 coughs, range 0–12) being sixfold higher than for the last 10 s of the challenge (mean 0.5 coughs, range 0–4). The continuous cough response showed a progressive decrease, which was highly significant (Page test $zL=2.87$, $p<0.002$).

On the second test day, one week after the first, the corresponding mean values for the first and last 10 s intervals were 2.7 coughs (range 0–8) and 0.7 coughs (range 0–6), respectively, a fourfold difference between first and last 10 s of the challenge (fig. 1). The difference in the continuous cough response was again highly significant for the second test (Page test $zL=2.71$, $p<0.003$). The response to distilled water tended to be greater on the first test day; however, this difference was not significant (Wilcoxon signed ranked test).

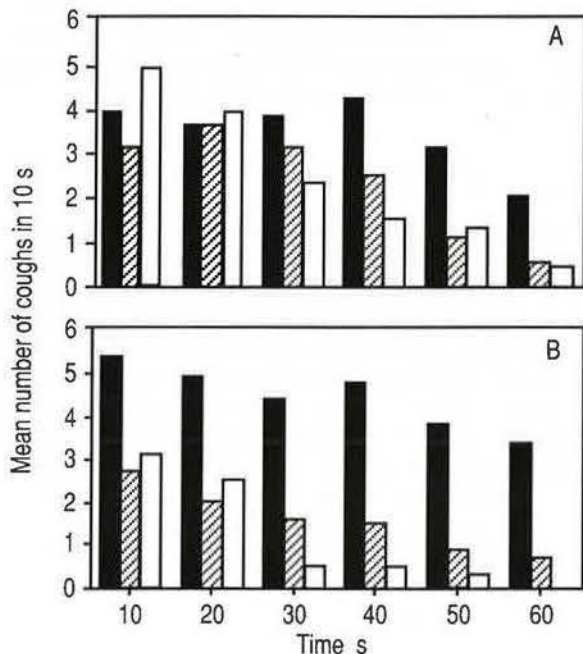


Fig 1. — Mean cough response at 10 s intervals during a one minute challenge with 0.68% citric acid, distilled water and capsaicin *via* nebulizer. First challenge (A) and second challenge (B) performed one week apart. ■: capsaicin 2 μ M; ▨: distilled water; □: citric acid.

Capsaicin. Individual cough response to capsaicin showed considerable variability: the maximum tolerable concentration of capsaicin being 1 μ M in three subjects, whilst in three other subjects this dose of capsaicin failed to elicit any cough response. We have, therefore, analysed data from two concentrations in the middle of the dose range to include all subjects studied. Figure 1 shows the results for the mean cough response ($n=10$ subjects) elicited by capsaicin at 2 μ M. On the first test day, the mean cough response for the first 10 s and last 10 s of each challenge were as follows; 2.7 and 2.5 coughs, respectively, for 1 μ M, and 3.9 and 2.0 coughs,

respectively, for 2 μ M. The results for the second test day were: 3.3 and 1.3 coughs, respectively, for 1 μ M and 5.4 and 3.4 coughs, respectively for 2 μ M. The 2 μ M challenge showed a significant reduction in response throughout the 60 s on both test occasions (Page test $zL=1.83$, $p<0.03$ one-tailed; $zL=2.55$, $p<0.005$ one-tailed, respectively). For capsaicin 1 μ M a significant reduction in response was demonstrated during the second test only (Page test $zL=0.89$, $p>0.18$ one-tailed; $zL=3.24$, $p<0.0007$ one-tailed). There was no significant difference between the two test days at the doses of 1 μ M and 2 μ M (Wilcoxon signed ranked test).

Long-term tachyphylaxis

Citric acid: cough challenge. The results of the citric acid study ($n=10$ subjects) are shown in figure 2. The mean cough response for three doses 100 mM, 300 mM and 1 M are shown, as the two doses 10 and 30 mM were below the cough threshold of the subjects tested. A significant degree of tachyphylaxis ($p<0.02$) was present between baseline and the first repeat challenge at 10 min, total mean cough response decreasing from 1.8 to 1.0 coughs. The cough responses were attenuated for the three active doses over a period of 40 min (mean cough response $t=0$, 2.5; $t=40$, 1.1 coughs for 1 M citric acid), but returned to near baseline levels by 240 min. The observed attenuation up to 40 min was significant for all three active doses of citric acid (100 mM, $L=479$, $p<0.05$ one-tailed; 300 mM, $L=505$, $p<0.001$ one-tailed; 1 M, $L=505$, $p<0.001$ one-tailed). Comparison of baseline challenge with that at 240 and 360 min, revealed no significant difference except at 300 mM when the 360 min challenge was found to be significantly attenuated compared with baseline (Wilcoxon signed rank test $p<0.05$).

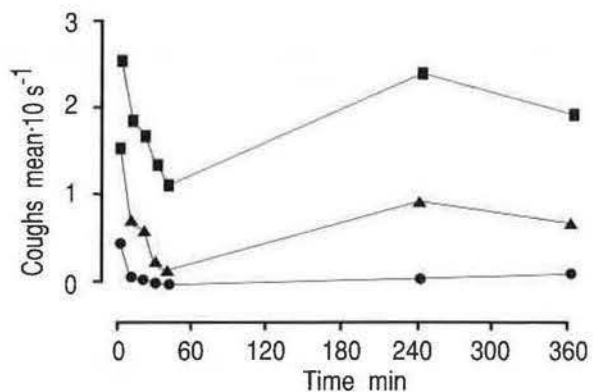


Fig 2. — Mean cough response recorded within 10 s of inhalation of 100 mM, 300 mM and 1 M of citric acid at seven time points from $t=0$ to $t=360$ min. $n=10$. Significant attenuation of response is seen at the three doses for the first 40 min. By 360 min only a dose of 1 M was associated with a significantly diminished response ($p<0.05$). —■—: 1 M; —▲—: 300 mM; —●—: 100 mM.

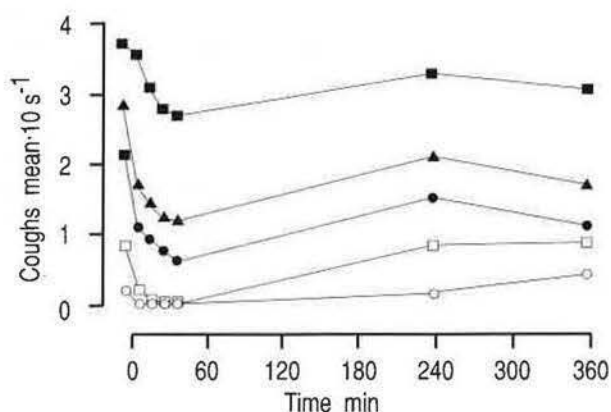


Fig 3. — Mean cough response recorded within 10 s of inhalation of 1, 3, 10, 30 and 100 μM of capsaicin at seven time points from $t=0$ to $t=360$ min. $n=9$. Significant attenuation of response is seen at the four higher doses for the first 40 min. By 360 min only a dose of 100 μM was associated with a significantly diminished response ($p<0.001$). —■—: 100 μM ; —▲—: 30 μM ; —●—: 10 μM ; —□—: 3 μM ; —○—: 1 μM .

Capsaicin: cough challenge. The results of the capsaicin study ($n=9$ subjects; one subject failed to complete the study) are shown in figure 3. A significant degree of tachyphylaxis ($p<0.02$) was present between baseline and the first repeat challenge at 10 min, total mean cough response decreasing from 1.9 to 1.3 coughs. The cough responses were significantly attenuated for the four higher doses over a period of 40 min (mean cough response $t=0$, 3.7; $t=40$, 2.7 coughs for 100 μM capsaicin), but returned to near baseline levels by 240 min. The observed attenuation up to 40 min was significant for the four higher doses of capsaicin (3 μM , $L=444$, $p<0.01$ one-tailed; 10 μM , $L=458$, $p<0.001$ one-tailed; 30 μM , $L=460$, $p<0.001$ one-tailed; 100 μM , $L=469$, $p<0.001$ one-tailed).

Using the Wilcoxon signed rank test comparison of baseline challenge with that at 240 and 360 min revealed significant attenuation of cough response for 30 and 100 μM challenges. Mean cough response fell from 2.8 at baseline to 2.0 (240 min, $p<0.08$) and 1.6 (360 min, $p<0.04$) for 30 μM challenge. For 100 μM challenge mean cough response fell from 3.7 at baseline to 3.2 (240 min, $p<0.04$) and 2.9 (360 min, $p<0.008$). There was no significant difference between the 240 and 360 min challenge at any dose.

Spirometry. With citric acid a small change of less than -5% was observed for FVC and FEV_1 throughout the test. The changes were more pronounced for PEFR but remained small with mean values throughout the time-course of the study ranging between -8% at 30 min to -10% at 40 min. Following capsaicin challenge there was a small rise in FVC of about 1% throughout the test. As with citric acid small changes were observed for FEV_1 averaging less than -3%. The changes for PEFR were generally smaller than that observed following citric acid, with mean values ranging between -5% at 10 min to -7% at 40 min.

Crossover tachyphylaxis

There was no significant difference between total cough response for both test days indicating that there was no treatment-period interaction. Analysis of the order of cough challenge indicated that both citric acid and capsaicin, when administered, first influence the subsequent challenge. When citric acid was given first the total cough response per subject was 22.5 compared with 17.1 when citric acid was given second (treatment effect=2.81, $p<0.05$). Similarly, when capsaicin was administered first total cough was 42.5 and reduced to 28.6 when capsaicin was given second (treatment effect=3.03, $p<0.02$) (table 1).

Table 1. — Tachyphylaxis observed during repeated challenge: a) with the same tussive agent; and b) with different agents during the crossover study.

	Baseline	Repeat challenge	% fall	(95% CI)
a) Single agent-tachyphylaxis				
Capsaicin	38.6	26.0	33%	(17–48%)
Citric acid	18.0	10.2	43%	(10–77%)
b) Crossover-tachyphylaxis				
Capsaicin	42.5	28.6	33%	(10–56%)
Citric acid	22.5	17.1	24%	(6.0–42%)

Table shows mean cumulative cough response, and percentage fall from baseline after repeat challenge at 10 min. For the crossover study baseline refers to the day when the listed agent was administered first, and repeat challenge refers to the day when that agent is administered 10 min after the other. CI: confidence interval

There was no significant period effect for either citric acid (period effect 0.94) or capsaicin scores (0.89). No significant change in FEV₁, FVC or PEF_R occurred following tussive challenge. Mean FEV₁ remained within 1% of pre-challenge variables, mean FVC fell by 2% following capsaicin challenge but was identical when citric acid was administered first. PEF_R was again the most variable test increasing by 3% when capsaicin was used first, but decreasing by 6% when citric acid was used first.

Discussion

In these studies we have examined the effect of repeated challenge on the cough reflex and have demonstrated that significant tachyphylaxis occurs with both short-term exposure to tussive agents and with challenge repeated over an interval of several hours. The mechanism whereby inhalation of a tussive agent is converted into a cough is unknown but clearly a reflex arc is stimulated with its sensory limb ascending in the vagus nerve, as vagotomy leads to a dramatic diminution in cough-like responses in animals [12]. Which nerve fibres within the vagal nerve trunk carry the majority of the sensory information leading to cough in man is a matter of much debate [13]. Large myelinated fibres arising in the larynx and upper airways have been demonstrated to be responsible for cough-like reflexes in several animal species, using electrophysiological techniques [3, 14]. In these studies, administration of distilled water led to stimulation of a train of nerve impulses, which adapted rapidly to the continuing presence of the tussive stimulus [3, 14]. These rapidly adapting receptors (RARs) have not been demonstrated by histological techniques, but unmyelinated free nerve endings of myelinated fibres have been demonstrated in the larynx [15]. The rapid development of tachyphylaxis to the inhalation of citric acid and distilled water in our short-term tachyphylaxis study is reminiscent of the data seen in animal studies [2], and we believe that the cough response to these tussive agents may be mediated through RARs. The majority of sensory nerves in the vagus are, however, of the small unmyelinated C-fibre type and may be stimulated by the pungent extract of peppers capsaicin [16]. The specificity of this latter agent has, however, recently been questioned by JACKSON *et al.* [4], who suggest that at higher concentrations myelinated fibres are also stimulated to produce cough. At the doses inhaled (0.125–12.5 nmol) in this study we believe capsaicin is a highly potent tussive agent, particularly if one considers that approximately a third of the dose delivered to the mouth reaches the airways [17].

Acute tachyphylaxis. In our study of acute tachyphylaxis, subjects had not previously undergone cough challenge and a significant learning effect was demonstrated between first and second challenge days

for citric acid. Whilst cough response to distilled water on the second day was not significantly reduced, because of the clear trend present between different challenge days, we adopted a policy of an introductory challenge before formal testing in all subsequent studies. No period effect was demonstrated in any of our other studies when subjects had undergone a previous challenge. We suggest that to obtain reproducible cough responses the subjects should be accustomed to the procedure.

Tachyphylaxis during one minute inhalation was analysed using the Page test for ordered alternatives to determine whether the cough responses were ordered in a specific sequence according to time. A highly significant reduction was seen with both citric acid and distilled water, and was also demonstrated, but was much less pronounced, with doses of capsaicin which caused the majority of subjects to cough. The difference in the degree to which acute tachyphylaxis occurs with these agents may indicate that different mechanisms of adaptation exist.

That citric acid challenge demonstrates most tachyphylaxis may be due to a rapid adaptation of the extracellular pH surrounding the putative cough receptor; however, the response to distilled water, which mainly alters ionic composition [18, 19] but not the extracellular pH, also demonstrated marked tachyphylaxis. Thus, if restoration of the extracellular milieu is the cause of tachyphylaxis in these two modalities of challenge, then rapid readjustment of extracellular pH and ionic composition to suppress further cough must occur with a similar time course. It is, perhaps, more likely that both tussive agents are acting through the same neuronal pathway which, unlike the C-fibre, is characterized by a rapidly adapting response [2, 13].

The relative absence of tachyphylaxis over one minute seen with capsaicin may be due to the slightly different methodology used. However, the mean cough response observed in the first 10 s of challenge was similar for all three tussive agents (fig. 1) and suggests that methodological differences played little part. We suggest that the persistent cough seen with capsaicin may indicate that there is stimulation of a different neuronal pathway, which adapts more slowly.

Long-term tachyphylaxis. Repeated challenges of both citric acid and capsaicin led to highly significant tachyphylaxis over 40 min. Whilst the result for citric acid was compatible with the marked tachyphylaxis seen in the acute study, the response to capsaicin is surprising. It appears that repeated individual challenges with capsaicin cause much greater down regulation of the cough reflex, possibly because of a slower phase of adaptation occurring with repeated 10 min challenge. Some down regulation was still present at four and six hours when compared with baseline, but was similar at four and six hours.

The results of the acute and long-term tachyphylaxis

studies show that with citric acid tachyphylaxis is seen within one minute and continues to be observed with repeated challenge at 10 min intervals for 40 min. Recovery takes place within two hours but is incomplete (fig 2). Tachyphylaxis with capsaicin occurs between 1 and 10 min, and is observed consistently with repeated 10 min challenge for the first 40 min. Recovery is slower than with citric acid, particularly for the higher doses, and we were unable to show complete recovery at 3 h 20 min. It is possible that the adaptation seen in our long-term study indicates a common influence for down-regulation in both rapidly adapting receptors and capsaicin sensitive neurones.

In nociception the concept of a neuronal gate has been proposed [20], and whilst simple gating of C-fibre afferents by larger myelinated neurones has proved difficult to establish, discrete descending pathways from brain stem nuclei are important modulators of afferent neurotransmission [21]. A large body of evidence also points to modulation of nociception by endorphin-containing central neurones [21]. The analogy of cough with pain is not unexpected; both stimuli have similar afferent pathways, namely large myelinated fibres and unmyelinated C-fibres, and both respond to pharmacological manipulation by opiates [21, 22]. We propose that a similar central modulation underlies the tachyphylaxis observed in our long-term study (figs 2 and 3).

A further indication that repeat challenge causes adaptation to citric acid and capsaicin *via* a common pathway is the response observed during the cross-tachyphylaxis study. Both citric acid and capsaicin showed cross-tachyphylaxis, whether administered first or second (table 1b), and both showed a similar response to that induced by repeated single agent challenge (table 1a). Thus cough responses are decreased by about one third at 10 min, irrespective of the type of tussive agent or the order in which they are administered. An alternative hypothesis to explain these findings is that different tussive agents do not exclusively stimulate specific fibre types, and that citric acid, whilst mainly stimulating rapidly adapting receptors, may also stimulate capsaicin sensitive neurones and vice versa [4, 23]. Thus, diminished response seen during cross-tachyphylaxis may be due to adaptation of a receptor pool common to both agents. It is likely that both central modulation of evoked responses and nonspecific receptor stimulation at higher doses play a part in tachyphylaxis observed during repeated cough challenge.

Whilst a very brief increase in respiratory resistance in response to capsaicin challenge has been described [24], in this present study in normal subjects we have been unable to demonstrate any clinically significant changes in ventilatory function. Whether these findings can be extrapolated to patients with airways hyperactivity is unknown.

We conclude that significant adaptation of the cough reflex occurs with both citric acid and capsaicin,

and that knowledge of time-course of tachyphylaxis observed is important for proper experimental design and data interpretation in cough challenge studies. Citric acid and distilled water, when compared to capsaicin, appear to have a different pattern of adaptive response and may thus stimulate different receptor populations with separate afferent neuronal pathways. Adaptation in RARs and C-fibres may be modulated centrally by descending pathways, or as a result of nonspecific receptor stimulation, thus accounting for cross tachyphylaxis.

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