Rhinology, 42, 63-67, 2004

Increased nasal patency caused by smoking and contraction of isolated human nasal mucosa*

Yoh-ichiro Maeda[1], Wataru Okita[2], Keiichi Ichimura[3]

[1] Department of Otolaryngology-Head and Neck Surgery, Toshiba Hospital
[2] Department of Otolaryngology-Head and Neck Surgery Niigatarosai Hospital
[3] Department of Otolaryngology-Head and Neck Surgery, Jichi Medical School

SUMMARY

In this study, we investigated the effects of smoking and nicotine, an important constituent of cigarettes, on the nasal patency using acoustic rhinometry (AR) and an in vitro bioassay technique. In the AR study, the nasal cavity volume of volunteers classified into two groups, smoking and nicotine chewing gum groups, was measured. The nasal cavity volumes immediately after smoking and 5 minutes after smoking significantly increased compared with that before smoking ($P<0.05$), whereas the nasal cavity volume after chewing a nicotine gum was unchanged compared with that before chewing the gum. An in vitro study showed significant nicotine-induced contraction of the human nasal mucosa (50.2 ± 14.0% noradrenaline-induced contraction: n=10). The threshold nicotine level that can induce human nasal mucosa contraction was 3.0 x 10^{-7} M. Prazosin (10^{-6}M) inhibited nicotine-induced contraction incompletely (20.5 ± 7.5% of noradrenaline-induced contraction n=5). These results indicate that smoking increases nasal patency and that nicotine induces contraction of the human nasal mucosa. The nicotine-induced contraction is likely mediated, at least in part by alpha1-adrenoceptors.

Key words: acoustic rhinometry, in vitro bioassay, smoking, nasal mucosa, nicotine

INTRODUCTION

Some smokers often feel the increase in nasal patency while smoking. The mechanism underlying this experience has not sufficiently been clarified because few reports have addressed the effect of smoking on the human nasal mucosa. However, several changes in cerebral circulation, cardiac muscle tension and other systemic vessels after smoking have already been reported (Ball and Turner, 1974; Toda, 1975; Toda and Okamura, 1990; De Sousa et al., 1991). Nasal patency is mainly due to the smooth muscle tension around the nasal vasculature. Considering previous reports, the feeling of increased nasal patency seems to originate from the nasal mucosal contraction that is due to the smooth muscle contraction of nasal blood vessels (Cauna and Cauna, 1975).

In this study, the effects of smoking and nicotine, an important constituent of cigarettes, on the nasal mucosa were investigated using two methods. An *in vivo* method is acoustic rhinometry (AR) while the other method is the *in vitro* bioassay technique, which was performed using isolated human nasal mucosa preparations.

AR is an apparatus for assessing the geometry of the nasal cavity (Hilberg et al., 1989). After the invention of AR, the nasal cavity volume as an index of nasal patency can be easily evaluated. This method, which is based on sound reflection analysis, provides an estimate of the cross-sectional area and the volume of the nasal cavity as a function of the distance from the nostril. Using AR, the response after administration of some neurotransmitters to the human nasal cavity *in vivo* could be easily investigated (Nakamoto et al., 1997; Silkoff et al., 1999). In the AR study, the volume changes of the nasal cavity caused by cigarette smoking and chewing a nicotine gum, named Nicorette®, were measured respectively. Nicorette® was invented as a smoking cessation aid in Sweden (Pharmacia & Upjohn) in 1978. It includes 2.0 mg nicotine ((S)-3-(1-methyl-2-pyrrolidinyl) pyridine) bound to ion-exchange resin and emits nicotine that is gradually absorbed into the intraoral mucosa by chewing. The low peak concentration of nicotine in the serum and the serum concentration-time curve both suggested the efficacy of the smoking cessation aid curing for cigarette addiction. Approximately 1 mg of nicotine is dissolved within 30 minutes of chewing (MacNabb et al., 1982; Benowitz et al., 1987). There has been some debate about how nicotine behaves, particularly at the nicotinic cholinoreceptor and sympathetic nerve terminal levels in human organs. In this study, the human nasal mucosa obtained by resection during nasal surgery was used for the investigation of nicotinic vasoactivity on the human nasal mucosa.

*Received for publication: October 29, 2003; accepted: February 12, 2004

MATERIALS AND METHODS

Acoustic rhinometry (AR)

Subjects

All volunteers, who were informed of the aim and side effects of this experiment and consented, were free from nasal symptoms and diseases. As far as we checked the nasal cavity by anterior rhinoscopy before this measurement, there were no abnormal findings in all volunteers. The two groups of volunteers were the cigarette-smoking group (smoking group), 8 men whose age ranged between 24-35, and the nicotine-gum-chewing group (chewing-gum group), 10 men whose ages ranged between 24-38. The men in the smoking group smoked more than 20 cigarettes a day and had a smoking history of more than four years.

Methods and statistical analysis

The nasal volume of the smokers was measured using AR (model A1-Acoustic Rhinometer; G.M. Instruments Co., UK). Measurement of the nasal cavity volume by AR was carried out in a soundproof room. The temperature and humidity were 18-22°C and 40-70%, respectively. The nasal cavity volume of these subjects was measured as follows:

1. Smoking group: before smoking and every 5 minutes after smoking; smoking period was 5 minutes. The cigarette used contained 0.6 mg of nicotine.
2. Gum-chewing group: before chewing Nicorette® (nicotine gum) and every 5 minutes after chewing it; chewing period was 30 minutes. Nicorette® contained 2.0 mg of nicotine.

The volume of each nasal cavity was measured three times by AR, and the median values were adopted.

Experimental data were compared between the values before smoking and every 5 minutes after smoking. The collected data about the change in nasal volume was analyzed using a paired *t*-test. A probability of less than 0.05 was considered significant.

In vitro bioassay technique

Materials

The human nasal mucosa that had been obtained by resection during nasal surgery was used for this experiment with the patients' consent. The nasal mucosa was resected from the inferior turbinate because of hypertrophic rhinitis that is the characteristic feature of nasal mucosa hypertrophy and causes nasal obstruction in the patients. There were no abnormal pathological findings in each specimen. More than twenty specimens of the human nasal mucosa were obtained by this method. Each specimen was stored in an aerated Krebs' bicarbonate solution bottle in ice water immediately after resection.

The drugs used are as follows: (-)-noradrenaline bitartrate (Sigma, St. Louis, USA), (-)-nicotine: (-)-methyl-2- (3-pyridyl) pyrrolidine (Tokyokasei-Kogyo Co., Tokyo, Japan) and prazosin hydrochloride (Sigma, St. Louis, USA)

Preparation

The specimens were cut into pieces of approximately 5x15 mm at room temperature. A strip of mucosa was suspended in a muscle organ bath containing 10 ml of Krebs` bicarbonate solution ((in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 10.0). The strip was fixed at the lower end of the bath. The upper end was attached to a strain gauge transducer (model UL-10GR; Minebea Co., Tokyo) under a tension of 0.5 g at a room temperature of 18-20°C. The bath solution was constantly sparged with 95% O_2 and 5% CO_2.

Recording of responses

This *in vitro* method for detecting changes in the muscle tension was based on previous reports (Jackson, 1979; Ichimura and Jackson, 1984). The changes in tension were recorded isometically using a pen-writing recorder (model NEC Sanei Instruments, Tokyo) of an oscillograph (model DSA601B Minebea Co., Tokyo). Before the start of experiments, the strips were allowed to equilibrate for about 40 minutes. During this period, the bath medium was replaced at approximately 15-minute intervals.

Experiments with nicotine

First, the threshold of nicotine level that can induce contraction of the human nasal mucosa was measured by applying nicotine cumulatively (n=5).

Second, noradrenaline (10^{-5}M) was added to the organ bath and noradrenaline-induced contraction of human nasal mucosa was measured. After the measurement, the preparations were washed with fresh Krebs' bicarbonate solution more than four times and equilibrated for about 30 minutes. Nicotine (10^{-5}M) was added to the organ bath containing the same specimen and nicotine-induced contraction of the human nasal mucosa was measured. The maximum contraction of the human nasal mucosa induced by nicotine (10^{-5}M) was compared with that induced by noradrenaline (10^{-5}M)(=100%).

Third, noradrenaline-induced contraction (10^{-5}M) was measured and prazosin (10^{-6}M) was added after washing the preparation and equilibration. Twenty minutes after the administration of prazosin, nicotine (10^{-5}M) was added and contraction was measured. The nicotine-induced contraction (10^{-5}M) in the presence of prazosin (10^{-6}M) was also compared with noradrenaline-induced contraction (10^{-5}M) (n=5).

RESULTS

Acoustic rhinometry

The time course of the changes in the volume of each nasal cavity in the smoking group is shown in Figure 1. The values immediately after smoking increased compared with those before smoking. Statistical analysis of the values before smoking and the every 5 minutes after smoking was performed. Significant differences were present between the values before smoking and 0 or 5 minutes after smoking (immediately after

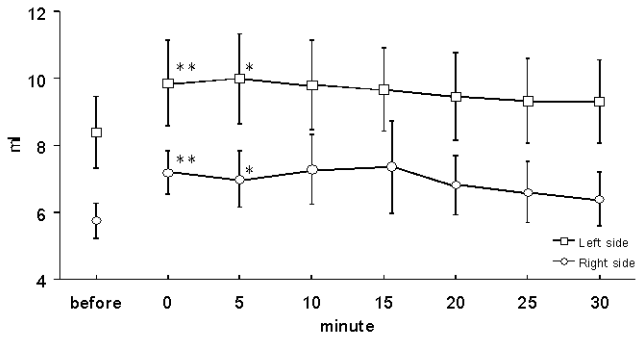

Figure 1. Time courses of changes in nasal volume after smoking. Difference in p values before and after smoking indicates as follows: * $p<0.05$, ** $p<0.01$ (paired *t*-test).

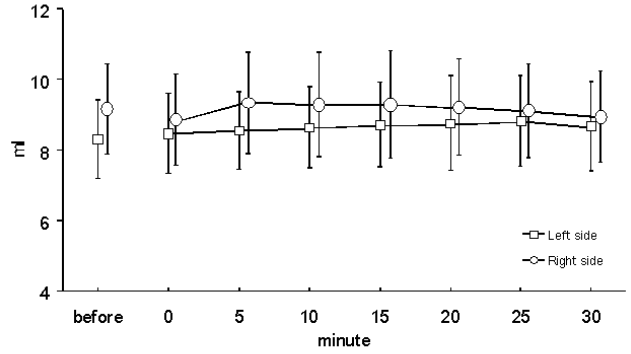

Figure 2. Time courses of changes in nasal volume after chewing nicotine gum. There is no difference in p values before and after chewingum.

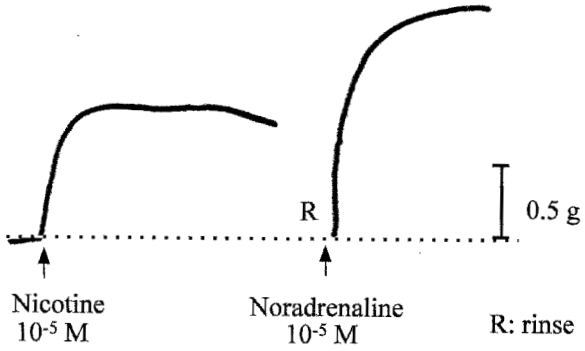

Figure 3. Nicotine-induced contraction of the human nasal mucosa.

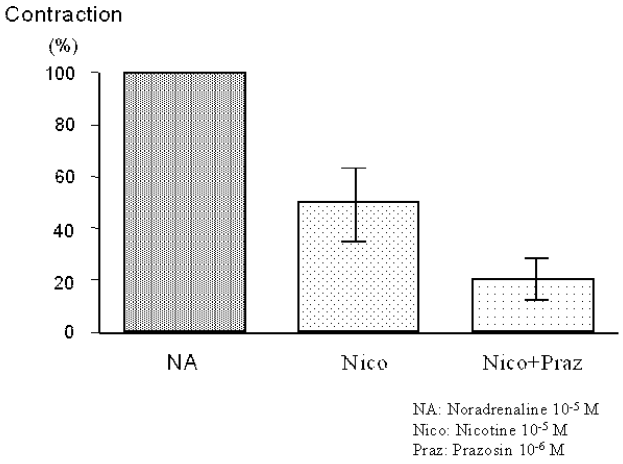

Figure 4. Effect of prazosin on the nicotine-induced contraction of the human nasal mucosa.

smoking: right $p<0.01$, left $p<0.01$; 5 minutes after smoking: right $p<0.05$, left $p<0.05$). This result indicates the increased nasal cavity volume caused by smoking.

The time course of the changes in nasal cavity volume in the gum-chewing group is shown in Figure 2. No significant change was observed in the gum-chewing group. The nasal cavity volume before chewing nicotine gum was compared with those every 5 minutes after gum-chewing. This indicates that the action of chewing nicotine gum had no effect on the human nasal patency.

In vitro bioassay experiment

The threshold concentration of nicotine for inducing nasal mucosal contraction was 3.0×10^{-7}M.

The contractile response of the human nasal mucosa to nicotine (10^{-5}M) is shown in Figure 3. The nasal mucosa contracted rapidly in response to nicotine and relaxed immediately after reaching the peak. Nicotine-induced contraction was expressed as a percentage of the contraction caused by noradrenaline (10^{-5}M), the average contraction being 50.2 ± 14.0% (SD), n=10. The values ranged from 23.1 to 68.4%. The average nicotine-induced contraction after administration of prazosin was 20.5 ± 7.5% (SD), n=5 (Figure 4). The values ranged from 0 to 27.3%.

DISCUSSION

This study demonstrated that the action of smoking led to an increased nasal patency, that is, the contraction of human nasal mucosa. This result is significant for smokers with nasal obstruction. The pharmacological change of the human nasal mucosa caused by smoking is interesting.

There are some problems in clarifying the mechanism of the nicotine-induced nasal mucosal contraction. First, it was hypothesized that stimulation of chemosensitive C-fiber afferents in the nasal mucosa results in local vasodilation and increased vascular permeability to macromolecules (Lundblad et al., 1982). After more detailed investigation, it became clear that the smoking-induced edema in the rat nasal mucosa was not caused by nicotine but by vapour-phase irritants, which activated capsaicin-sensitive C-fiber afferents (Lundberg et al., 1984; Stjärne et al., 1989). In our investigation, however, the

data supporting the reduction in nasal patency as a result of nasal mucosal edema after smoking had not been obtained. The reason for this discrepancy is as yet unknown.

Second, as particulate matters and the mainstream smoke of cigarettes contain a lot of chemical substances, it is difficult to specify the main substance that can considerably induce the contraction of the human nasal mucosa. Aside from nicotine, the total particulate matter in cigarette smoke contains carboxylic acids, aldehydes and ketones, alcohols and other compounds. Nicotine is the only one substance that has considerable possibility to induce nasal mucosal contraction. As the results of the *in vitro* bioassay experiment indicated, nicotine induced considerable contraction of the human nasal mucosa. The results of *in vitro* experiment support the phenomenon of increased nasal patency caused by smoking, specifically by nicotine.

Third, the nicotine-induced contractile response of the human nasal mucosa is probably mediated by noradrenaline. There are some reports, which indicated cardiovascular changes after smoking depended on changes in serum noradrenaline level (Westfall and Watts, 1964; Ball and Turner, 1974). The question remains which organ releases noradrenaline into the blood. Considering systemic changes after smoking, the original organ that releases a quantity of noradrenaline in the human blood serum seems to be the adrenal gland. However, some reports also indicated that nicotine causes the sympathetic nerve terminal to release noradrenaline at local organs (Cryer et al., 1976; Kirpekar et al., 1980; Wang et al., 2000). Others reported that the generation of an action potential in the postganglionic sympathetic nerve fibers is not the cause of noradrenaline release but a direct effect of nicotine (Loffelholz, 1970; Jayasundar and Vohra, 1978). The report indicating that the response of releasing noradrenaline changes depending on the concentration of nicotine is acceptable (Sarantos-Laska et al., 1981). A model of a resected adrenal gland is needed to solve this problem. Considering the *in vitro* result, it is reasonable to assume that noradrenaline released from the sympathetic nerve terminal in the nasal mucosa causes the local nasal mucosal contractile response.

The fourth problem is the chemical substances absorbed from the nasal mucosa after smoking. Absorption of nicotine from tobacco snuff through the nasal mucosa has been reported (Temple, 1976). This study proved that the ratio of metabolites in tobacco snuffers` urine is similar to that in smokers` urine. The potential capacity for chemical substance absorption from the nasal mucosa is quite excellent. The exposed surface of the nasal mucosa would easily absorb some kinds of chemical substances from the inspired gas. As the nasal mucosa is composed of erectile tissues, including numerous arterioles, venules and smooth muscles around it, some chemical substances affect these organs and eventually enter the circulation in nasal mucosa with ease. Therefore, substance in the nasal cavity can easily regulate the nasal mucosa contraction and circulation. In daily life, the sympathetic and parasympathetic nerves attached to smooth muscles are the most important factor in nasal patency. The chemical substances in the circulation also affect smooth muscle tones through the vessel walls. In this study, the absorbed nicotine in the nasal mucosa would directly influence smooth muscles or mediate the endothelium-through-nasal-mucosal circulation after absorption. From the results of the *in vitro* bioassay experiment, it is reasonable to conclude that the mucosal contraction after smoking is a localized action.

The prazosin administration study indicated that an alpha1-adrenergic antagonist reduced the nicotine-induced mucosal contraction. However, prazosin did not completely inhibit the nicotine-induced contractile response. This result suggests that the nicotine-induced mucosal contraction originates not only from alpha1-adrenoceptor stimulation but also from other receptor systems.

Although these experiments on the human nasal mucosa were limited in terms of the number of specimens and volunteers used, the results provide a clue for clarifying nicotine-induced human nasal mucosa contraction.

ACKNOWLEDGEMENTS

The authors thank Professor K. Kaga, N. Takeuchi and K. Ishio of the ENT Department, Tokyo University for extensive technical advice.

REFERENCE

1. Ball K, Turner R (1974) Smoking and the heart. the basis for action. Lancet 2: 822-826.
2. Benowitz NL, Jacob P, Savanapridi C (1987) Determinants of nicotine intake while chewing nicotine polacrilex gum. Clin Pharmacol Ther 41: 467-473.
3. Cauna N, Cauna D (1975) The fine structure and innervation of the cushion veins of the human nasal respiratory mucosa. Anat Rec 181: 1-16.
4. Cryer PE, Haymond MW, Santiago JV, Shah SD (1976) Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. N Engl J Med 295: 573-577.
5. De Sousa P, Cherian G, Thomas J, Thulesius O (1991) Coronary artery constriction is enhanced with nicotine and propranolol, particularly after endothelical damage. Clin Physiol 11: 143-152.
6. Hilberg O, Jackson AC, Swift DL, Pedersen OF (1989) Acoustic rhinometry, evaluation of nasal cavity geometry by acoustic reflection. J Appl Physiol 66: 295-303.
7. Ichimura K, Jackson RT (1984) Evidence of alpha2-adrenoceptors in the nasal blood vessels of the dog. Arch Otolaryngol 110: 647-651.
8. Jackson RT (1979) A new *in vitro* method of drug assay of nasal blood vessels. Arch Otolaryngol 225: 33-38.
9. Jayasundar S, Vohra MM (1978) Mechanism of nicotine-induced release of norepinephrine from adrenergic nerve endings: is generation and propagation of impulses necessary? Arch Int Pharmacodyn Ther 232:202-210.
10. Kirpekar SM, Gracia AG, Prat JC (1980) Action of nicotine on sympathetic nerve terminalis. J Pharmacol Exp Ther 213: 133-138.
11. Loffelholz K (1970) Nicotinic drugs and postganglionic sympathetic transmission. Naunyn-Schmiedergs Arch Pharmacol 267: 64-73.
12. Lundberg JM, Lundblad L, Saria A, Anggard A (1984) Inhibition of cigarette smoke-induced oedema in the nasal mucosa by capsaicin pretreatment and a substance P antagonist. Naunyn-Schmiedebergs Arch Pharmacol 326: 181-185.

13. Lundblad L, Anggard A, Lundberg JM (1982) Vasodilation in the cat nasal mucosa induced by antidromic trigeminal nerve stimulation. Acta Physiol Scand 115: 517-519.
14. MacNabb ME, Ebert RV, McCusker K (1982) Plasma nicotine level produced by chewing nicotine gum. JAMA 248: 865-868.
15. Nakamoto S, Sakakura Y, Pedersen OF, Ukai K (1997) Evaluation of nasal passage patency after antigen and histamine challenge in guinea pig by acoustic rhinometry. Am J Rhinol 11: 85-89.
16. Sarantos-Laska C, Mc-Culloch MW, Rand MJ, Story DF (1981) Nicotinic release of noradrenaline in the presence of tetrodotoxin from sympathetic nerve terminals in rabbit isolated atria. J Pharm Pharmacol 33: 315-316.
17. Silkoff PE, Roth Y, McClean P, Cole P, Chapnik J, Zamel N (1999) Nasal nitric oxide dose not control basal nasal patency or acute congestion following allergen challenge in allergic rhinitis. Ann Otol Rhinol Laryngol 108: 368-372.
18. Stjärne P, Lundblad L, Lundberg JM, Anggard A (1989) Capsaicin and nicotine-sensitive afferent neurones and nasal secretion in healthy human volunteers and in patients with vasomotor rhinitis. Br J Pharmacol 96: 693-701.
19. Temple DJ (1976) The absorption of nicotine from tobacco snuff through the nasal mucosa. Arch Pharm 309: 984-987.
20. Toda N. (1975) Nicotine-induced relaxation in isolated canine cerebral arteries. J Pharmacol Exp Ther 193: 376-384.
21. Toda N, Okamura T (1990) Modification by L-N^G-monomethyl arginine (L-NMMA) of the response to nerve stimulation in isolated dog mesenteric and cerebral arteries. Jpn J Pharmacol 52: 170-173.
22. Wang M, Okada S, Murakami Y, Yokotani K (2000) Nicotine-induced noradrenaline release from the isolated rat stomach by activation of L- and N-type calcium channels. Jpn J Pharmacol 83: 102-106.
23. Westfall TC, Watts DT (1964) Catecholamine excretion in smokers and non-smokers. J Appl Physiol 19: 40-42.

Yoh-ichiro Maeda
Department of Otolaryngology-Head and Neck Surgery
Toshiba hospital,
6-3-22 Higashiooi, Shinagawa-ku,
Tokyo 140-8522
Japan

Tel: +81-3-3764-0511
Fax: +81-3-3764-3415
E-mail: rhinology-tky@umin.ac.jp