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Central nicotinic stimulation reduces vascular conductance in the gingiva in anesthetized rats

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Objective: The central effects of nicotine on gingival blood flow and vascular conductance were investigated.

Background: Nicotine is absorbed in cigarette smoking, which is a risk factor for periodontal disease. Although studies have shown peripheral effects of nicotine on gingival blood flow and gingival vascular conductance, little is known about its central effects.

Methods: We used laser Doppler flow measurements to investigate the changes of gingival blood flow produced by 5 min intracerebroventricular (i.c.v.) injection of (–)-nicotine ditartrate in rats anesthetized by urethane and α-chloralose, along with occasional measurements of the blood pressure from the femoral artery.

Results: The i.c.v. injection of nicotine at 15, 50, and 150 μg reduced the gingival blood flow significantly, compared with saline. The maximal reduction was dose-dependent. Next, we measured the blood pressure and gingival blood flow in the i.c.v. injection of nicotine at 50 μg, to calculate the gingival vascular conductance. The blood pressure was reduced, along with the change of gingival blood flow immediately after the injection, whereas the gingival vascular conductance fell with a time-lag.

Conclusion: These results suggest that the reduction of gingival blood flow by central nicotinic stimulation is accompanied in part by a change of vascular tonus in the gingiva.

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Cigarette smoking is a known risk factor for periodontal disease (1–3), as well as for lung cancer and cardiovascular disease. Several studies have suggested some putative periodontopathic influences of smoking, for example, morphological changes of the blood vessels (4, 5), the dysfunction of oxygen sufficiency (6), and changes of the immune system (7, 8) in the gingiva.

The data on possible changes of gingival blood flow, from the results of human and animal experiments, are contradictory. In smokers, gingival blood flow is significantly increased by cigarette smoking (9). However, intravenous administration of nicotine reduces the marginal temperature of a gingival site in the anesthetized rabbit, suggesting a decrease in gingival blood flow (10, 11). In other studies, the vascular conductance of the calf and the blood flow of forearm skin in smokers decreased after cigarette smoking (12, 13).

Nicotine in the plasma after cigarette smoking effects not only the peripheral organs quickly, but also the brain. Subcutaneous nicotine injection increases expression of *c-fos* in the hypothalamus, suggesting that peripheral nicotine administration stimulates the central nervous system (14). Recently, we reported that some of the neurons in the subfornical organ, which has efferent connections to the hypothalamus, were activated by nicotine (15). However, it is unclear whether

central nicotinic stimulation changes gingival blood flow and gingival vascular conductance.

In this study, the gingival blood flow and blood pressure were measured in anesthetized rats after intracerebroventricular (i.c.v.) injection of nicotine, and the changes of the gingival vascular conductance were evaluated.

Material and methods

Animals

The experiments were conducted on male Wistar rats weighting 350–450 g. All were individually housed in plastic cages in a temperature- and humidity-controlled room under regular light/dark conditions (light on from 8.00 AM to 8.00 PM). All were maintained on water and pellets ad libitum. The experimental procedures were approved by the animal experiment committee of Kyushu Dental College.

Cannulation procedure

A 24-gauge stainless-steel guide cannula for i.c.v. injection of drugs was implanted in the lateral cerebral ventricle of each rat under sodium pentobarbital anesthesia (60 mg/kg of body weight, i.p.), as described in our previous study (16). A stainless steel guide cannula was implanted into the lateral cerebral ventricle 0.8 mm caudal to the bregma, 1.4 mm to the right lateral side to midline, and 2.5 mm below the dura matter. The cannula was fixed to the cranium by means of dental resin and screws.

Drug application

Drugs were purchased from the following pharmaceutical companies: (–)-nicotine ditartrate (described as 'nicotine' in this report) from Research Biochemicals International (Natick, MA, USA) and hexamethonium chloride from Sigma (St. Louis, MO, USA). Nicotine and hexamethonium were dissolved in isotonic saline. Isotonic saline was used as the control. The concentrations of nicotine were 5 μg, 15 μg, 50 μg, and 150 μg per 20 μl. Hexamethonium chloride (100 μg) was mixed with 50 μg nicotine. A microinfusion pump (Microprocessor-Controlled Syringe Pump, Stoelting Co., Wood Dale, IL, USA) was used for the i.c.v. injection at the rate of 4 μl/min for 5 min.

Gingival blood flow and blood pressure measurement

At least 3 days after the cannulation procedure, rats were anesthetized with a mixture of urethane (800 mg/kg, i.p.) and α-chloralose (70 mg/kg, i.p.) by intraperitoneal injection. Rectal temperature was monitored and body temperature was maintained at 37 ± 0.2°C by using a heating pad. All data were collected after 0.5–1 h from the injection of the anesthetics. We measured gingival blood flow in the anesthetized rats with a laser Doppler flowmeter (LDF; BRC, BRL-100, Nagoya, Japan). To set the LDF probe vertically on the gingival site behind the incisors without, as much as possible, exerting any pressure on the tissue, rats were placed on their back in a stereotaxic frame with ear bars. Further, the upper jaw was placed on a home-made frame and the lower jaw pulled with a string, to keep the mouth open and to insert and set the LDF probe. For better presentation of gingival blood flow change, we chose to use the averaged values every 1 min. But, they did not always indicate the maximal. Therefore, the average values for 10 s in the maximal response of gingival blood flow were used to indicate the peak. To measure blood pressure, rats were prepared surgically with an indwelling catheter that was filled with heparin (10 U/ml) in saline via the right femoral artery. The catheter was connected to a pressure-transducer (Nihon Kohden, Tokyo, Japan).

Data analysis

Gingival blood flow, blood pressure, and body temperature were monitored continuously and stored by a PowerLab system (PowerLab AD Instruments, Castle Hill, NSW, Australia). We analyzed gingival blood flow and/or blood pressure for a period of 30 min (5 min before the i.c.v. injection, 5 min during the injection, and 20 min afterwards). The LDF values represent the blood flow of the superficial vessels in tissue by means of Doppler effects against moving blood cells. The values are, by nature, in arbitrary units. The changes of the gingival blood flow were expressed as percentage changes every 1 min vs. the averaged values of the gingival blood flow for 5 min before the i.c.v. injection. The one exception was that the maximal responses of the gingival blood flow were expressed as the percentage changes for 10 s. The change of gingival vascular conductance was calculated as follows:

$$\begin{aligned}&\text{change of GVC}(\%)\\&= \frac{\text{GVC}}{\text{GVC(control)}} \times 100\\&= \left(\frac{\text{GBF}}{\text{GBF(control)}} \Big/ \frac{\text{BP}}{\text{BP(control)}}\right)\\&\times 100,\end{aligned}$$

where GVC is gingival blood flow, GBF is gingival vascular conductance, and BP is blood pressure. GVC (control), GBF (control), and BP (control) indicate the average of GVC, GBF, and BP, respectively, for 5 min before i.c.v. injection.

The differences between the control and nicotine injection groups were evaluated using two-way ANOVA and the Bonferroni post-test for the whole-experimental period. Further, the gingival blood flow values in the maximal response were analyzed statistically by *t*-test. Differences were considered significant at the level $p < 0.05$. For the statistical analyses and the graphs, Graph Pad Prism version 3.03 (GraphPad Software, Inc, San Diego, CA, USA) was used. All values are expressed as the mean ± SEM.

Results

The time courses of the percentage changes of gingival blood flow following the i.c.v. injection of nicotine at 5, 15, 50, and 150 μg, and saline ($n = 6$, in all cases) are shown in Fig. 1(A). Although the gingival blood flow was obviously reduced by the i.c.v. injection of 5 μg nicotine ($n = 4/6$), the difference, compared with saline by two-way

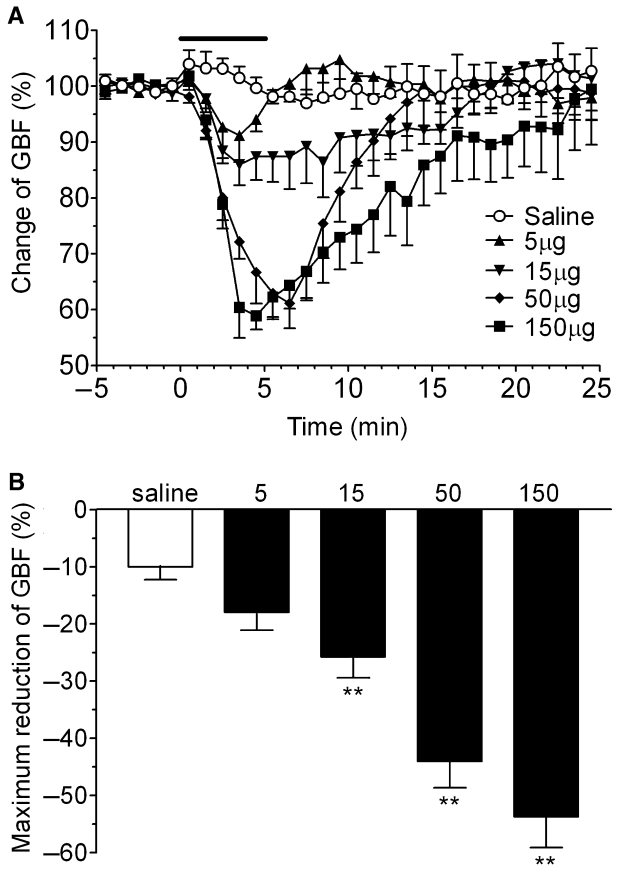

Fig. 1. The changes of gingival blood flow (GBF) produced by intracerebroventricular (i.c.v.) injection of nicotine. The error bars indicate SEM. (A) Time courses of the effects of the i.c.v. injection of nicotine on the gingival blood flow. The changes of gingival blood flow are indicated as the change of percentage in each 1 min vs. the 5 min values in mean arbitrary units before i.c.v. injection of nicotine. Saline, $n = 6$; nicotine: 5 μg, $n = 4$; 15 μg, $n = 6$; 50 μg, $n = 6$; 150 μg, $n = 6$. (B) Maximal reductions of the changes of gingival blood flow (GBF) after the i.c.v. injection of nicotine. The responses were dose-dependent. Numbers on the bar graph indicate the concentrations. $^{**}p < 0.001$ by *t*-test, compared with saline.

ANOVA, was not significant. The gingival blood flow was significantly reduced by 15, 50, and 150 μg nicotine ($p < 0.05$ at 15 μg, $p < 0.0001$ at 50 and 150 μg, by two-way ANOVA) (Fig. 1A). Further, there were significant decreases of gingival blood flow from 2 min to 9 min at 50 μg and from 2 min to 11 min at 150 μg (by Bonferroni post-test).

The values of the percentage changes of gingival blood flow every 1 min (Fig. 1A) did not always indicate the maximal responses. The percentage changes in the maximal response of gingival blood flow over 10-s periods showed better the peak values of the response. The maximal reductions of gingival blood flow after 5, 15, 50, and 150 μg nicotine and saline are shown in Fig. 1(B). The gingival blood flows were reduced significantly by 25.8 ± 3.7% at 15 μg, 44.1 ± 4.6% at 50 μg, and 53.7 ± 5.4% at 150 μg ($p < 0.0001$, by *t*-test, compared with saline).

The gingival blood flow and blood pressure were measured in other rats to evaluate the changes of vascular conductance. The mean blood pressure in anesthetized rats was 79.5 ± 4.4 mmHg ($n = 16$) before the i.c.v. injection. The typical responses of gingival blood flow and blood pressure at 50 μg are shown in Fig. 2(A). The gingival blood flow and blood pressure were decreased significantly by 41.6 ± 6.5% and 39.0 ± 3.7% by the i.c.v. injection of nicotine at 50 μg ($n = 5$), compared with saline (15.4 ± 5.2% in gingival blood flow and 5.9 ± 3.2% in blood pressure, $n = 6$). The values of the maximum reductions and the time courses of gingival blood flow in Fig. 3(A) were similar to those in the measurements of gingival blood flow only, seen in Fig. 1(A) (at 50 μg nicotine). The gingival vascular conductance calculated from the gingival blood flow and blood pressure was significantly decreased from 4 to 9 min (by Bonferroni post-test, Fig. 3B). The reduction began after a slight delay from those of blood pressure and gingival blood flow.

The effects of the selective nicotinic receptor antagonist hexamethonium 100 μg on the changes in gingival blood flow and blood pressure by the application of nicotine 50 μg ($n = 5$) were also examined. The maximal reductions of gingival blood flow (14.6 ± 6.1%) and blood pressure (6.9 ± 6.3%) by the two drugs were significantly less ($p < 0.01$, by *t*-test, Fig. 2B), compared with that produced by the i.c.v. injection of nicotine only.

Discussion

In this study, we found a reduction of gingival blood flow and blood pressure by the i.c.v. injection of nicotine and a slightly delayed reduction of gingival vascular conductance after the injection. As after i.c.v. injection nicotine diffuses slowly in the brain, the different nuclei may be activated after various time delays. A study has reported that when a hypertonic saline was injected into the ventricle of the brain, the concentration in the parenchyma of the brain lately and slowly changed, compared with the i.c.v. concentration, and has suggested that this regional difference of the NaCl concentration in the brain reflects the long-lasting blood pressure response (17). According to this concept, we think that i.c.v.-injected nicotine slowly diffuses in the brain and affects the different nuclei in a similar manner to NaCl. The early cardiovascular responses might begin with the parallel reduction of both blood pressure and vascular tonus, followed by the vasoconstriction or resistant dilatation of blood vessel.

Nicotine levels in the plasma during cigarette smoking increase up to

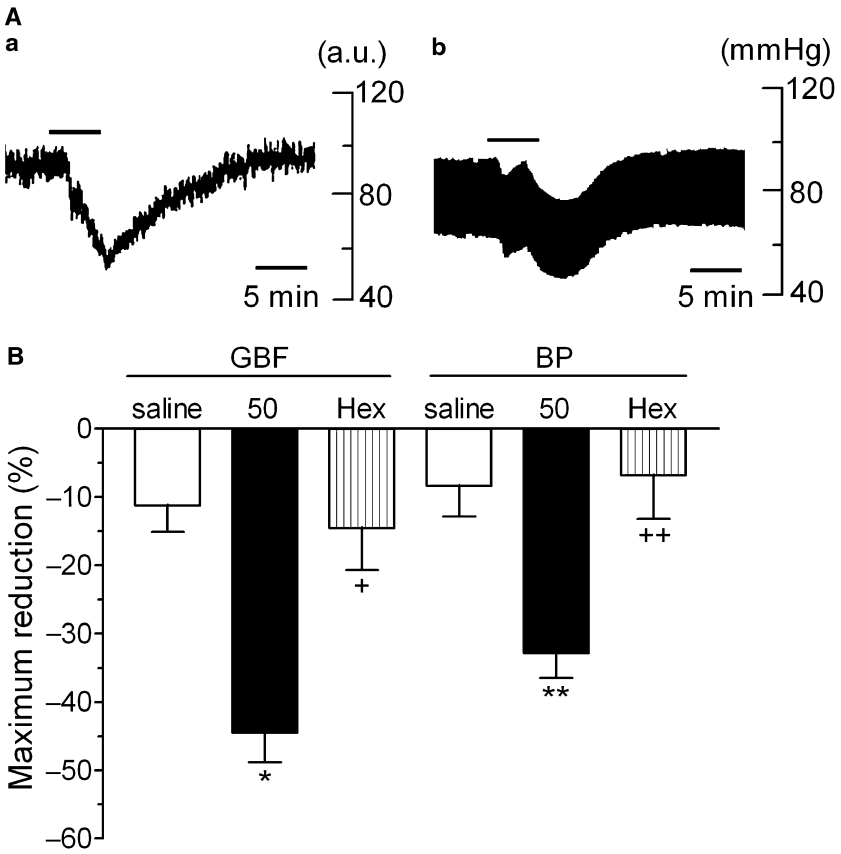

Fig. 2. The changes of gingival blood flow and blood pressure produced by the intracerebroventricular (i.c.v.) injection of nicotine and the effects of hexamethonium. (A) The typical responses of gingival blood flow (a) and blood pressure (b) at 50 μg nicotine. (B) Maximal reductions of the changes of gingival blood flow (GBF) and blood pressure (BP) following application of 50 μg nicotine and 100 μg hexamethonium (Hex) after the i.c.v. injection of nicotine. The error bars indicate SEM. $*p < 0.01$ and $**p < 0.001$ by *t*-test, compared with saline. $+p < 0.01$ and $++p < 0.001$ by *t*-test, compared with 50 μg nicotine only.

approximately 0.5 μM (18). We have recently reported that neurons in the subfornical organ, which lack the blood–brain barrier, are excited by 1 μM nicotine (15). In the present study, significant responses by nicotine injection in gingival blood flow were obtained at 15 μg/20 μl. In other words, the numerical concentration was 75 μM. Just after the i.c.v. injection, nicotine rapidly diffused in the ventricle and might be diluted 20 times [the concentration was estimated according to a previous study (17)]. But, even when diluted, the concentration was still several times higher than mentioned above. Meanwhile, the nicotine concentration would be further diluted in the parenchyma of the brain, in which nicotine affected. In the present study, anaesthetized animals were used. Under the anesthesia, the central nervous system should be suppressed. Therefore, to observe significant responses, relatively higher concentration would be needed.

The subfornical organ connects to the paraventricular and the supraoptic nuclei that control the autonomic nervous system and secrete vasopressin. The number of *c-fos* immunopositive neurons in the hypothalamic nuclei increases after subcutaneous nicotine injection (14). These results suggest that the systemic administration of nicotine stimulates the central nervous system as well as the peripheral organs. Further, it has been demonstrated that intravenous administration of nicotine in rabbits decreases gingival blood flow (10, 11). Taken together, it is reasonable to consider that the reduction of gingival blood flow by the intravenous administration of nicotine is due to actions not only on the peripheral regions, but also on the central nervous system.

Central nicotinic stimulation induces vasopressin release from the hypothalamus into the plasma (19). Similarly, smoking increases the plasma vasopressin concentration (12). The increased level of vasopressin contracts the blood vessels and possibly reduces the blood flow. A study has reported that the reduction in skin blood flow after smoking is suppressed by the administration of vasopressin antagonist (13). Thus, there is the possibility that the reduction of gingival vascular conductance in this study was induced by vasopressin in the plasma.

The present study shows the reduction of blood pressure by the i.c.v. injection of nicotine. This is consistent with the observations that blood pressure in chloralose-anesthetized cats was decreased the i.c.v. injection of nicotine (20). However, it is inconsistent with observations that blood pressure in urethane-anesthetized rabbits was raised by intravenous injection of nicotine (10, 11). In conscious rats, the blood pressure was raised by i.c.v. injection of nicotine (21). We think that the most important difference between these studies is whether experiments were accomplished under conscious conditions, and the kinds of anesthetics that were used. As we used chloralose together with urethane, we could obtain similar depressor responses by the i.c.v. injection of nicotine to those in chloralose-anesthetized cats. However, we have no clear explanation on these differences.

The vascular resistance is a physical variable to show obstruction of blood flow in the vessel. It is changed by the cross section of blood vessel and the blood viscosity. In the present study, it is considered that the reduction of gingival vascular conductance was due to the constriction of peripheral arteriole, because the gingival vascular conductance change was transient. In smokers, gingival blood vessels tend to show stenosis and their numbers decrease (4, 5). In rats chronically nicotine-medicated for 2 weeks, the

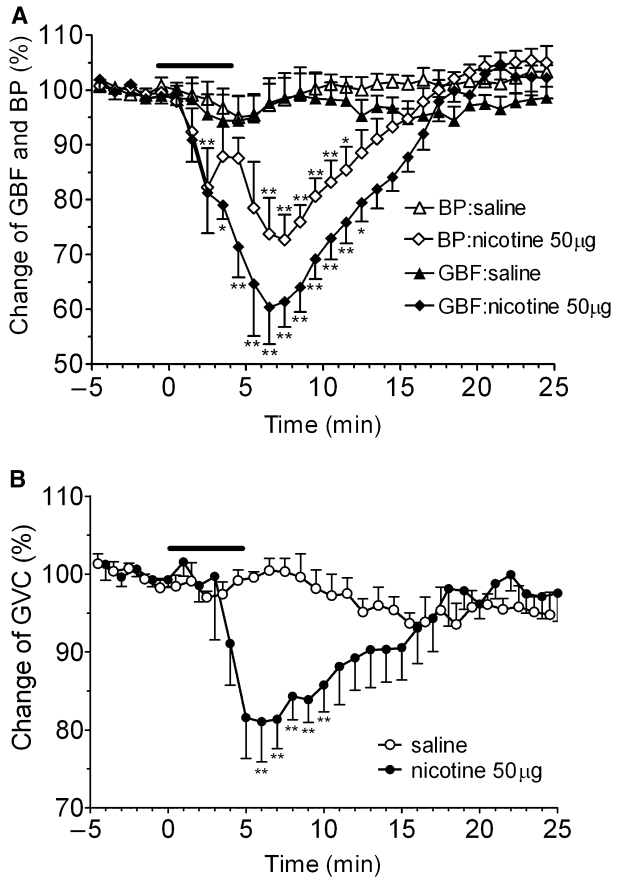

Fig. 3. (A) Time courses of the effects produced by the intracerebroventricular (i.c.v.) injection of 50 μg nicotine on gingival blood flow (GBF) and blood pressure (BP). The error bars indicate SEM. $*p < 0.01$ and $**p < 0.001$ by Bonferroni post-test following two-way AVOVA, compared with saline. (B) Time courses of the changes of gingival vascular conductance (GVC) at 50 μg nicotine and saline. The error bars indicate SEM. $**p < 0.001$ by Bonferroni post-test following two-way ANOVA, compared with saline. Notice that although the blood pressure and gingival blood flow were reduced immediately after nicotine injection, the gingival vascular conductance was decreased with a certain time-lag.

widths of gingival blood vessels decrease (22). A similar chronic reduction by smoking of gingival blood flow would be elicited by a long-term uptake of nicotine in humans. Clinically, it is well known that bleeding in probing, gingival exudates and redness and swelling in gingival sites of smokers are less than in non-smokers (23–25). These phenomena are tightly related to blood circulation in the gingival site. We think that chronic application of nicotine elicits constriction of the blood vessels, by acting both centrally and peripherally, and that gingival blood flow is continuously reduced in smokers. On the other hand, the controversial effects of smoking on gingival blood flow in human smokers, such as a temporary increase of gingival blood flow after cigarette smoking, has been demonstrated (9). The following observation may be of some help in considering the mechanisms underlying the two different responses. Parotid salivary secretion in non-smokers decreases after smoking, whereas it increases in smokers (26). This suggests that there are differences in parotid salivary secretion after cigarette smoking between non-smokers and smokers. Possibly, chronic nicotine exposure may modify the responses to a nicotinic application. In the present study, we used rats unmedicated by nicotine. The results might be different in rats chronically medicated by nicotine. To certify this, we will need another experiment about the effects of intracerebroventricular administration of nicotine on rats chronically exposed by nicotine.

In conclusion, it is suggested that nicotine taken up by smoking affects the central nervous system and influences periodontal disease by changing the tonus of the blood vessels in the gingiva.

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References

1. Bergström J, Preber H. Tobacco use as a risk factor. *J Periodontol* 1994;**65:**545–550.
2. Haber J, Wattles J, Crowley M, Mandell R, Joshipura K, Kent RL. Evidence for cigarette smoking as a major risk factor for periodontitis. *J Periodontol* 1993;**64:**16–23.
3. Tomar SL, Asma S. Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. *J Periodontol* 2000;**71:**743–751.
4. Mirbod SM, Ahing SI, Pruthi VK. Immunohistochemical study of vestibular gingival blood vessel density and internal circumference in smokers and non-smokers. *J Periodontol* 2001;**72:**1318–1323.
5. Bergström J, Persson L, Preber H. Influence of cigarette smoking on vascular reaction during experimental gingivitis. *Scand J Dent Res* 1988;**96:**34–39.
6. Hanioka T, Tanaka M, Ojima M, Takaya K, Matsumori Y, Shizukuishi S. Oxygen sufficiency in the gingiva of smokers and non-smokers with periodontal disease. *J Periodontol* 2000;**71:**1846–1851.
7. Fraser HS, Palmer RM, Wilson RF, Coward PY, Scott DA. Elevated systemic concentrations of soluble ICAM-1 (sICAM) are not reflected in the gingival crevicular fluid of smokers with periodontitis. *J Dent Res* 2001;**80:**1643–1647.
8. Boström L, Linder LE, Bergström J. Smoking and cervicular fluid levels of IL-6 and TNF-alpha in periodontal disease. *J Clin Periodontol* 1999;**26:**352–357.
9. Baab DA, Öberg PÅ. The effect of cigarette smoking on gingival blood flow in humans. *J Clin Periodontol* 1987;**14:**418–424.
10. Clarke NG, Shephard BC, Hirsch RS. The effects of intra-arterial epinephrine and

nicotine on gingival circulation. *Oral Surg Oral Med Oral Pathol* 1981;**52:**577–582.
11. Clarke NG, Shephard BC. The effects of epinephrine and nicotine on gingival blood flow in the rabbit. *Arch Oral Biol* 1984;**29:**789–793.
12. Grassi G, Seravalle G, Calhoun DA *et al.* Mechanisms responsible for sympathetic activation by cigarette smoking in humans. *Circulation* 1994;**90:**248–253.
13. Waeber B, Schaller MD, Nussberger J, Bussien JP, Hofbauer KG, Brunner HR. Skin blood flow reduction induced by cigarette smoking: role of vasopressin. *Am J Physiol* 1984;**247:**H895–H901.
14. Seppä T, Salminen O, Moed M, Ahtee L. Induction of Fos-immunostaining by nicotine and nicotinic receptor antagonists in rat brain. *Neuropharmacology* 2001;**41:**486–495.
15. Ono K, Honda E, Toyono T, Kataoka S, Nakamura T, Inenaga K. Evidence for the presence of nicotinic receptors on rat subfornical organ neurons. *Auton Neurosci* 2003;**108:**87–90.
16. Ito K, Morikawa M, Inenaga K. Suppression of reflex saliva from rat parotid gland following intracerebroventricular injection of hypertonic NaCl and sucrose. *Arch Oral Biol* 2002;**47:**93–97.
17. Hirose M, Nose H, Chen M, Yawata T. Cerebrospinal fluid sodium concentration and osmosensitive sites related to arterial pressure in anaesthetized rats. *Pflügers Arch* 1996;**431:**807–813.
18. Pidoplichko VI, DeBiasi M, Williams JT, Dani JA. Nicotine activates and desensitizes midbrain dopamine neurons. *Nature* 1997;**390:**401–404.
19. Iitake K, Share L, Ouchi Y, Crofton JT, Brooks DP. Central cholinergic control of vasopressin release in conscious rats. *Am J Physiol* 1986;**251:**E146–E150.
20. Armitage AK, Hall GH. Further evidence relating to the mode of action of nicotine in the central nervous system. *Nature* 1967;**214:**977–979.
21. Buccafusco JJ, Yang X. Mechanism of the hypertensive response to central injection of nicotine in conscious rats. *Brain Res Bull* 1993;**32:**35–41.
22. Johnson GK, Fung YK, Squier CA. Effects of systemic administration of nicotine on capillaries in rat oral mucosa. *J Oral Pathol Med* 1989;**18:**230–232.
23. Bergström J, Preber H. The influence of cigarette smoking on the development of experimental gingivitis. *J Periodont Res* 1986;**21:**668–676.
24. Haber J. Smoking is a major risk factor for periodontitis. *Curr Opin Periodontol* 1994:12–18.
25. Haber J. Cigarette smoking: a major risk factor for periodontitis. *Compendium* 1994;**15:**1004–1008.
26. Winsor AL. The effect of cigarette smoking on secretion. *J General Psychol* 1932;**6:**190–195.