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 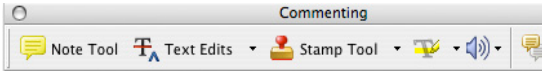

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 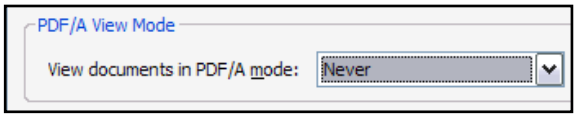

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 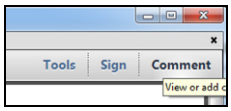

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 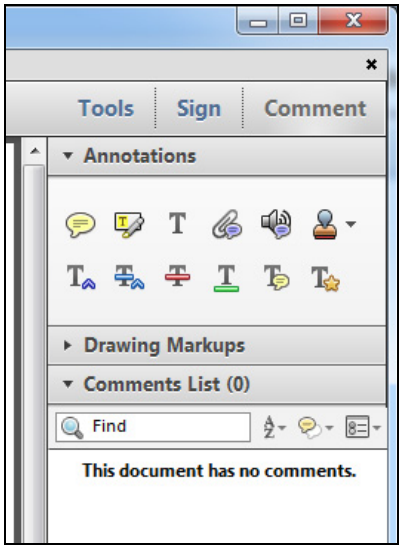
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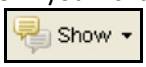
Review	To review your changes, click on the 'Show' button  on the Commenting tool bar. Choose 'Show Comments List'. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.	Your changes will appear automatically in a list below the Comment tool bar. Navigate by clicking on a correction in the list. Alternatively, double click on any mark-up to open the commenting box.
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[Original Research]

CHEST

A Randomized Clinical Trial Comparing the Effects of Antitussive Agents on Respiratory Center Output in Patients With Chronic Cough

Claudia Mannini, MD; Federico Lavorini, MD; Alessandro Zanasi, MD; Federico Saibene, MD; Luigi Lanata, MD; and Giovanni Fontana, MD

BACKGROUND: Cough is produced by the same neuronal pool implicated in respiratory rhythm generation, and antitussive drugs acting at the central level, such as opioids, may depress ventilation. Levodropropizine is classified as a nonopioid peripherally acting antitussive drug that acts at the level of airway sensory nerves. However, the lack of a central action by levodropropizine remains to be fully established. We set out to compare the effects of levodropropizine and the opioid antitussive agent dihydrocodeine on the respiratory responses to a conventional CO_2 rebreathing test in patients with chronic cough of any origin.

METHODS: Twenty-four outpatients (aged 39-70 years) with chronic cough were studied. On separate runs, each patient was randomly administered 60 mg levodropropizine, 15 mg dihydrocodeine, or a matching placebo. Subsequently, patients breathed a mixture of 93% oxygen and 7% CO_2 for 5 min. Fractional end-tidal CO_2 (F_{ETCO_2}) and inspiratory minute ventilation ($\dot{V}_I$) were continuously monitored. Changes in breathing pattern variables were also assessed.

RESULTS: At variance with dihydrocodeine, levodropropizine and placebo did not affect respiratory responses to hypercapnia ($P < .01$). The ventilatory increases by hypercapnia were mainly accounted for by a rise in the volume components of the breathing pattern.

CONCLUSIONS: The results are consistent with a peripheral action by levodropropizine; the assessment of ventilatory responses to CO_2 may represent a useful tool to investigate the central respiratory effects of antitussive agents.

TRIAL REGISTRY: European Union Clinical Trials Register (EudraCT No.: 2013-004735-68); URL: https://www.clinicaltrialsregister.eu/

CHEST 2017; ■(■):■-■

KEY WORDS: breathing pattern; chronic cough; CO_2 rebreathing; dihydrocodeine; levodropropizine

ABBREVIATIONS: F_{ETCO_2} = fractional end-tidal carbon dioxide; P0.1 = mouth inspiratory pressure measured 0.1 s after mouth occlusion; TT = total duration of the respiratory cycle; $\dot{V}_I$ = inspiratory minute ventilation; V_T = tidal volume

AFFILIATIONS: From the Department of Experimental and Clinical Medicine (Drs Mannini, Lavorini, and Fontana), University of Florence, Florence; Pneumology Unit (Dr Zanasi), University of Bologna, S. Orsola Malpighi Hospital, Bologna; and Medical Department (Drs Saibene and Lanata), Dompè SPA, Milan, Italy.

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There is growing interest in the development of drugs that are effective in controlling cough.[1] However, we feel that attention should be paid not only to efficacy but also to the safety of such drugs, particularly when they are being administered to patients with respiratory disorders. The cough motor pattern originates from the neuronal pool that also generates the eupnoeic respiratory rhythm,[2,3] and most of the widely used antitussive drugs act centrally. Thus, it should be questioned whether their pharmacologic effect is devoid of the potential risk of ventilatory disturbances, since they may also depress the physiological responses to stimuli that activate breathing. Of note, the centrally acting antitussive agent codeine has been reported to depress the central response to hypercapnia more markedly in male subjects than in female subjects[4]; however, this more pronounced depressant effect by codeine in female subjects turned out to actually reflect differences in smoking habits rather than sex.[4]

Years ago, Read[5] developed a simple, inexpensive, effective, reproducible, and safe method for clinically assessing changes in the sensitivity of the mechanisms generating the central respiratory output. The methodology has been used in many clinical trials, and we trusted that the use of it would offer the unique possibility of reliably assessing if and to what extent a drug interacts with respiratory rhythmogenesis in humans. Previous animal studies have demonstrated no change in baseline respiratory activity following codeine in pentobarbitone-anesthetized cats.[6] In contrast, Adcock et al[7] demonstrated a significant depression of ventilation by high-dose IV codeine in anesthetized guinea pigs. It should be noted, however, that only a few studies have considered the use of the central CO_2 response as a measure of neural response to a respiratory drug in humans.[8]

Levodropropizine is an oral antitussive agent[9] prescribed worldwide. A meta-analysis suggested that levodropropizine provides better antitussive effects than do placebo and centrally acting antitussive agents in children and adults.[10] Levodropropizine may act through inhibition of C fibers[11] modulating the release of protussive neuropeptides.[9] Accordingly, clinical trials have demonstrated that levodropropizine causes less somnolence than do central antitussive agents,[12] with well-established depressant action on respiratory activity. Although published evidence confirms the peripheral action of levodropropizine,[9] its effects at the neuronal level have been poorly investigated. A previous study in patients with COPD suggested that levodropropizine does not inhibit the respiratory response to hypercapnia,[9] but CO_2 sensitivity in these patients may be blunted as a result of the disease.[13]

Therefore, by using the rebreathing method devised by Reid,[5] we set out to compare the effects of levodropropizine and dihydrocodeine, an opioid antitussive agent, on respiratory center output in patients with chronic cough and no evidence of airway obstruction. We also assessed short-term changes in breathing patterns, minute ventilation ($\dot{V}_I$), and respiratory drive during CO_2 rebreathing[5] prior to and after administration of the recommended doses of these agents. No attempt was made at further examining the well-established antitussive action of both active agents.

Methods

Patients

Twenty-four nonsmoking outpatients (16 women aged 39-70 years) (Table 1) with chronic cough (cough duration ranging from 8-100 months) of any origin referred to the Florence Cough Clinic from September 2014 to May 2015 were enrolled in the study. They had normal airway caliber (mean ± SD FEV_1/FVC, 0.78 ± 0.04) and reported no recent (< 4 weeks) airway infections. All patients reported at least one symptom suggestive of a gastroesophageal disturbance (n = 19) or an upper airway cough syndrome (n = 8), or both (n = 15). However, none of them showed any improvement following treatment of these most common causes of chronic cough.[14] Thus, in agreement with the current understanding of the chronic cough paradigm, all patients examined fit the criteria for classification into the so-called cough hypersensitivity syndrome.[15] All patients were free of any chronic treatment for their cough at the time of the study.

This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Azienda Ospedaliero Universitaria Careggi, Florence, Italy (approval: AOUC-001-2013; EudraCT 2013-004735-68). All adult participants provided written informed consent to participate in this study.

Protocol and Recording Procedures

This study was performed in a single-blind crossover fashion (Fig 1). Under control conditions (ie, in no-drug trials), the ventilatory responses to a standard CO_2 rebreathing test[5] were assessed in each patient. Subsequently, on each of three separate (48-72 h) occasions, after clinical assessment, patients were randomly administered a single oral dose of levodropropizine (60 mg [20 drops]), dihydrocodeine (30 mg [90 drops]), or placebo (20 drops of a multivitamin compound). The order of administration of the three agents was obtained by using an online software program (www.randomization.com), which creates random permutations of treatments for studies in which subjects are to receive all the treatments in random order. Two hours later, patients breathed a mixture of 93% oxygen and 7% CO_2 for 4 min.[5] The inspired air mixture was warmed and humidified and flowed through a 6 L reservoir bag past the inspiratory port of a two-way nonrebreathing balloon shutter occlusion valve (Hans Rudolph

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No. 4). During 5 min of relaxed air breathing and during CO_2 rebreathing, we measured, on a breath-by-breath basis, the tidal volume (V_T), the inspiratory time, the V_T/inspiratory time (an index of respiratory drive), and the duration of the respiratory cycle (TT).[2] The $\dot{V}_I$ and respiratory frequency (respiratory frequency, 60/TT) were subsequently calculated; the fractional end-tidal CO_2 (F_{ETCO_2}) was also monitored (Normocap CD 102; Datex).[2] In a subgroup of eight randomly selected patients, the value of mouth inspiratory pressure measured 0.1 s after mouth occlusion (P0.1), which is another index of respiratory drive,[8] was also recorded. In each set of four to eight breaths, the inspiratory line was silently and randomly closed during expiration by inflating the balloon. The mouth pressure during the following occluded inspiration was measured at a side port on the occlusion valve connected to a pressure transducer with a noncompliant catheter. Reported values of P0.1 were the mean of at least six measurements of occlusion pressure, the lowest and the highest values being discarded.

Data Analysis

Based on previous investigations,[16] the study, with a sample of 24 patients, was to have a > 80% statistical power of detecting a 10% between-treatment difference in CO_2 sensitivity, with a significance level of .05. The primary outcome of the study was the CO_2 sensitivity calculated as the slope of the $\dot{V}_I/F_{ETCO_2}$. Comparisons of breathing pattern variables, P0.1 values, and slopes were performed by repeated-measure analysis of variance followed by Dunn tests. $P < .05$ was taken as significant.

Results

All patients completed the study; no adverse effect were reported by patients, except some discomfort caused by the hyperpnea that occurred during rebreathing.

Individual mean values of baseline breathing pattern variables observed on each study day were similar and were not influenced by placebo or active agents (Table 2). Rebreathing consistently induced a marked increase ($P < .01$) in baseline $\dot{V}_I$, irrespective of the previously administered agent; these increases were proportional ($r = 0.98 \pm 0.01$) to F_{ETCO_2} (Fig 2, Table 2) and were always within the normal range.[17] Analysis of

TABLE 1] Patient Anthropometric, Functional, and Clinical Characteristics

Patient No.	Sex	Age, y	BMI	FEV_1/FVC	Cough Duration, mo
1	M	50	24.07	0.79	72
2	M	62	28.36	0.73	96
3	F	51	20.81	0.75	24
4	F	43	21.59	0.77	24
5	F	50	25.40	0.79	24
6	F	68	31.25	0.88	18
7	F	43	23.59	0.73	60
8	M	65	24.91	0.79	60
9	F	56	25.39	0.77	12
10	M	63	27.26	0.72	36
11	M	40	22.38	0.79	8
12	F	55	30.64	0.77	10
13	M	65	26.10	0.86	8
14	M	31	23.50	NA	9
15	F	61	25.18	0.73	12
16	F	60	27.12	0.77	84
17	F	65	28.09	0.73	72
18	F	68	25.71	0.79	7
19	F	50	25.10	0.81	100
20	F	70	24.91	0.74	96
21	M	50	25.12	NA	12
22	F	45	22.53	0.80	24
23	F	63	26.44	0.80	18
24	F	39	27.55	0.81	36
Mean		54.71	25.54	0.78	38.42
SD		10.76	2.57	0.04	32.28

NA = not available.

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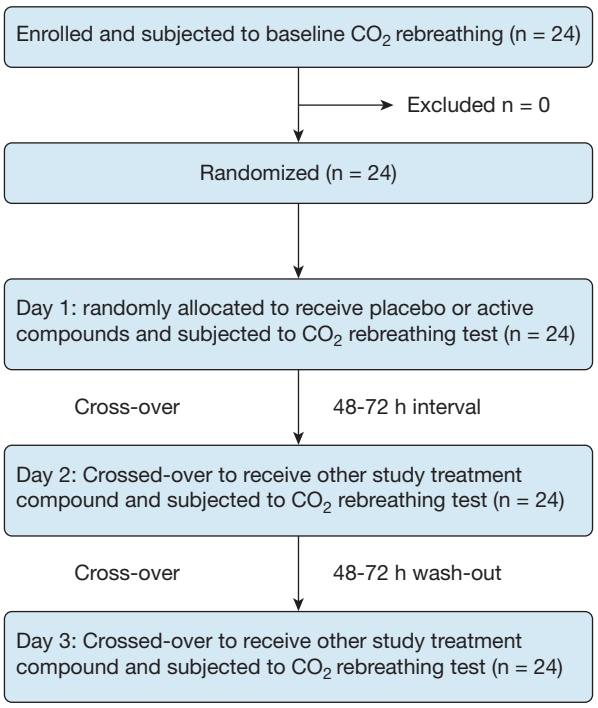

Figure 1 – *CONSORT flow diagram outlines the design and conduct of the study.*

the breathing pattern (Table 2) revealed that the ventilatory increases were mainly accounted for by a rise in V_T and by less marked increases in respiratory frequency (Fig 2, Table 2). CO_2 sensitivity after levodropropizine and placebo administration was similar and higher ($P < .05$), respectively, than after dihydrocodeine administration (Fig 2). In eight patients, P0.1 consistently increased significantly during rebreathing; however, compared with placebo and levodropropizine, these increases were less prominent ($P < .05$) following dihydrocodeine administration (Table 2).

Discussion

The results demonstrate that levodropropizine does not affect the ventilatory response to CO_2 in patients with chronic cough, supporting the lack of any significant central neuronal respiratory action. Conversely, with a standard and safe dose of dihydrocodeine, we have established a difference in ventilatory response to CO_2. Since the vast majority (about 70%) of patients in our study were women, we believe it is inappropriate to attempt at evaluating any sex-related difference in the CO_2 ventilatory responses.

It has long been known that IV morphine administration profoundly depresses eupneic breathing,[18] and the

TABLE 2] Mean (SD) Values of Breathing Pattern Variables (24 Patients) and Mouth Inspiratory Pressure Measured 0.1 s After Mouth Occlusion (Eight Patients) Recorded in Control Condition as well as at Various Values of P_{ETCO_2} Following Administration of Placebo, Levodropizine or Dihydrocodeine in Chronic Coughers

Variable	Placebo				Levodropizine				Dihydrocodeine			
P_{ETCO_2}, mm Hg	Control	50	60	70	Control	50	60	70	Control	50	60	70
V_T, L	0.80 (0.18)	1.41 (0.12)	2.00 (0.15)	2.39 (0.18)	0.84 (0.15)	1.38 (0.11)	1.91 (0.12)	2.21 (0.14)	0.9 (0.10)	1.32 (0.18)	1.78 (0.19)	2.18 (0.21)
Respiratory frequency, breaths/min	11.05 (2.55)	20.30 (3.01)	19.12 (2.99)	21.15 (2.79)	11.32 (1.99)	21.15 (2.05)	20.64 (1.91)	21.55 (2.42)	12.01 (3.05)	16.59. (2.79)	16.85 (2.46)	18.42 (2.18)
V_T/T_I, L/s	0.65 (0.05)	0.72 (0.04)	0.85 (0.05)	0.95 (0.04)	0.64 (0.04)	0.75 (0.03)	0.84 (0.08)	0.91 (0.07)	0.60 (0.03)	0.67 (0.04)	0.7 (0.04)	0.79 (0.05)
$\dot{V}_I$, L/min	8.83 (1.84)	28.50 (4.51)	38.15 (3.79)	52.60 (4.01)	9.48 (2.05)	29.05 (2.99)	39.25 (3.79)	50.05 (4.76)	10.75 (1.78)	22.12 (3.01)	30.46 (2.45)	40.08 (3.04)
P.01, cm H_2O	2.55 (0.68)	3.19 (0.60)	4.59 (0.58)	6.43 (0.60)	2.60 (0.58)	3.15 (0.68)	4.68 (0.57)	6.35 (0.62)	2.51 (0.59)	2.89 (0.72)	4.05 (0.49)	5.11 (0.54)

P.01 = mouth inspiratory pressure measured 0.1 s after mouth occlusion; P_{ETCO_2} = partial pressure of end tidal CO_2; T_I = inspiratory time; $\dot{V}_I$ = inspiratory minute ventilation; V_T = tidal volume; V_T/T_I = mean inspiratory flow.

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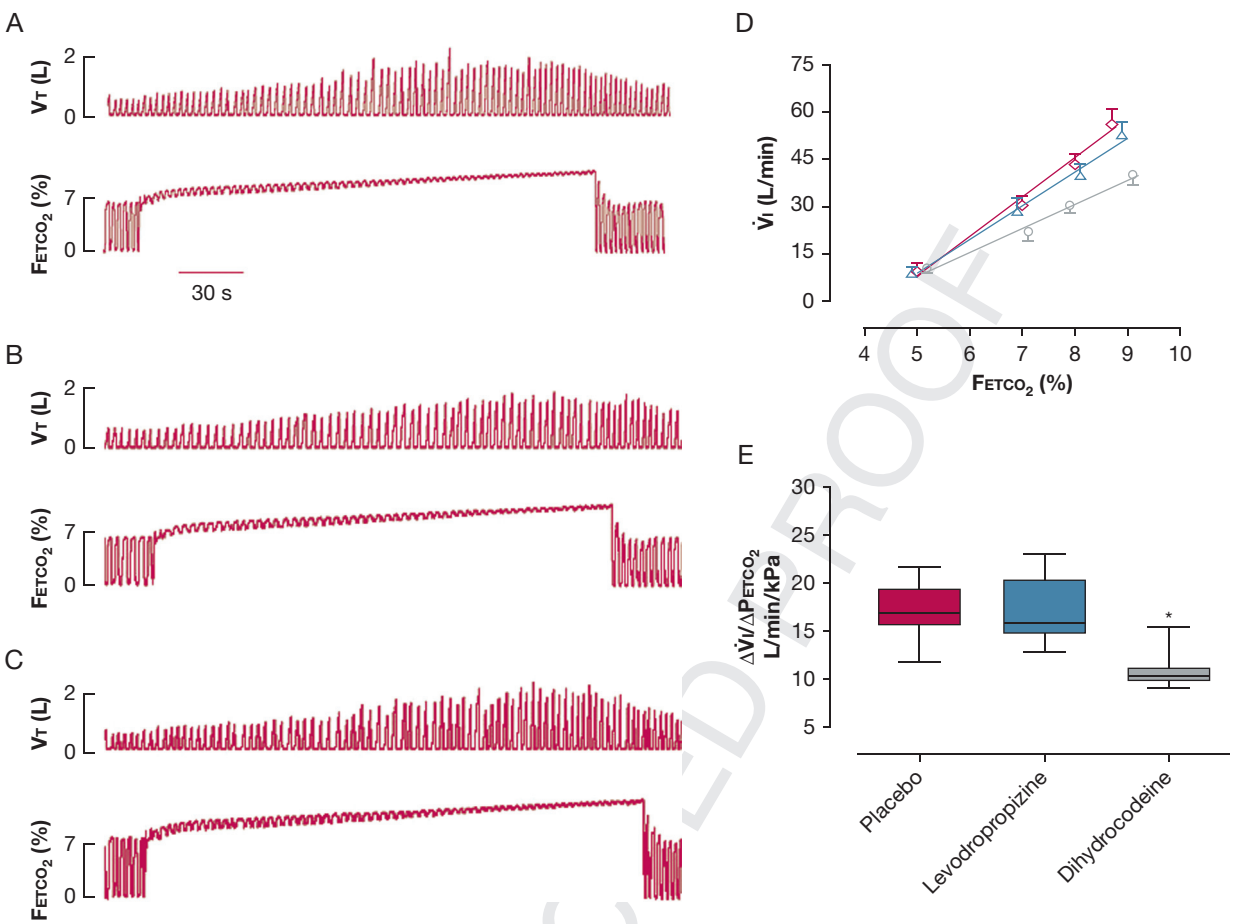

Figure 2 – *A-C, Original recordings of tidal volume (V_T) and fractional end-tidal CO_2 (F_{ETCO_2}) observed during 4-min CO_2 rebreathing tests performed after administration of (A) levodropropizine, (B) dihydrocodeine, and (C) placebo in one representative patient. D, Relationship between F_{ETCO_2} values and the corresponding inspiratory minute ventilation ($\dot{V}_I$) values observed after levodropropizine (diamonds), dihydrocodeine (circles), and placebo (triangles) administration. Data are mean ± SD. E, Box and whiskers plots showing median (minimum/maximum) values of CO_2 sensitivity observed after placebo, levodropropizine, and dihydrocodeine administration. *P < .05. P_{ETCO_2} = partial pressure of end tidal CO_2.*

neurophysiological mechanisms that subserve this inhibitory response have been reevaluated in more recent years. Indeed, animal studies have shown how sensory inputs to the brainstem reconfigure the pontomedullary respiratory central pattern generator so that normal respiration is converted to that of a cough motor pattern.[19-22] Ionotropic glutamate receptors located in the caudal aspect of the nucleus tractus solitarii, especially those within the commissural subnucleus of the nucleus tractus solitarii, have been implicated in the mediation of the cough reflex evoked by the mechanical stimulation of the tracheobronchial tree in the rabbit.[23] Conversely, in the guinea pig, Canning and Mori[24] provided evidence for a more distributed termination of the cough receptor endings innervating the extrathoracic trachea within the nucleus tractus solitarii. Species differences may account, at least to some extent, for the different results between laboratories. Whatever the specific sites of termination of cough receptors within the nucleus tractus solitarii, it seems likely that various aspects of this region play an important role in the integration of peripheral inputs regulating the cough reflex, and therefore they could be the site of action of antitussive drugs.[25,26]

Notably, Bolser et al[25] demonstrated that intravertebral artery administration of opioids reduced, in a dose-dependent manner, the number of coughing episodes and rectus abdominis burst amplitude during coughing induced by mechanical stimulation of cat trachea. In light of these studies, it seems well established that the respiratory medullary areas are also involved in cough mediation and that depression of respiratory neurons may occur following administration of cough suppressants.

The present findings further demonstrate the robustness and sensitivity of the CO_2 rebreathing method[5] and reliably demonstrate that levodropropizine does not have a central depressant action in subjects with chronic cough, normal CO_2 sensitivity, and no evidence of

airway obstruction. In addition, the result is in keeping with previous observations[8] obtained in patients with COPD showing no significant change in CO_2 sensitivity after therapeutic doses of levodropropizine. In this study,[8] however, no comparisons were done with central antitussive drugs nor were attempts made at detecting changes in the pattern of breathing, possibly pointing at more subtle effects on respiratory control by levodropropizine. In this study, we demonstrated that, at variance with dihydrocodeine, which affects both the frequency and the volume components of the breathing pattern, levodropropizine does not influence breathing pattern or $\dot{V}_I$ compared with control conditions.

The antitussive action of levodropropizine is not fully characterized, but it seems independent of bronchodilation or muscarinic receptor antagonism,[27] since at doses that inhibit induced coughing, it does not block methacholine-induced bronchoconstriction in asthma.[27] Previous animal studies suggest that its antitussive action may depend on blockade of a large proportion of pulmonary C fibers.[11] Most importantly, however, the results confirm the lack of any detectable action by levodropropizine, but not dihydrocodeine, at the level of the human respiratory network.

This was a single-blind study, that is, patients were unaware of the administered agents. However, blinding becomes less important for reducing observer bias, as the outcomes are assessed objectively, thus leaving little opportunity for bias.[28]

In conclusion, levodropropizine does not affect the ventilatory response to CO_2 in subjects with chronic cough and normal CO_2 sensitivity; this suggests the lack of any depressant central action. We propose that the clinical study of respiratory control during CO_2 rebreathing should routinely be used when one needs to investigate the central respiratory effects of drugs, especially antitussive agents.

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