ORIGINAL ARTICLE

cAMP and cGMP in nasal mucus: relationships to taste and smell dysfunction, gender and age

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Abstract

Purpose: To evaluate the presence and concentration of cAMP and cGMP in human nasal mucus in normal volunteers, to relate these findings to age and gender, and to compare normal levels with those in patients with taste and smell dysfunction.

Methods: Nasal mucus was collected over one to four days in 66 normal subjects and 203 patients with smell loss (hyposmia). Samples were centrifuged at 20,000 rpm, the supernatant removed and analyzed for cAMP and cGMP by using a 96 plate technique with a specific spectrophotometric colorimetric ELISA assay.

Results: Both cAMP and cGMP were present in human nasal mucus with both cAMP and cGMP significantly higher in normal women than in normal men [men vs. women; cAMP, 0.23 ± 0.002 vs. 0.34 ± 0.05 ($P < 0.05$); cGMP, 0.28 ± 0.03 vs. 0.63 ± 0.12 ($P < 0.01$)]. Both cAMP and cGMP changed with age; both moieties increased in a U shaped, parabolic pattern reaching a peak at age 41-50 with cAMP diminishing thereafter and then increasing to its highest level over age 70. Both cAMP and cGMP were lower in patients with taste and smell dysfunction than in normal subjects [normals vs. patients; cAMP, 0.31 ± 0.05 vs. 0.15 ± 0.02 ($P < 0.01$); cGMP, 0.56 ± 0.07 vs. 0.025 ± 0.02 ($P < 0.001$)] suggesting a relationship to olfactory pathology.

Conclusions: This is the first definitive study to demonstrate the presence of these cyclic nucleotides in nasal mucus and the first to reveal decreased levels in patients with impaired taste and smell function. Since olfactory receptor

sensitivity decreases with age increased nasal mucus cAMP over age 70 may appear incongruous but suggests one role of cAMP in olfactory function may relate to feedback mechanism(s) whereby its increase over age 70 yr reflects a physiological attempt to enhance diminishing olfactory function through growth and development of olfactory receptor activity.

It is well known that both cAMP and cGMP are present in human parotid saliva¹⁻³, that levels of both moieties change with various physiological conditions^{4, 5} and with some pathological states.⁶⁻⁸ Previous investigators also established roles for both of these moieties in taste^{9,10} and smell^{11,12} function. We demonstrated that saliva levels of both moieties were lower than normal in patients with taste and smell dysfunction.¹³ These results corroborated a role for these moieties in taste and smell function since these lower than normal levels were associated with taste and smell pathology¹⁴; treatment with phosphodiesterase (PDE) inhibitors, which biochemically enhanced levels of these moieties, improved taste and smell function among these patient.^{15,16}

We also demonstrated in preliminary studies that both moieties were present in human nasal mucus.¹⁷

Since previous investigators established roles for these moieties in both taste^{9,10} and smell^{11,12} function their presence in nasal mucus was understandable. Our previous results in saliva led to the hypothesis that patients with smell loss (hyposmia) might also exhibit lower than normal levels of cAMP and cGMP in nasal mucus since most moieties present in saliva are also present in nasal mucus¹⁸ albeit their concentrations may vary considerably. This hypothesis suggested that a more extensive study of these cyclic nucleotides in nasal mucus was indicated. In order to test this hypothesis we measured cAMP and cGMP in nasal mucus of both normal subjects and of patients with taste and smell function. We also studied several physiological parameters of these moieties including changes within sex and age. The results indicated that both of these moieties were present in human nasal mucus, that there were differences with respect to gender and that there was a specific pattern of change with age.

Methods

Studies were performed at The Taste and Smell Clinic in Washington, DC between June 2004 and November 2006 and constitute studies on consecutive normal subjects and patients with taste and smell dysfunction. Studies were performed consistent with a protocol approved by the IRB of the Georgetown University Medical Center and all subjects agreed to participate in the study consistent with the requirements of this protocol.

Nasal mucus was collected from 66 normal volunteers, aged 16-79 yr [(53±2) mean±SEM], 40 men, aged 19-74 yr (54±3) and 26 women, aged 16-79 yr (53±2). All normal subjects were in good health without acute or chronic disease and were not taking any medication at time of study. Taste and smell function was within normal limits in each subject.

Nasal mucus was also collected from 203 patients, aged 18-86yr (55±1). Patients were 86 men, aged 23-86 yr (54±2) and 117 women, aged 18-85 yr (55±2). Patients were consecutive subjects who presented to

The Taste and Smell Clinic, Washington, DC for evaluation and treatment of taste and smell dysfunction of various etiologies. Dysfunction included those who developed abnormalities following an influenza-like illness (97 patients, age 53±2 yr, 63 women, 34 men), post allergic rhinitis (42 patients, age 56±2 yr, 15 women, 27 men), post head injury (26 patients, age 51±6 yr, 15 women, 11 men) and associated with a variety of other etiologies of sensory dysfunction (38 patients, age 58±7 yr, 24 women, 14 men).

Smell loss was determined in each patient by psychophysical measurements by use of a standard three stimuli, forced choice, staircase, nasal sniff technique in a fixed, controlled design previously described^{15,19}; techniques and results of these methods were documented in a double blind clinical trial.²⁰ Four odors were used: pyridine (dead fish-like), nitrobenzene (almond extract), thiophene (petroleum) and amyl acetate (banana oil). These techniques resulted in determination of detection and recognition thresholds and magnitude estimation values for these four odors

Nasal mucus was collected in 50ml plastic tubes using spontaneous nasal discharge over one-four days. No blood was present in any sample. Amounts collected varied from 1-40 ml. In the laboratory, mucus was centrifuged at 20,000 rpm in a Sorvall RC5 plus refrigerated centrifuge for 20-50 min, the supernatant transferred to 500 µl plastic tubes and stored at -20°C until assayed.

cAMP and cGMP were determined by a 96 plate spectrophotometric colorimetric assay using kits supplied by R&D Systems (Minneapolis, MN) by methods previously described.^{20,21} Protein was determined by measurement of Δ215 (absorbance at 215-225nm) with use of an extinction coefficient as previously described.²³ Results were expressed as pmol/ml nasal mucus and pmol/mg protein.

Reliability of assay techniques was previously reported.^{21, 22} Reliability of present results were also determined in several studies. To determine methodological reliability cAMP and cGMP were determined

TABLE 1. cAMP and cGMP concentrations in nasal mucus in normal subjects

	cAMP		cGMP		Total protein mg/dl	Age y
	pmol/ml	pmol/mg protein	pmol/ml	pmol/mg protein		
Normals						
Total (66)	0.31±0.05*	0.12±0.04	0.56±0.07 ^{c1}	0.19±0.03	3.25±0.20	49± 7
Men (40)	0.23±0.002 ^d	0.07±0.04	0.28±0.03 ^c	0.09±0.03 ^{c1}	3.28±0.11	42±18
Women (26)	0.34±0.05	0.14±0.06	0.63±0.12	0.20±0.04	3.15±0.43	49± 9

() subject number

* Mean ± SEM

With respect to women: ^a $P<0.001$, ^c $P<0.01$, ^d $P<0.05$ With respect to cAMP: ^{c1} $P<0.01$

TABLE 1A. Range of cAMP and cGMP concentrations in nasal mucus in normal subjects and patients with hyposmia

	cAMP		cGMP		Total protein mg/dl	AGE y
	pmol/ml	pmol/mg protein	pmol/ml	pmol/mg protein		
Normals						
Total	0.02 – 1.33	0.01 – 0.40	0.10 – 1.25	0.02 – 0.90	2.00 – 4.98	16 – 79
Men	0.02 – 1.20	0.01 – 0.21	0.20 – 1.13	0.02 – 0.90	2.26 – 4.98	
Women	0.02 – 1.33	0.01 – 0.40	0.10 – 1.25	0.03 – 0.60	2.00 – 3.35	
Patients						
Total	2.00 – 3.35	0.00 – 0.27	0.01 – 0.89	0.00 – 0.57	1.67 – 4.34	18 – 86
Men	0.00 – 0.80	0.00 – 0.12	0.00 – 0.89	0.00 – 0.31	1.82–4.34	
Women	0.00 – 0.69	0.00 – 0.27	0.00 – 0.55	0.00 – 0.57	1.67 – 3.92	

in several ways. Duplicates of 12 nasal mucus samples were determined on 15 occasions; standard deviation of these samples varied from 0.007-0.038 for cAMP and 0.005-0.018 for cGMP respectively; mean coefficients of variation varied from 1-10% for both moieties. cAMP and cGMP from one subject were determined on 17 separate occasions over a period of three years; the standard deviation for these determinations for cAMP (pmol/ml) was 0.29 with a mean coefficient of variation of 3%; for cAMP/ (pmol/mg protein), 0.13 with a mean coefficient of variation of 4%; for cGMP (pmol/ml), 0.02 with a coefficient of variation of 4%; for cGMP (pmol/mg protein) 0.007 with a coefficient of variation of 5%.

Mean±SEM of results were obtained for normal subjects and patients for both men and women and for age. Significance of differences were determined by Student t test.

Results

Nasal mucus cAMP and cGMP in normal subjects

cAMP and cGMP are both found in nasal mucus in normal subjects (Table 1). Mean levels of cGMP were higher than that of cAMP. This result differs from findings in saliva in which cGMP was lower than cAMP in both normal subjects and in patients with taste and smell dysfunction.¹³ Mean levels of both cAMP and cGMP were significantly higher in women than in men (Table 1). There were no differences in total protein or in age between men and women (Table 1).

Nasal mucus cAMP and cGMP in patients with taste and smell dysfunction compared with normal subjects

cAMP and cGMP are both found in nasal mucus from patients and were significantly lower than in normal subjects (Table 2). As in normal subjects, mean levels

TABLE 2. cAMP and cGMP concentrations in nasal mucus in normal subjects and in patients with hyposmia.

	cAMP		cGMP		Total protein mg/dl	Age y
	pmol/ml	pmol/mg protein	pmol/ml	pmol/mg protein		
Patients (203)	0.15±0.02*, e, b ¹	0.06±0.02	0.25±0.02 ^a	0.10±0.01 ^d	2.56±0.12 ^e	54±2
Normals (66)	0.31±0.05 ^{c1}	0.12±0.04	0.56±0.07	0.19±0.03	3.25±0.20	49±7

() subject number

* Mean ± SEM

With respect to normals: ^a $P<0.001$, ^b $P<0.005$, ^d $P<0.05$, ^e $P<0.01$ With respect to cGMP: ^{b1} $P<0.005$, ^{c1} $P<0.01$

TABLE 3. cAMP and cGMP in nasal mucus in normal men and women and men and women with hyposmia.

	cAMP		cGMP		Total protein pmol/ml	Age y
	pmol/ml	pmol/mg protein	pmol/ml	pmol/mg protein		
Patients						
Total (203)	0.15±0.02*, b	0.06±0.02	0.25±0.02 ^d	0.10±0.01 ^d	2.56±0.12 ^e	54±2
Men (86)	0.14±0.04 ^{d1}	0.05±0.03	0.28±0.03	0.10±0.02	2.77±0.15 ^{d1}	56±2
Women (117)	0.16±0.05 ^{e2}	0.06±0.02	0.23±0.02 ^{b2}	0.09±0.01 ^a	2.60±0.08	54±2
Normals						
Total (66)	0.31±0.05	0.12±0.04	0.56±0.07	0.19±0.03	3.25±0.20	49± 7
Men (40)	0.23±0.002	0.08±0.04	0.28±0.03	0.09±0.03	3.28±0.11	42±18
Women (26)	0.34±0.05	0.14±0.06	0.63±0.12	0.20±0.04	3.15±0.43	49± 9

() subject number

* Mean ± SEM

With respect to normals: ^a $P<0.001$, ^b $P<0.005$, ^d $P<0.05$, ^e $P<0.01$ With respect to normal men: ^{d1} $P<0.05$, ^{e1} $P<0.01$ With respect to normal women: ^{a2} $P<0.001$, ^{b2} $P<0.005$, ^{e2} $P<0.01$

of cGMP in patients were less (by 20%) than mean levels of cAMP (Table 2). Nasal mucus protein in patients was less (by 19%) than in normal subjects (Table 2). These latter results influenced all calculations of cAMP and cGMP expressed per mg protein. There were no age differences between the two groups (Table 2).

Categorized by sex, both men and women patients exhibited lower levels of both nasal mucus cAMP and cGMP than did normal men and women; differences were significant only when comparing women patients with normal women (Table 3). Mean cAMP in nasal mucus from men patients was less than in normal men (by 33%). Mean cAMP levels were also less in women patients than in normal women (by 43%) (Table 3). cGMP levels in men patients were higher than in women patients but were lower when expressed as pmol/ml protein, as in normal men and women (Table 3). Levels of nasal mucus cGMP were significantly lower in women patients than in normal women. Mean protein levels in both men and women

patients were less than in normal men and women but differences were significant only when comparing normal men with men patients (Table 3).

Nasal mucus cAMP and cGMP with respect to age

Patterns of change in both cAMP and cGMP with age (Fig. 1, 2) were similar to patterns previously observed in saliva.¹³ For cAMP (expressed per ml nasal mucus) there was an U shaped, parabolic function with age increasing up to age 50, then decreasing until age >70 yr (Fig.1); at age >70 yr cAMP increased dramatically to levels higher than among all younger patients (Fig. 1). Expressed per mg protein these changes were also observed but were much less robust than expressed per ml nasal mucus. For cGMP expressed per ml nasal mucus a pattern similar to that observed with cAMP through age 51-60 yr was demonstrated but after this period little change was observed (Fig. 2). Expressed per mg protein there was very little change in cGMP with age (Fig. 2). A prob-

FIGURE 1. Nasal mucus cAMP with respect to age. Hatched bars reflect cAMP, in pmol/ml nasal mucus, with lines indicating ± 1 SEM. Speckled bars reflect cAMP/protein, pmol/mg protein, with lines indicating ± 1 SEM.

FIGURE 2. Nasal mucus cGMP with respect to age. Hatched bars reflect cGMP, in pmol/ml nasal mucus, with lines indicating ± 1 SEM. Speckled bars reflect cGMP/protein, in pmol/mg protein, with lines indicating ± 1 SEM.

lem with interpreting these latter data (expressed per mg protein) was the consistent decrease in nasal mucus protein as patients aged (Table 4).

Discussion

These results indicate that both cAMP and cGMP are present in human nasal mucus and that women have higher levels of both moieties than do men. These latter results are consistent with the well known observation that women have higher olfactory sensitivity compared to men.^{24,25}

These results also indicate that patients with taste and smell dysfunction who exhibit hyposmia also exhibit decreased levels of cAMP and cGMP in nasal

mucus compared to normal subjects. Since nasal mucus is the physiological medium which bathes the olfactory epithelium²⁶, since both moieties play critical roles in both taste^{9,10} and smell^{11,12} function and in several aspects of neural function^{27,28}, including acting as putative growth factors in both gustatory^{9,10} and in olfactory systems^{15,29} and in neural development in several systems³⁰⁻³², these findings suggest that some physiological mechanism(s) related to function of these moieties and perhaps others moieties as well³³ relate to loss of olfactory acuity. This suggestion is confirmed by results in which treatment of these patients with PDE inhibitors increased these moieties in nasal mucus^{19,34} and also improved their smell function.^{14,16,19}

Changes in both cAMP and cGMP with age suggest the presence of a specialized signaling system related to taste and olfactory function.^{25,26,35} Both cAMP and cGMP secretion in nasal mucus mirror age related changes in the olfactory system up to age 50 yr. Until age 50, smell acuity increases^{36,37} as do nasal mucus cAMP and cGMP [35, Figs. 1, 2]. After age 50 several aspects of smell acuity decrease^{36,37} as do levels of both of these moieties [35, Figs. 1, 2]. Previous anatomical studies^{38,39} supported by recent functional brain magnetic imaging (fMRI) studies of odor identi-

TABLE 4. Nasal mucus protein with respect to age in patients with taste and smell loss

Age yr	Age mean	Prot mg/dl
21-30 (13)	27 $\pm$ 1	3.01 $\pm$ 0.04
31-40 (38)	35 $\pm$ 1	2.79 $\pm$ 0.27
41-50 (39)	45 $\pm$ 1	2.73 $\pm$ 0.17
51-60 (41)	56 $\pm$ 1	2.41 $\pm$ 0.13 ^a
61-70 (39)	66 $\pm$ 1	2.40 $\pm$ 0.15 ^a
71-80 (33)	78 $\pm$ 1	2.42 $\pm$ 0.13 ^a

() subject number;

* Mean $\pm$ SEM;

With respect to age 21-30: ^a $P < 0.001$

fication⁴⁰ demonstrated atrophy of olfactory neural structures^{38,39} and decreased fMRI activation⁴⁰ in older compared with younger subjects. These changes are consistent with decreased olfactory acuity after age 50 yr with decreased smell function consistent with decreased olfactory receptor function.⁴¹ However, the dramatic increase in cAMP over 70 yr is inconsistent with this knowledge and suggests other roles for this moiety in olfactory function. Several hypotheses for changes in nasal mucus cAMP with age are possible. cAMP may be a growth factor involved with olfactory receptor development^{17, 42}; increases after age 70 yr may reflect an olfactory-receptor feedback mechanism whereby, as olfactory receptor number diminishes with age, nasal mucus cAMP secretion increases reflecting an attempt to increase diminished receptor activity in an effort to maintain homeostasis in this critically important sensory system. Since cAMP plays a role in olfactory signaling its increase after age 70 may reflect a role in direct or indirect signaling to increase neural or receptor activity to enhance system function. Other roles related to age related changes in neural transduction or secretion of apoptotic factors such as TNF α and/or TRAIL⁴³ may also be involved. Preliminary studies of changes in nasal mucus TNF α with age demonstrate a similar U shaped pattern to that of cAMP with age but without this dramatic increase after age 70 yr (unpublished observations, Velicu, I., Schmidt, L., Henkin, R.I., 2007).

Treatment of patients with the PDE inhibitor theophylline improved smell function in patients with hyposmia¹⁵⁻¹⁷ presumably by increasing nasal mucus cAMP. Similar improvement in smell function has also been demonstrated using the specific PDE 3 inhibitor cilostazol (unpublished data, RI Henkin, I Velicu, 2006). These findings continue to define the roles that cAMP (and perhaps cGMP) play in smell function although mechanism(s) of these roles is presently unclear.

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