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Decreasing nasal mucus Ca^{++} improves hyposmia*

Georgios Panagiotopoulos[1], Stefanos Naxakis[2], Antonios Papavasiliou[3], Konstantinos Filipakis[1], Georgios Papatheodorou[4], Panagiotis Goumas[2]

[1] ENT Department, 401 General Military Hospital of Athens, Greece
[2] Department of Otorhinolaryngology Head and Neck Surgery, University of Patras Medical School, Patra, Greece
[3] ENT Department, Psychico Medical Center, Athens, Greece
[4] Research Unit, 401 General Military Hospital of Athens, Greece

SUMMARY

It is well documented that cytoplasmic Ca^{++} regulates sensitivity to cyclic adenosine monophosphate (cAMP). There is also evidence that Ca^{++} in the mucus may also modulate sensitivity to cAMP in vivo. Assuming that mucosal Ca^{++} could significantly change the excitability of the receptor neurons, we examined the alterations in the olfactory sensitivity by creating small changes in mucosal Ca^{++}. Thirty one patients complaining of olfactory loss were examined and their olfactory acuity was measured before and after the administration of a sodium citrate buffer solution in the nasal cleft. Thirty patients (96.8%) improved their scores in less than an hour period of time. Furthermore, 23 of them (74.2%) realized an improvement in their own sense of smell.

Key words: olfaction, odor identification, Ca^{++}, hyposmia, nasal mucus

INTRODUCTION

The sense of smell is one of the most important means through which our environment communicates with us. From an evolutionary standpoint, it is one of the most ancient of senses and a primal one as well, for both humans and animals. Nevertheless its significance is surprisingly neglected compared to other senses and treatment for olfactory disorders is more or less limited.

A critical role of Ca^{++} in vertebrate olfactory receptor neurons (ORNs) is to couple odor-induced excitation to intracellular feedback pathways that are responsible for the regulation of the sensitivity of the sense of smell [1, 2].

It is well documented that cytoplasmic Ca^{++} regulates sensitivity to cyclic adenosine monophosphate (cAMP) [1, 3]. By entering the cilium during the odorant response together with calmodulin (CaM) and / or an endogenous factor, Ca^{++} reduces the sensitivity of the cyclic nucleotide gated (CNG) channels to cAMP [4 – 6]. Exposure of olfactory receptor cells to odorant molecules stimulates the influx of Ca^{++} through cyclic nucleotide gated channels into the small volume within the cilia, the site of olfactory transduction. One of the effects of the consequent rise in intraciliary Ca^{++} is a negative feedback action on various stages of the odor transduction mechanism [3, 7] However, there is little evidence to indicate whether Ca^{++} in the mucus may also modulate sensitivity to cAMP in vivo.

A rise in mucosal Ca^{++} may contribute to the above-mentioned consequent influx of Ca^{++} inside the cell. There is evidence that odorous stimulation induces secretion from supporting cells. If this secreted material increases mucosal Ca^{++}, this could also be a mechanism for longer term adaptation to the continued presence of an odorant [5], taking into account the fact that odor adaptation in vertebrate olfactory receptor neurons (ORNs) is commonly attributed to feedback modulation caused by Ca^{++} entry through the transduction channels [8]

In order to determine the role of the mucosal Ca^{++} as far as the sensitivity of the olfactory receptor neuron in vivo is concerned, we examined the alterations in the olfactory sensitivity by creating small changes in mucosal Ca^{++} with the help of a sodium citrate - sodium acid buffer solution. This solution actually binds free Ca^{++} ions in the nasal mucus and therefore diminishes mucosal Ca^{++}.

A question that arises is if small changes in mucosal Ca^{++} can significantly change the sensitivity of CNG channels and thus the excitability of the receptor neurons. More specifically, as long as an increase in mucosal Ca^{++} may desensitize olfactory receptor neurons, can we actually improve hyposmia simply by decreasing mucosal Ca^{++}?

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MATERIALS AND METHODS

Thirty-one patients complaining of hyposmia-anosmia volunteered to take part in this study. Each one of them was asked to complete a questionnaire (Table 1) concerning critical information about their olfactory dysfunction [9 - 11].

The etiological diagnosis of olfactory loss was based on the patient's history, inspection of the nasal cavity, and examination of the olfactory cleft by endoscopy (rigid 30o endoscope 2,7mm). X-ray tomography was carried out when necessary such as when rhino-sinus inflammation was present [12], and/or ostiomeatal complex pathology was observed. Our primary aim was to exclude any condition associated with mechanical obstruction in order to ensure adequate administration of the buffer solution onto the mucus of the olfactory cleft. The characteristics of the study group are shown in Table 2 in total.

In order to volunteer in our study, the patients were instructed to read a form (Table 3) containing information about the aim of the investigation and the characteristics of the buffer solution that was to be administered into the olfactory clefts of their nose (Ethics approval given by the Scientific Council, University of Patras Medical School). Then they were invited to participate in our research as below:

Table 1. Questionnaire regarding patient's profile and medical history.

Name:
Age: height: weight:
Loss of smell: light? Moderate? Severe?
Loss of taste also: yes? No?
Olfactory capability before current change:
Duration of olfactory loss:
Precipitating incident around the time of olfactory loss:
Intermittent or continuous olfactory loss?
If continuous sudden or gradual?
Bilateral or unilateral loss?
Bilateral or unilateral nasal obstruction symptoms?
History of trauma? (describe)
Other symptoms?
Past medical – surgical history, medication:
Job related environmental exposure?
Smoke – alcohol consumption:
Duration of subjective improvement:

Table 2. Particular characteristics of the studied group. The numbers in the right column refer to the exact number of the patients with percentage in parenthesis.

Gender	
Female	20 (64.5%)
Male	11 (35.5%)
Smoking status	
0 cigarettes per day	16 (51.6%)
1-20 cigarettes per day	8 (25.8%)
>21 cigarettes per day	7 (22.6%)
Alcohol Consumption	
No	16 (51.6%)
Low	13 (41.9%)
Moderate	1 (3.2%)
High	1 (3.2%)
History	
Unspecified	5 (16.1%)
Head Trauma	1 (3.2%)
Nasal Surgery	7 (22.6%)
Upper Respiratory Infection	18 (58.1%)
Onset (median and range)	
4 months (0.5-420 months)	
Therapy in the past	
Yes (Decongestant therapy + steroids)	22 (71%)
No	9 (29%)
Endoscopic findings	
Unspecified	20 (64.5%)
Deviated Nasal Septum	2 (6.5%)
Deviated Nasal Septum with Turbinate Hypertrophy	2 (6.5%)
Turbinate Hypertrophy	7 (22.6%)
CT scan	
Yes	12 (38.7%)
No	19 (61.3%)

Table 3. Acceptance form.

ACCEPTANCE FORM

You are asked to take part in an experimental investigation concerning the administration of a aqueous solution in your nose. This particular solution consists of sodium citrate and sodium acid with similar PH and osmolarity to your own nasal mucus. We believe that the administration of this solution may improve your own sense of smell. Nevertheless, although we do not have any evidence that this solution may actually harm you, we can not reassure you about it. We can not inform you neither with any possible side effects which may include a further weakening or perhaps a total lost sense of smell or the duration of the benefits or the side effects of the administration, should they happen. If you are willing to take part in this investigation at your own risk you are kindly requested to sign in the box below.

Day 1: each patient's olfaction was evaluated 2 times; firstly with no medication and secondly after the administration of 1.0 cc normal saline (2.0 cc in total) in each nostril's olfactory cleft.
Day 2: each patient's olfaction was reevaluated before and after the administration of 1.0 cc in each nostril (2.0cc in total) of adrenaline (Epinefrine bitartate 1,8mg/ml equivalent to Epinefrine 1mg/ml (DEMO.GR 10027/03)). Scores were collected every 15 minutes for an hour in order to ensure that there were no alterations of their olfactory capability, to exclude any type of obstructive pathology that might affect the appropriate administration of the buffer solution into the olfcatory cleft. All thirty-one patients appeared to have considerably stable scores during both days' evaluations, and they were invited for the 3rd day.
Day 3: Each patient's olfactory function was evaluated again and compared with the previous days' scores. All scores were almost the same allowing us to ensure the reliability of our measurements.
Their olfactory acuity was then measured after the administration of a sodium citrate buffer solution in the nasal cleft. The buffer solution was composed of sodium citrate – citrate acid (3.5gr / 140ml, pH 7.4, osmolarity 298). The patients were instructed to indicate whether they experienced any alteration in their olfactory acuity which was evaluated every 15 minutes for a period of 1 hour or more frequently if we or the patient experienced any change.
The 12-item odor identification test of the "Sniffin' Sticks" [13] was used to determine olfactory capability.
In order to ensure that the nasal drops went mainly into the olfactory cleft, the patients were instructed to adopt the 'head down and forwards' position [14] for 1 minute. During the first 5 to 10 seconds of the one-minute period each solution was administered towards the olfactory cleft by the patient themselves with the use of a 2.5cc syringe
The patients were pre-informed that on all three days the same buffer solution would be administered into their nose with slight alterations in its osmolarity, to achieve the best possible results. No other details were provided.

Statistical analysis
Each day, before giving any nasal solution, the score of each patient was measured in advance. These scores were considered to be the baselines for the subsequent analysis.
Scores are expressed as median and range. Before and after administration of any substance, changes were evaluated by Wilcoxon signed ranks tests. All tests were performed at a= 0.05 significance level. Statistical analysis was performed based on the best values provided by the olfactory test results using the SPSS 10.0 for windows statistical package.

RESULTS

Thirty-one individuals (20 females and 11 males) volunteered to take part in our pilot study and completed the three days. Female scores were better than male scores, which corroborate previous observations by other authors [15, 16] indicating that female olfactory function as measured by identification testing is superior throughout life [17] Perhaps this is the reason why females more frequently seek medical advice regarding their dysfunction.
During the period of measuring patients' acuity we realized that the buffer solution needed a certain amount of time to take effect. Thus it was necessary to allow several minutes (median time period 266 minutes (range 15-50 minutes)) for the patients to achieve maximum scores. All these scores once they reached their highest value were stable throughout the rest of the study period as they were evaluated again at the end of one hour.
Thirty patients (96.7%) improved their scores during a period of less than an hour. Furthermore, 23 of them (74.2%) experienced an improvement in their own perceived sense of smell. More specifically, 8 patients (25.8%) felt there was no improvement after the buffer administration, 5 (16.1%) patients observed a minimal improvement, 15 (48.4%) noticed a substantial improvement while 3 (9.7%) patients felt there was a significant improvement of their sense of smell as Table 4 illustrates.

Statistical analysis revealed that median patients' score before administration of normal saline (day 1) was 7 (0-9). After administration of normal saline 3 patients had a higher score, 1

Table 4. Effect of normal serum, adrenaline and treatment, standard scale.

	Median (range)	p-values
Before (day 1)	7 (0-9)	0.317
Normal serum	7 (0-9)	
Before (day 2)	7 (0-9)	1
Adrenaline	7 (0-9)	
Before (day 3)	7 (0-9)	<0.0001
Treatment	11 (0-12)	

p-values from the corresponding wilcoxon signed rank.

patient had a lower score and 27 patients had a stable score (7 (0-9)). On day 2, before administration of adrenaline, median patients' score was 7 (0-9). After administration of adrenaline 3 patients had a higher score, 3 patients had a lower score and 25 patients had a stable score (7 (0-9)). Before administration of the buffer solution (day 3), median patients' score was 7 (0-9). After administration of the solution 30 patients had a higher score, and 1 patient had a stable score (11 (0-12)). This particular patient was the only one who suffered from a complete loss of smell, providing us with no answers at all, before and after the administration of each solution.

No differences were observed between patients' scores before and after the administration of normal saline and adrenaline (p-values of 0.319 and 1, respectively). On the other hand, patients' scores after administration of the buffer solution differ highly from the baseline patients' score (p-value$<$0.0001) (Table 5).

All patients were requested to contact the investigators one month later in order to be reevaluated. Twenty seven patients (including the only patient who had a stable score before and after the administration of the buffer solution), actually visited us after a period of time ranging from 29 to 33 days. Their olfactory acuity was measured again and found to match the pre-testing levels. Four patients informed us by phone that their subjective improvement of sense of smell lasted for a period of 8 to 48 hours though obviously this was not validated by any olfactory testing. The median time period for the rest of the patients' subjective sense of smell improvement was 3 hours, ranging from 0 to 336 hours.

No severe side effects were reported. Eleven patients observed no side effects at all (35.5%), 1 patient reported hyperosmia (3.2%), 14 patients (45.2%) reported light itching, 1 patient (3.2%) mentioned itching and nasal blockage for a period of 20 minutes, 2 patients (6.4%) felt itching followed by nasal secretions for a period of half an hour and finally 2 patients (6.4%) reported nasal secretions only for the same period of time (Figure 1).

Table 5. Scores before and after the administration of normal saline, adrenaline and buffer solution.

	Median score (range)	p-values
Before (day 1)	7 (0-9)	0.317
Administration of Normal saline	7 (0-9)	
Before (day 2)	7 (0-9)	1
Administration of Adrenaline	7 (0-9)	
Before (day 3)	7 (0-9)	$<$0.0001
Administration of Buffer solution	11 (0-12)	

DISCUSSION

"Sniffin' Sticks" is an olfactory test based on pen-like dispensing devices. Although the results of this test are limited regarding accurate estimation of olfactory sensitivity [18], they are capable of providing us with the basis for the routine clinical screening for the olfactory dysfunction [19, 20].

The patients were pre-informed that on all three days the same buffer solution would be administered into their nose with slight alterations in its osmolarity, to achieve the best possible results. Although this introduces significant bias, statistically significant improvement occurred only after the administration of the buffer solution itself. It is also clearly understood that the buffer solution caused an indefinable improvement in the sense of smell (as measured with the use of "Sniffin' Sticks"), particularly in those patients who provided us with answers indicating that there is a hypofunction rather than a complete inactivation regarding olfactory receptor neurons.

Twenty-six patients had experienced conditions mainly associated with smell deficiency, such as head injury, nasal surgery, or an upper respiratory infection before the onset of their olfactory dysfunction, known causes of olfactory loss [21, 22].

We asked all subjects to contact us regarding the duration of

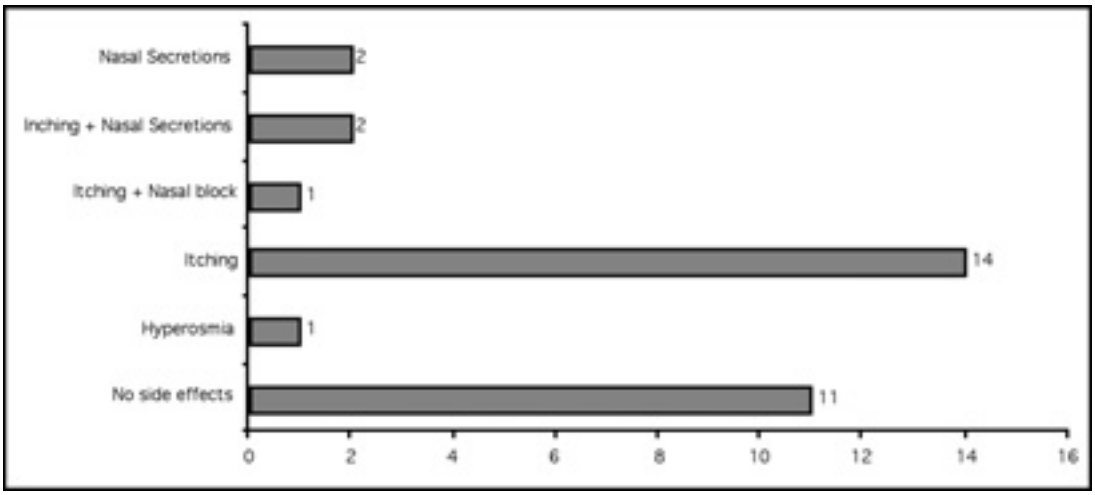

Figure 1. Side effects as reported by the patients themselves, after the administration of buffer solution.

the subjective improvement in their sense of smell and one of our patients surprisingly informed us that the improvement in his sense of smell lasted for a period of two weeks.

We strongly believe that small changes in mucosal Ca++ can significantly change the sensitivity of CNG channels and thus the excitability of the receptor neurons in vivo. Simply by diminishing mucosal Ca++ with the help of a sodium citrate - sodium acid buffer solution we actually managed to improve hyposmia, albeit temporarily. There are plenty of issues to be answered and of course we know that our selection of patients is particularly limited for the time being. However these results strongly indicate that excess in mucosal Ca++ is indefinably related with a diminished sense of smell and perhaps an alternative way of treating hyposmia may in the future include medically induced alterations in the mucosal Ca++.

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Dr Georgios Panagiotopoulos
Makrigianni 52 P.C.
15344 Gerakas, Athens
Greece

Tel: +30-69-4466-8844
E-mail: gporl@hotmail.com