Hoarseness as a Sign of Possible Non-Specific Mucosal Hyperreactivity in Vocal Tract

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ABSTRACT

The aim of the present paper was to evaluate the possible impact of methacholine challenge on the voice and vocal tract in ten patients reporting hoarseness due to presumable hyperreactivity to some environmental factors. Ten age- and gender-matched healthy subjects served as controls. Methacholine was used for hypersensitivity challenge and saline solution (0.9% NaCl) as control substance. Subjects were examined on two separate challenge occasions testing either methacholine in increasing doses (3, 6, 12 mg) or NaCl. Voice recordings, videolaryngoscopy, and measurement of nasal secretion were performed before and after each sniffing session. Subjective complaints were documented. Data were statistically analyzed with three-way analysis of variance (ANOVA) and group comparisons performed. Perceptual analysis of voice recordings showed an increase of the grade of voice disorder in patients after exposure to both substances. Moreover, one of the patients became aphonic and another severely dysphonic after NaCl. No voice quality changes were detected in controls. The videolaryngoscopy findings divergent from normal did not increase in any group. Nasal secretion was significantly higher after sniffing of methacholine than NaCl in both groups. The frequency of subjective complaints was equal in both groups after both substances. However, there was a qualitative difference in the character of the symptoms: the patients complained of throat, vocal and nasal symptoms while the controls complained exclusively of nasal symptoms.

The study supports the view that vocal dysfunction after exposure to non-specific environmental irritating factors may be triggered mainly by emotional mechanisms such as off-warding reaction or dissociative disorder.

BACKGROUND AND AIM

Recurrent voice disorders, hoarseness or loss of voice, with or without breathing difficulties and chronic cough are sometimes reported by patients exposed to certain irritating factors in their environment, mainly at their workplace. For a professional voice user these vocal reactions can be especially bothersome and should, in that context, also be regarded as occupational voice disorders (1, 2). The possible interplay between the place of work and the voice problems might also give rise to medico legal aspects (3). Thus, to evaluate possible causal relationships between the environmental irritating factors and patients' voice disorders is a matter of importance for the voice clinic.

Hoarseness in patients with this kind of problem is often attributed to an allergic reaction at the laryngeal level (2, 4, 5). However, the causative factors reported by many patients are not the ordinary airborne allergens but less well-defined irritants such as scents, smoke, car exhausts, etc. (6) Hence, nonspecific hyperreactivity in the airway may also be considered as

an important causal factor of mucosal reactions, similar to those evoked by allergy responses (7).

It would appear that the relationship between nonspecific hypersensitivity in vocal tract mucosa and hoarseness has been little systematically studied, probably because of practical difficulties in the testing of vocal reactions. Namely, the patient experiences hoarseness after exposure to not well-defined irritating agents and hitherto in certain environments. This combination of agent and environment is hardly possible to replicate in the clinic. Therefore, we aimed to design an appropriate method to study this reaction, using a substance known to provoke hyperreactivity. Methacholine, commonly applied as an agent for triggering hyperreactivity in the lower airways (8) and also to evaluate nasal hyperreactivity (9), seems to be a possible agent for provoking hypersensitivity reactions in the vocal tract.

The aim of this saline-controlled, single-blind study was to evaluate the possible effects of methacholine challenge on the vocal tract and vocal function in people with suspected nonspecific hyperreactivity.

SUBJECTS AND METHODS

Patients

Eleven consecutive patients referred to the Voice and Speech Department, University Hospital of Lund were initially included. The patients complained of hoarseness after exposure to some environmental factors, mainly at their workplace. Before the referral to the phoniatric department no patient had tested positive to standard tests for IgE-mediated allergy. One patient (pat 7) became aphonic and reported breathing difficulties after challenge with physiological saline solution (0.9% NaCl). Hence, this person could not be motivated to undergo further challenge with methacholine and therefore data for methacholine are missing for this patient. Consequently, this patient had to be excluded from statistical analyses and the results are therefore based on analyses obtained from the remaining ten patients. Four of the remaining patients were occasional/regular smokers. Patient characteristics are shown in Table 1.

Controls

Eleven unpaid control subjects, individually gender and age matched, without asthma and allergy and without any medication, were recruited. The controls had no voice problems; however, occasional hoarseness in connection to upper respiratory tract infection may have occurred in the past. All controls were nonsmokers.

TABLE 1. *Summary of patients and background data*

PAT	M/F	AGE	PROFESSION	SMOKING HABITS	ALLERGY TESTING	REPORTED TRIGGERING SUBSTANCE	SUBJECTIVE SYMPTOMS AT EXPOSURE
P1	F	22	Preschool teacher	Nonsmoker	Neg Skin-pricktest	Washing detergents, tomatoes	Hoarseness, Episodes of voice loss, Itching in throat and mouth
P2	M	33	Engineer	Nonsmoker	Neg Skin-pricktest	Smoke, perfumes, bananas	Hoarseness, coughing, rhinitis, laryngitis
P3	F	35	Industrial worker	5 cig/day	Neg Skin-pricktest	Gluing substances	Hoarseness, rhinitis, itching in eyes, dryness in eyes and nose,
P4	F	44	Nursing assistant	5–10/day	Neg Skin-pricktest Neg RAST**	Dust mites, dog and cat, strongly scented flowers	Hoarseness, episodes of voice loss
P5	F	46	Seamstress	Nonsmoker	Neg Skin-pricktest Neg RAST	Strong scents, fabrics, various foods	Hoarseness, episodes of voice loss, stuffed nose, breathing difficulties
P6	F	47	Cashier	20/day	Neg Skin-pricktest	Perfumes, washing detergents, oranges	Hoarseness, breathing difficulties, rash
P7*	F	47	Cleaner	10/day	Neg Skin-pricktest	Various scents, working environment	Hoarseness, episodes of voice loss, breathing difficulties
P8	F	48	Teacher	Nonsmoker	Neg Skin-pricktest Neg RAST	Damp air, cold air	Hoarseness, coughing, breathing difficulties
P9	F	56	Physiotherapy-assistant	Nonsmoker	Neg. RAST test	Smoke, cigarette smoke, exhaust, chemical substances	Hoarseness headache, smarting in nose
P10	F	63	Teacher	Nonsmoker	Neg skin-pricktest Neg RAST	Working environment, damp air, mold.	Hoarseness, episodes of voice loss
P11	M	63	Industrial worker	>5/day	Neg Skin-prick test	Strong scents	Hoarseness, dryness and itching in throat and palate, stuffed nose

*The patient was later excluded from methacoline testing ** RAST: Radio-Allergo-Sorbent-Test

Procedures

General design of challenge study

Before routine phoniatric examination, all patients and controls were orally informed about the test procedures. They were informed that they were going to sniff a solution containing a substance that might cause an allergic reaction. They were also informed of the repeated voice recordings and repeated examinations of the larynx and nose. However, they were not informed about the sniffing of NaCl. Medication was stopped 24 hours before the challenge and smoking was not allowed for at least two hours before testing. Neither patients nor controls had signs of upper respiratory tract infection or allergic manifestations before testing.

The challenge was performed on two occasions with at least one week in between.
Each of the two occasions started with a base examination consisting of voice recording and videolaryngoscopy, followed by three *challenge sessions* (S1, S2, S3), i.e. sniffing of test substance with 15 min rest in between. During the rest period a nasal secretion was collected and possible subjective symptoms were freely reported.
On the first occasion subjects sniffed 0.9% NaCl in all three sessions. On the second occasion the subjects sniffed methacholine in increasing doses (3, 6, and 12 mg/ml). The subjects were asked to sniff the respective substances given in one to two puffs in front of each nostril with deVilbiss nebulizer (10). Each time 4 ml of the test substance was administered.

Nasal inhalation was intended to imitate the natural intake of airborne substances. In this manner, most of the inhaled substances should be deposited in the upper vocal tract.

Voice recordings and analyses
The voice recordings were performed with the patient sitting in a soundproof room, reading a standard Swedish text "Nordanvinden och solen". In the initial stage of the study the recordings were registered on an Otari tape recorder and in later stages on Sony MDS-101, with the microphone (Sennheiser) placed on constant, but not standardized, distance. The switch in recording routines during the study was unfortunate but unavoidable due to ongoing modernization of our clinic. Nevertheless, every single subject's recordings were performed with the same equipment. Furthermore, spectral analysis did not reveal any significant differences in the sound quality between the two recording methods (unpublished data, Lyberg Åhlander V, Rydell R).

Each recording was coded and copied to cassette tape in randomized order. Eight recordings were duplicated to test intrajudge reliability (8 × 20 recordings + 8 duplicates).

Three experienced voice clinicians performed perceptual voice analysis independently of each other. Ten voice parameters according to Hammarberg's protocol Swedish Voice Evaluation Approach (SVEA) (11) were judged. In addition, Grade of Voice Disorder (Grade) was estimated in analogy with the GRBAS scale (12). The Visual Analog Scale (VAS) 100 mm was used to judge the voice parameters. Mean VAS value was calculated for each parameter, and the variability and interaction of the measurements of each voice parameter were analyzed.

Videolaryngostroboscopy (VLSS)
Laryngostroboscopy was performed according to routine at the time of the examination, either with a laryngeal mirror and Zeiss surgery microscope (with 300 mm lens, magnification × 11.8) or with 70° rigid laryngoscope (Storz, WQ 075). It was performed without local anesthesia, with the patient/control sitting upright in the examination chair. Examination of the vibratory capacity of the vocal folds was performed with a stroboscope (Bruel & Kjaer 4914, trigging frequency of 1.0/s). The images of the laryngeal findings were videotaped with either U-matic or VHS-equipment.
In analogy with the changing of the resources for the recordings of voice, mentioned above, the equipment for the recording of laryngeal findings also had to be changed, due to the modernization of our clinic. Laryngeal microscopy technique enables at least as high quality of the recorded image as does a rigid laryngoscope; however, it is usually less well tolerated by the examined subject. The U-matic equipment broke down and could only then be replaced with the new technique. Nevertheless, every single subject's recordings were performed with the same equipment. We believe that the quality of laryngeal recordings with both U-matic and VHS meets the international standards permitting equally good estimation of the laryngeal parameters used in this study.

The VLSS recordings were judged in consensus by a panel of three phoniatricians who assessed the structure of vocal folds; pattern of ab–adduction; characteristics of mucosal wave; symmetry/asymmetry in posterior larynx according to protocol ad mod. Hirano & Bless (13). In addition, increased secretion and appearance of blood vessels were recorded.

Nasal secretion
After each challenge, the participants collected nasal secretions in a test tube, for 15 min, sitting in a bent-over position.

Subjective symptoms
After each sniffing, the participants were asked to freely report their subjective symptoms, such as hoarseness, breathing difficulties, and stuffy nose. The symptoms were registered in a protocol.

Statistics
The statistics for all analyses were calculated with SPSS for Windows (vers 12), SPSS Inc (Chicago, IL, USA).

Three-way analysis of variance (ANOVA) (14) was used in the analysis of the voice parameters as well as for nasal secretion. For the ANOVA the factors for the voice and nasal parameters were: Group (G) with two levels (Patients/Controls), Treatment (T) with two levels (methacholine/NaCl) and Session (S) with four levels (base/1/2/3).

The intrajudge correlation for the voice judges was analyzed calculating intraclass correlation (ICC), which calculates the accordance between two or more judges. This method compares the variance between individuals with the variance within individuals.
The data in this material contain a large number of parameters perceived and judged close to zero, which is not ideal for estimating the intrajudge reliability. Such problems are commonly known and met in the analysis of voice, especially in populations with slight vocal pathology or with so-called normal voices.
It was not possible to make a statistical analysis of the laryngostroboscopic findings due to a very small number of abnormal findings in each group.

A probability level of less than 0.05% was considered to be significant.

Ethical aspects
The study was vetted and approved by the ethical committee at Lund University (No LU 352-95).

RESULTS

Voice – perceptual analysis
Among the 11 patients there was one who became aphonic after exposure to NaCl and so it was only possible to analyze the data in the remaining ten patients. One of ten also became severely dysphonic after NaCl and six of ten patients reported hoarseness after methacholine as well as NaCl. Three of ten did not experience any voice symptoms. None of the controls reported any voice disturbances.

The mean values of the ratings of the 10 voice parameters are shown in Table 2a (NaCl) and 2b (methacholine) and the mean values of Grade of Voice Disorder are given in Fig 1. Summary of three-way ANOVA for 10 voice parameters and Grade of Voice Disorder are given in Table 3. As can be seen from Table 3 the differences were significant for all voice parameters with respect to the factor Group (G), except for aphonic episodes, reduced pitch, and unstable register. However, concerning Grade of Voice Disorder the three-way ANOVA revealed significant interaction between both Groups and Treatments.

TABLE 2a,b *Perceptual voice analysis in ten patients and ten controls, before (base) and after consecutive sniffing of NaCl. Mean values of ratings of ten voice parameter, according to SVEA, judged on a 100 mm VA-Scale by three judges. Standard deviation within parenthesis.*

2a NaCl

Aspects	**Base**		**NaCl 1**		**NaCl 2**		**NaCl 3**	
	Pat	**Contr**	**Pat**	**Contr**	**Pat**	**Contr**	**Pat**	**Contr**
Breathiness	1,3	0,16	2	0,63	3,5	0,9	5,55	1,16
	(1,2)	(0,3)	(1,1)	(0,3)	(1,2)	(0,8)	(2)	(1)
Hyperfunction	12,87	3,35	9,8	2,23	20,8	2,46	18,8	4,35
	(1,4)	(2,7)	(3,5)	(2,0)	(8,5)	(2,5)	(10,3)	(4,4)
Vocal fry	24	6,7	21,9	4,03	21,95	5,16	22,72	8,3
	(9,8)	(1,7)	(13)	(1,7)	(11)	(1)	(10,1)	(0,8)
Roughness	11,13	0	10,23	0	12,11	0	20,22	0
	(6,1)		(4,7)		(3,8)		(5,9)	
Increased pitch	3,4	0,43	1,43	0,93	11,18	2,03	4,66	0,66
	(0,3)	(0,7)	(1,7)	(1,6)	(4,5)	(0,9)	(4,7)	(1,1)
Reduced pitch	4,86	0,36	6,1	1,5	5	1,96	5,66	0,96
	(5)	(0,6)	(9)	(2,2)	(4,5)	(2,3)	(5)	(0,8)
Aphonic episodes	0,41	0	0,9	0	0	0	1,77	0
	(0,7)		(1,5)				(1,6)	
Hard glottal attacks	4,85	3,63	9,11	2,3	3,4	2,1	5,34	3,53
	(7,6)	(4,3)	(8,6)	(2,9)	(5,2)	(3,4)	(7,6)	(5,6)
Unstable register	2,2	0	1,76	0	2,77	0,5	2,25	1,53
	(1,2)		(2,4)		(2,5)	(0,9)	(2,8)	(1,8)
Reduced sonority	18,8	3,6	17,4	0,96	28,51	2,86	32,07	4,86
	(4,4)	(0,5)	(6,2)	(1)	(3,8)	(1,9)	(8,3)	(3,6)
Grade of voice disorder	16,9	2,16	19,1	1,1	29,7	2,3	32,5	3,5
	(6,5)	(1,5)	(8,8)	(1,3)	(8)	(2,8)	(7,6)	(4,7)

2b Methacholine

Aspects	**Base**		**Meta 1**		**Meta 2**		**Meta 3**	
	Pat	**Contr**	**Pat**	**Contr**	**Pat**	**Contr**	**Pat**	**Contr**
Breathiness	0,58	0,46	5,53	1,1	1,44	0,9	3,1	1,36
	(0,5)	(0,8)	(5)	(1,9)	(2,2)	(0,8)	(2,8)	(1,3)
Hyperfunction	12,42	2,23	17,4	2,13	10,43	4,03	23,2	1,75
	(4)	(2,1)	(4)	(2,0)	(6,1)	(3,7)	(6)	(2,4)
Vocal fry	17,53	6,2	21,06	8,5	17,53	7,83	23,33	7,64
	(15,2)	(1,8)	(10,1)	(1,8)	(9,3)	(1,5)	(7,1)	(0,5)
Roughness	13,37	0	13,16	0	12,87	0	10,1	0
	(5,7)		(8,8)		(7,9)		(5,4)	
Increased pitch	2,3	1,16	9,2	2,34	8,42	2,16	12,16	2,5
	(0,2)	(0,8)	(4,8)	(2,2)	(3,3)	(2,6)	(5,4)	(2,5)
Reduced pitch	3,37	1,68	1,26	1,11	1	1,23	0,9	3,4
	(1,5)	(2,4)	(1,2)	(1)	(1,7)	(1)	(1,5)	(4)
Aphonic episodes	0	0	2,06	0	0	0	7,4	0
			(3,6)				(2,8)	
Hard glottal attacks	5,14	3,43	6,75	2,96	6,75	2,96	7,29	1,45
	(4,7)	(4,11)	(5,6)	(5,1)	(7)	(3,6)	(8,1)	(2,2)
Unstable register	0	1,33	1,03	0,74	1,48	0,46	6,23	0,33
		(2,3)	(1,8)	(1,3)	(2,6)	(0,8)	(5,6)	(0,6)
Reduced sonority	17,7	5,0	20,2	3,75	25,37	1,73	28,86	3,85
	(1,4)	(4,4)	(3,9)	(1,5)	(8,3)	(0,9)	(4,2)	(3,3)
Grade of voice disorder	15,8	3,0	19,8	1,8	20,9	2,4	27,2	1,2
	84)	(3,5)	(6)	(1,8)	(5,1)	(1,9)	(4)	(1,4)

As can be seen from Fig 1 the Grade of Voice Disorder was judged higher in patients than in controls, already at base recordings. In controls, this parameter was rated equally low through all challenge procedures. In patients, however, the grade of voice disorder tended to increase

TABLE 3. *Summary of significance values, F-values and degrees of freedom of three-way analysis of variance of perceptual analysis. Ten voice parameters and Grade of Voice Disorder, with relation to factors group (G) treatment (T) and session (S). For details see text.*

Variable	*G*		**T**		**G+T**			**S**			**G+S**			*T+S*			**G+T+S**		
	S	s	F	df	s	F	df	s	F	df	s	F	df	s	F	df	s	F	df
Breathiness	0,018	0,539	0,391	1,000	0,368	0,850	1,000	0,193	1,688	2,253	0,431	0,884	2,253	0,671	0,431	2,197	0,658	0,451	2,197
Hyperfunctio n	0,006	0,350	0,916	1,000	0,411	0,705	1,000	0,347	1,092	2,080	0,371	1,022	2,080	0,069	2,860	2,016	0,015	4,665	2,016
Vocal fry	0,013	0,680	0,175	1,000	0,221	1,594	1,000	0,400	0,957	2,221	0,757	0,310	2,221	0,699	0,443	2,641	0,890	0,179	2,641
Roughness	0,040	0,435	0,635	1,000	0,435	0,635	1,000	0,701	0,252	1,412	0,701	0,252	1,412	0,275	1,333	2,288	0,275	1,333	2,288
Increased pitch	0,039	0,801	0,065	1,000	0,898	0,017	1,000	0,114	2,143	2,613	0,213	2,143	1,564	0,275	1,331	2,384	0,695	0,419	2,384
Reduced pitch	0,212	0,047	4,493	1,000	0,014	7,175	1,000	0,632	0,570	2,908	0,242	1,436	2,908	0,837	0,209	2,256	0,500	0,737	2,256
Aphonic episodes	0,135	0,564	0,345	1,000	0,564	0,345	1,000	0,477	0,642	1,364	0,477	0,642	1,364	0,280	1,296	1,485	0,280	1,296	1,485
Hard gl attacks	0,030	0,390	0,773	1,000	0,602	0,281	1,000	0,145	1,965	2,341	0,028	3,615	2,341	0,689	0,443	2,507	0,225	1,523	2,507
Unstable register	0,286	0,104	2,908	1,000	0,623	0,250	1,000	0,260	1,393	1,673	0,226	1,560	1,673	0,625	0,481	2,036	0,161	1,906	2,036
Reduced sonority	0,001	0,382	0,801	1,000	0,296	1,154	1,000	0,333	1,127	1,931	0,241	1,478	1,931	0,632	0,454	1,929	0,658	0,412	1,929
Grade of Voice Dis.	0,006	0,012	33,71	1,000	0,011	0,532	1,000	0,118	53, 52	1,692	0,089	1,919	1,692	0,223	9,711	1,978	0,650	0,150	1,978

in time after subsequent challenges, for both substances tested. The three-way ANOVA showed that there were significant differences between the groups and also an interaction between group and treatment where the difference was significant only for the patient group (p=0.047).

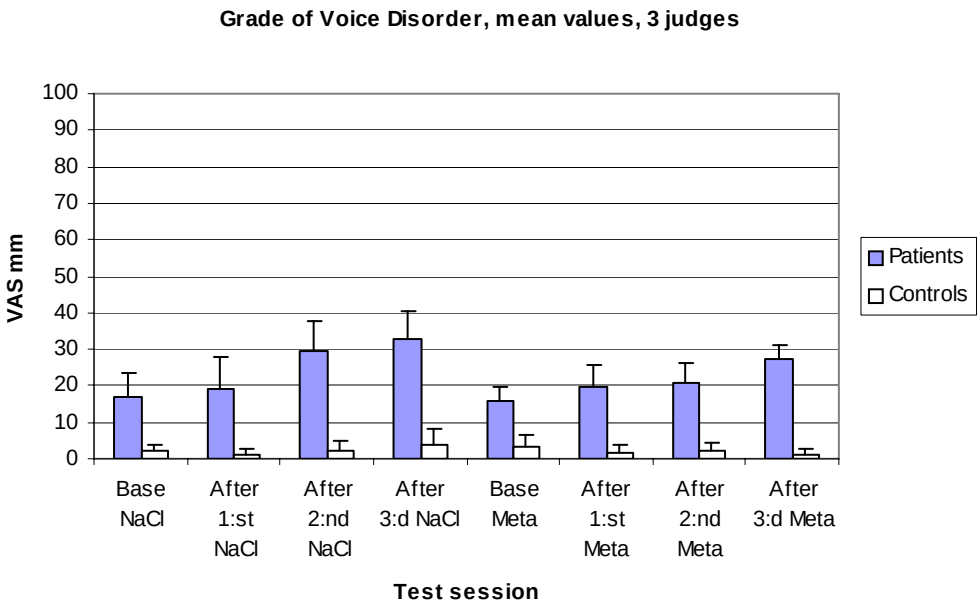

FIGURE 1: Perceptual voice analysis in patients (n=10) and controls (n=10). Mean values of ratings of Grade of Voice Disorder before (base) and after consecutive NaCl and methacholine sniffing sessions, judged on a 100 mm VA-Scale

Some minor observations on laryngeal motility aspects were seen in the patient group after exposure to both substances (Table 4). Apart from increased activity of false vocal folds in one subject after methacholine sessions 1, 2, and 3, no systematic changes were found. In the control group no observations on any aspect of laryngeal motility were recorded.

TABLE 4. *Videolaryngostroboscopy in **patients**. Laryngeal **motility aspects** divergent from ideal stroboscopic findings before (base) and after consecutive NaCl and methacoline sniffing sessions.*

	Motility							
Patient	**Base**	**NaCl 1**	**NaCl 2**	**NaCl 3**	**Base**	**Meta 1**	**Meta 2**	**Meta 3**
1								
2								
3	-	-	-	-			-	
4						F	F	F
5		R						
6								
8								
9								
10	-			-				
11	R	R	R, F	R	R	R	R	R

Empty box= normal glottal activity, incl. normal mucosal wave.
R= reduced motility (pat 11 reduced left vf.) incl reduced mucosal wave
F= activity of ventricular folds
-= not accessible

Nasal secretion

As shown in Fig. 2 the amount of nasal secretion was significantly higher after sniffing methacholine than after NaCl, in both groups. Neither after methacholine- nor NaCl challenge there were any significant differences in amount of secretion between the groups as shown by t-Test (Table 5). Also the three-way ANOVA showed no difference in nasal secretion with respect to factor Group (p= 0,1 , F=1,91 df=1,69). However the amount of nasal secretion increased significantly with respect to consecutive challenges (factor Session, p=,000 F= 53,52 df= 1,69) and there was also interaction between sessions and tested substances (factor Treatment and Session, p=,000 F= 9,71 df=1,97), i.e. the amount of secretion increased with every next session of tested substance.

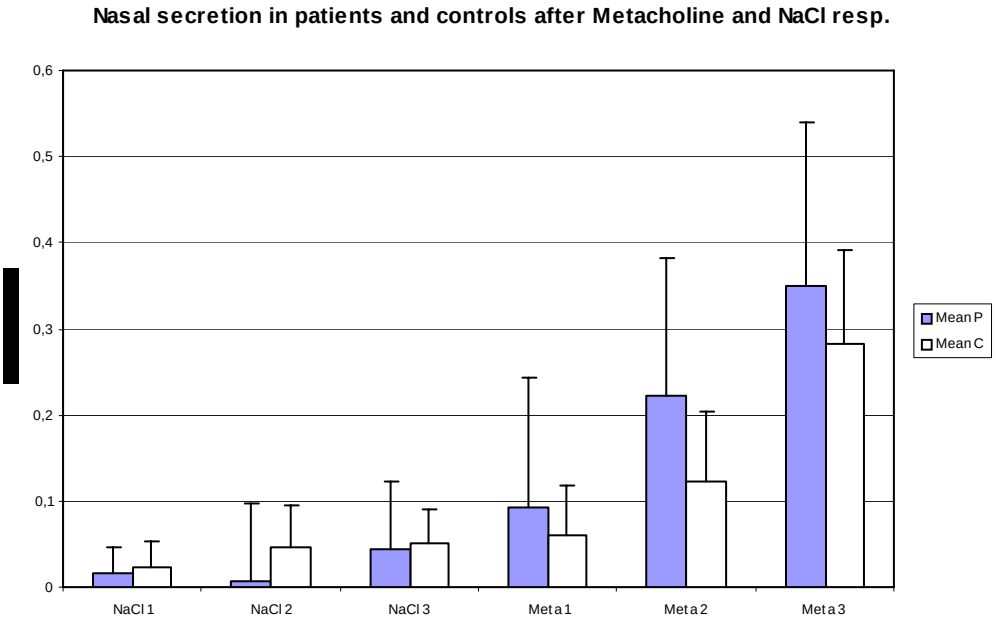

FIGURE 2. Mean values of nasal secretion in milliliters before- and after consecutive NaCl and methacholine sniffing sessions.

TABLE 5

(A) Summary of T-test; comparison of Nasal secretion in 10 patients and 11 controls after three sessions with saline (NaCl) challenge (B)

Summary of T-test; comparison of Nasal secretion in 10 patients and 11 controls after three sessions with methacholine challenge

A **Session**	t	df	P (two tailed)
NaCl 2	0,669	19	0,511
NaCl 3	0,318	19	0,754
NaCl 4	-0,546	19	0,592

B **Session**	T	df	P (two tailed)
Methacholine 2	-0,528	19	0,603
Methacholine 3	0,029	-1,415	0,173
Methacholine 4	-10,375	19	0,185

Subjective symptoms

As shown in Table 6, neither controls nor patients reported any nasal or vocal symptoms before the challenges (base recordings). The patient group reported similarly frequent symptoms after exposure to both NaCl and methacholine. The controls reported few symptoms after NaCl but frequent symptoms after methacholine, in fact, equally frequent as the patient group. Interestingly, the character of symptoms was different: the controls reported exclusively nasal symptoms (stuffed nose, runny nose) whereas the patient group, in contrast, reported voice symptoms (hoarseness and voice loss) and throat symptoms (itching, soreness) as well as nasal symptoms.

TABLE 6. *Number of patients and controls reporting subjective symptoms before (base) and after consecutive NaCl and methacholine sessions.*

	NaCl, Base	NaCl 1	NaCl 2	NaCl 3	Meta Base	Meta, 1	Meta, 2	Meta, 3
Patients (n=10*)	0	5	9	8	0	6	9	10
Controls (N=10)	0	0	2	2	0	7	9	7

TABLE 7. *Videolaryngostroboscopy. Laryngeal **structural aspects** divergent from normal stroboscopic findings before (base) and after consecutive NaCl and methacoline sniffing sessions*

A Patients

	Structure							
Patient	**Base**	**NaCl 1**	**NaCl 2**	**NaCl 3**	**Base**	**Meta 1**	**Meta 2**	**Meta 3**
1	Œ			Œ	Œ		Œ	Œ
2				S, V			S, V	S, V
3	-	-	-	-	-	V	-	S, V
4	S, V	Œ, S, V	Œ, S, V	Œ, S, V	Œ, V	Œ V	Œ, S	Œ, V
5						S, V	Œ, S, V	Œ, S
6			V	V			V	
8			V	V	S		S	V
9					Œ		S	
10	-	S	S		S			
11					S	S		S

B Controls

	Structure							
Control	**Base**	**NaCl 1**	**NaCl 2**	**NaCl 3**	**Base**	**Meta 1**	**Meta 2**	**Meta 3**
1			Œ	Œ				
2	V	V	V	V, S	V	V	V	V
3								
4								
5								
6	S			S				
8								-
9						Œ	Œ	
10	Œ	Œ	Œ	Œ	Œ	Œ	Œ	Œ
11								

Œ= marginal edema of vocal fold
V= visible blood vessels on vocal fold
N= vocal fold nodules
S= secretion
-= not accessible
Empty box denotes no abnormal finding

DISCUSSION

This single-blind study revealed that any challenge with methacholine or with saline, in patients with presumed hyperreactivity of the vocal tract, had an impact on voice quality. The frequency of the subjective symptoms did not differ between the patients and controls. However there was an interesting difference as to the character of symptoms: the patients reported hoarseness and symptoms of throat and nose, while the controls reported exclusively the expected nasal symptoms. No significant impact on structural or functional laryngeal aspects was recorded in either group after any challenge. The amount of nasal secretion was significantly increased in both groups after sniffing of methacholine as compared to NaCl but the amount of nasal secretion was not higher in the patients.

Methodological considerations

Design of methacholine challenge in evaluation of suspected hyperreactivity within vocal tract

Methacholine is expected to act upon the bronchial and nasal muscarine receptors. It is not known whether any similar mechanism exists in the vocal tract. To the best of our knowledge, methacholine has not previously been used systematically within the context of voice disorders without breathing difficulties. Within pulmonary medicine, however, methacholine challenge is well documented for provoking obstructive reactions within the airways (8). In the present study we intended to provoke hoarseness under controlled circumstances and the challenge was designed for testing of the vocal tract, without penetration to the lower respiratory tract. In order to fit these premises, the provoking substances (methacholine or saline solution) were nasally inhaled. A common effect of nasal intake of methacholine is a secretary response, and also a common response in allergy (9). As expected, in the present study the nasal secretion increased after sniffing of methacholine in both groups studied.

Although a negative impact on the vocal quality in patients was shown by statistical analyses, the results must be interpreted with caution. The tests were performed thoroughly but the small size of tested groups does not permit validation of the test. We believe, however, that occurrence of dysphonia after provocation with methacholine is sufficient evidence to support the suspicion of nonspecific hyperreactivity in the vocal tract.

Saline as a control substance

Our aim was to provoke a vocal reaction in patients complaining of hoarseness after exposure to certain elusive substances such as car exhausts, scents, and detergents. In cases with defined allergens it is possible to use the specific agents for stimulus and it is also possible to make up an appropriate placebo (2, 5). However, in our study, without a specific allergen as the stimulating substance, there is no obvious choice of placebo. A control substance to methacholine was, however, required and we adopted a model with physiologic saline solution (0.9% NaCl) (9). Methacholine, given orally, in doses used in the present study, may be perceived as very slightly sweet. However, when it is sniffed, an average person cannot perceive this taste and or tell the difference from saline.

Interestingly, in controls, it was found that saline produced only expected reactions, i.e. increase of nasal secretion. In patients, in contrast, provocation with saline solution also caused deterioration of voice and other symptoms of the vocal tract. Thus, the choice of saline as a control substance seemed to be appropriate for the purpose of our study.

Matching of the tested subjects
The subjects included in the control group were voice-healthy and gender and age matched to the patients. However, we were unable to match the groups as to smoking habits. Among the patients, four of ten individuals were smokers. Unfortunately it was not possible to find appropriate smokers willing to participate in our study as controls. Consequently, all the controls were nonsmokers. Smoking is a significant factor in evaluating voice quality, and smoking habits might explain the elevated grade of voice disorder present already at the base recordings in our patient group.

For evaluation of the results it is important to find out whether smoking habits in some of our patients may explain an additional deterioration of their voices after provocation with both methacholine and saline? It would appear that smokers do not seem to be more prone to react to methacholine or saline than a nonsmoking population in routine pulmonary challenge with methacholine. By analogy we presume that the vocal reaction in our population may be ascribed rather to a hypersensitivity mechanism.

Evaluation of subjective symptoms
Evaluation of subjective symptoms is an important part of clinical assessment of voice disorders. Questionnaires on voice function, such as the VHI (15), are currently used for that purpose, covering a defined but still limited view of symptoms. In our study subjective symptoms were reported freely but were registered by the examining doctor in the protocol. With this approach it was hoped the subjects would express their own spontaneous "hierarchy" of self-perceived discomfort. Some bias in our interpretation of the replies could be expected and, therefore, to try to decrease the possible bias, a semi-standardized protocol of the reported symptoms was used. A more standardized approach with VHI seemed less suitable in our design. Namely, our subjects had to report on their symptoms repeatedly with short time intervals during the one hour testing procedures. The subjects' learning the content of a form would presumably cause a still greater bias.

The tested participants' expectations of their own reactions to the test should also be considered as an important factor of possible bias when interpreting one of the findings in our study, i.e. the qualitative difference between the groups in reporting on subjective complaints. The controls reported exclusively nasal symptoms whereas the patients complained about symptoms from voice as well as throat and nose. We presume that controls had no particular expectations on voice alteration after nasal application of any substance. Patients, on the other hand, as being more predisposed to voice problems, could have been more focused on their voices, being their *locus minoris resistentiae* (4, 6). Moreover, due to ethical demands, the patients had to be informed of all possible effects of the sniffed substance, including hoarseness. This information *per se* also gave rise to negative expectation on vocal function during the test. It would appear that it is not possible to avoid these sources of bias in clinical trials.

Possible mechanisms of dysphonia in our patients
Deteriorated voice quality and increased reports of subjective symptoms from the larynx and pharynx occurred in our patients after subsequent challenges and with both substances tested, methacholine and NaCl.
However, analyses of recordings of videolaryngoscopy did not reveal any significant impact on either structural or functional aspects of the larynx. How can these apparently contradictory findings be interpreted? Which are the possible mechanisms of deviant voice in our patients?

Structural changes in the larynx due to allergic inflammatory reaction have often been discussed as a possible cause of dysphonia. A causal relationship between claimed allergy on the laryngeal level and dysphonia is, however, not obvious. Indeed, the human vocal folds seem to be less prone to allergic reactions since the mast cells involved in allergic response are present mostly in the epiglottis and subglottic regions in the adult larynx (16). Moreover, according to some previous studies, allergic adults rarely complain of hoarseness and evidence for laryngeal allergic reaction triggered by airborne allergens seems rare (17). Thus voice disorders in allergic adults were interpreted to be secondary to impairment of nasal breathing and resonance (17). Other authors of more recent studies (18, 19) interpreted dysphonia in allergic patients as sign of laryngeal reaction since they reported vocal fold edema as being a common feature in an allergic population.

In controlled provocation studies, structural alterations such as edema or increased mucus were observed after either inhalation of airborne allergens (5, 6) or intake of food allergens (4). However, these studies still did not lend enough support for a causal relationship between the laryngeal manifestations and allergy. Reidy et al. (5) stated that their provocation study failed to demonstrate a direct causal relationship between antigen exposure to *dust mite* and physical or functional changes in the larynx, although there were signs of considerable laryngeal inflammation in the studied allergic population. Obviously, it cannot be excluded that microscopic or molecular changes occur in the laryngeal mucosa due to direct contact with allergen or chemical agents, but such reactions are difficult to confirm with current clinical methods. Future examination techniques, such as high speed filming, may probably better reveal impact on the mucosal structure.

In our patients, some minor laryngeal findings deviant from normal had already been found before any challenge. However, those could scarcely be considered as the manifestation of allergy, except for in the patient with edema (Table 7a). Moreover, none of our subjects had proven immune-based allergy prior to the present study and their vocal symptoms appeared after exposure to nonspecific chemical agents (Table 1). We may rather hypothesize that the vocal disorder in our patients was caused by a nonspecific hyperreactivity (7), defined as an exaggerated or altered reaction in a goal organ to otherwise harmless stimuli. Thus, some kind of alteration of the functional state within the vocal tract due to nonspecific hyperreactivity should be taken into account in this context.

A nonspecific chemical stimulus, applied to any part of vocal tract mucosa, may trigger a variety of motor responses at the glottal or supraglottal level, which will modify the functional state of the vocal tract with increased effort in phonation (18). In our cases such altered vocal behavior may have occurred, as indicated by the anamnesis (with repeated incidences of hoarseness in specified environments) and confirmed by the results of the provocation with methacholine. Yet, for the clinician, it is important to try to understand how those responses are elicited, not least with respect to future therapy. Two possible mechanisms should be considered: either an off-warding reaction or a dissociative motor reaction.

Haapanen (2) proposed that an *off-warding reaction,* induced by supraglottal stimulation and defending larynx and lungs, would create a “pharyngeal dysphonia” by activating the supraglottal laryngeal sphincter. Our results from videolaryngostroboscopy, performed using the rigid laryngoscope, failed to reveal any systematic alteration in laryngeal or supralaryngeal motility aspects in patients after any challenge. A flexible fiber optic instrument would probably be a better tool to detect possible alterations of motor behavior of

the vocal tract, especially supraglottal and pharyngeal. On the other hand, our results of perceptual voice analysis revealed an increase of “hyperfunction”, an aspect of voice quality that might be interpreted as a sign of increased adductor pattern in the vocal tract during phonation (11). This, in turn, may indicate a tendency to some compression within the vocal tract following the challenges. Hence, it may be presumed that methacholine, nasally inhaled, could have given rise to an off-warding reaction, modifying motor behavior in the vocal tract and, consequently, being responsible for deviant voice quality in some of the patients.

Two of our patients showed an obviously exaggerated reaction pattern already after saline challenge, with either aphonia lasting for a few hours (pat 7, who had to be excluded from further testing with methacholine) or unstable fundamental frequency (pat 3). According to common daily life experience, momentary aphonic episodes or instability of fundamental frequency may also occur in nonhyperreactive persons after stimulation of pharyngeal and/or laryngeal sphincter (e.g. intake of pepper!). It would appear that such reactions do not normally last for as long as in our patients and in those cases we came to the conclusion that, at least in those two patients, a *dissociative motor laryngeal disorder* (20) occurred. The neural mechanism of dissociative disorder is not known but there is a consensus that this vocal motor dysfunction on an emotional basis can be triggered in prone individuals by a variety of stimuli. In our two patients this diagnosis was additionally confirmed by excellent results of logopedic intervention with prompt normalization of voice function and quality.

At present, the CNS mechanisms of either off-warding or dissociative disorder are not known in detail but emotional aspects of etiology of the two conditions should be taken into consideration while planning preventive steps, treatment strategies, and consideration of prognosis of voice disorder in hyperreactive people.

Therapeutic considerations

Our results confirm previous results (5) that patients with hyperreactive hoarseness might have a predisposition for voice disorders. In voice therapy it is important to identify the possible trigging agent of dysphonia and to focus on enabling the patients to handle the anticipated vocal reactions on various levels. Mental training through, e.g. cognitive therapy is important to identify triggering situations and prevent reactions as an effect of expectancy. The training of adequate voice technique enables the patient to handle the voice disorder in the actual situation of hyperreaction.

CONCLUSION

This study indicates that, people who experience hoarseness after exposure to elusive airborne substances in their daily life, will also respond with vocal and throat symptoms to nasally sniffed inert substance i.e. saline. Our study suggests that a non-specific hyperreactivity of the vocal tract may be a sign of either an off-warding reaction or a dissociative motor voice disorder, indicating the psychological basis for that disorder.

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