Arachidonic Acid–Induced COX-1 and COX-2–Mediated Vasodilation in Rat Gingival Arterioles *In Vivo*

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Abstract: The roles of cyclooxygenase (COX) and prostaglandins (PGs) in the regulation of vasoreactivity of rat gingival arterioles *in vivo* were evaluated by using an intravital microscope. The superfusion of indomethacin (a nonselective COX inhibitor) or SC-560 (a selective COX-1 inhibitor) onto the gingiva significantly constricted the arterioles, though NS-398 (a selective COX-2 inhibitor) did not affect the diameter of the arterioles. The SC-560–mediated constriction of the arterioles was completely reversed by an additional treatment with arachidonic acid (AA). The superfusion of AA, beraprost-Na (an analogue of PGI₂) or PGE₂ onto the gingiva significantly dilated the arterioles dose-dependently. The AA–

induced dilation of the arterioles was significantly reduced by the treatment with SC-560 or NS-398. The expression of COX-1 and COX-2 were positive in the endothelium, but not the smooth muscles, of the arterioles. The expression of PGE synthase (PGES) was found only in the smooth muscles, but not the endothelium, of the arterioles. Neither the endothelium nor the smooth muscles of the arterioles expressed PGI synthase (PGIS). These findings suggest that the COX-2–mediated PG cascade may collaborate with the COX-1 pathway in the regulation of arteriolar myogenic activity in rat gingiva in the case of the supply of a large amount of AA. [The Japanese Journal of Physiology 55: 293–302, 2005]

Key words: gingiva, arterioles, indomethacin, SC-560, NS-398.

Local gingival blood flow plays a crucial role in maintaining dental health care in physiological conditions [1–3]. For evaluating local blood flow in the gingival tissues *in vivo*, laser-doppler and ultrasonic flowmetry have been used on animals and humans [4–7], but they are both disadvantageous for evaluating continuous changes in the diameter of gingival microvessels *in vivo*. On the other hand, an intravital microscope system enables us to continuously measure changes in the diameter of resistant microvessels, including small arteries and arterioles *in vivo*.

PGs are produced via the arachidonic acid–COX pathway. COX is a limiting enzyme in this cascade and has been classified into two isoforms, COX-1 (constitutive type) and COX-2 (inducible type) [8–11].

Recently, it has become clear that periodontitis is involved in the dysfunction of gingival microcirculation in humans [12], and PGE₂ plays a major role in the incidence of periodontitis. It is known that the COX-2 pathway involves the PGE₂–mediated inflammatory responses [13–18]. There is, however, no study for evaluating the roles of COX-1 and COX-2 in the regulation of *in vivo* gingival microcirculation in physiological conditions.

Thus the purpose of the present study was first to establish an experimental apparatus with an intravital microscope system suitable for investigating rat gingival microcirculation *in vivo*. And then, by using this experimental apparatus, we investigated the effects of AA, COX inhibitors, or PGs on changes in the diam–

Received on Nov 7, 2005; accepted on Nov 11, 2005; released online on Nov 12, 2005; DOI: 10.2170/jjphysiol.R2137

A. Nakatsuka deceased Oct 15, 2005.

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Fig. 1. An experimental layout for investigation of changes in the diameter of arterioles in rat gingiva by using an intravital microscope (A). B (yellow circle), C (yellow circle), and D indicate a low, middle, and high magnification of the measuring field in rat gingival microcirculation, respectively. White and black arrowheads in D show arteriole

and venule of rat gingival tissues, respectively. Bars in B–C and D are 1 mm and 100 μ m, respectively. Representative pictures of immunohistochemical study for smooth muscle α -actin expression in rat gingival tissue (E; bar 50 μ m). White and yellow arrowheads in E show arteriole and venule in rat gingival tissue, respectively.

eter of rat gingival arterioles *in vivo*. Moreover, the immunohistochemical localization of COX-1 and COX-2, PGIS, and PGES in gingival tissues were also evaluated.

METHODS

Animals. Seven-week-old male Wistar rats ($n = 55$, SLC Japan, Japan) were used for the present study. They were housed in an environmentally controlled vivarium and fed a standard pellet diet and water *ad libitum*. The Animal Ethics Committee, Shinshu University School of Medicine, in accordance with the principles and guidelines of the Japanese Physiological Society, approved all experimental protocols.

Surgical procedures. The rats were anesthetized through a subcutaneous injection containing a mixture of 2% α -chloralose and 10% urethane (0.7 ml/100 g body weight) for the *in vivo* experiments [19]. Additional anesthetics were administered (0.1 ml) when the rats showed body movement, hyperven-

tilation, or increment arterial blood pressure. Each rat was tracheotomized, and polyethylene tubing (No25, Igarashi, Japan) was inserted to ensure spontaneous respiration in room air (Fig. 1A). To measure arterial blood pressure, the left femoral artery was cannulated with an elongated polyethylene tubing (No15, Igarashi, Japan) that was connected to a pressure transducer (Becton, Dickinson and Company, USA) and an amplifier (6M52, Sanei, Japan). Changes in arterial blood pressure during experiments (Fig. 1A) were monitored with a MacLab data acquisition system (AD Instruments, Australia) and a personal computer (Power Macintosh 8500/1200, Apple, USA). A warm heat pad maintained the body temperature ($37.0 \pm 0.5^\circ\text{C}$) of the rats. Experiments were terminated if mean arterial blood pressure fell below 80 mm Hg and were discarded from analyses.

Evaluation of vasoreactivity in rat gingival microcirculation. Figure 1A also shows a schematic illustration for investigating changes in the diameter of rat gingival arterioles *in vivo*. The rats were placed

on the stage of an intravital microscope (E600, Nikon, Japan), and the gingival tissue was examined *in vivo*. Figure 1, B, C, and D, are photographs of low, middle, and high magnification of rat gingival tissue, respectively. The photographs were taken with a stereo zoom microscope (SZ60, Olympus, Japan) and a digital camera (Coolpix 4500, Nikon, Japan). To prevent moving the observation field of rat gingiva, we fixed the lower jaw by using a silk suture (Fig. 1B) at a lower incisor. We measured changes in the diameter of the gingival arterioles ($13.7 \pm 0.1 \mu\text{m}$, $n = 55$) in Fig. 1D (white arrowhead). The images of gingival arterioles were obtained through a water-immersion objective lens (10 $\times$, Nikon, Japan), a photo-eyepiece lens (2.5 $\times$, Nikon, Japan), and a monochrome chilled charge-coupled device (C-CCD) camera (C5985, Hamamatsu Photonics, Japan). The images were also recorded on a videocassette recorder (VCR; BR-S800, Victor, Japan) through a video timer (VTG-33, For A, Japan) and then displayed on a TV monitor (Trinitron super pitch, Sony, Japan). A video caliper [20] enabled us to measure changes in the diameter of the gingival arterioles. In our preliminary study, the superfusion of 37°C Krebs-bicarbonate solution onto the surface of rat gingival tissue caused a significant constriction of the arterioles. Thus in the present study the gingival surface of rats was superfused with 37°C distilled water with or without drugs because the water is a natural solution for the oral cavity. The rate of superfusion was maintained at 6 ml/min during experiments.

Experimental protocols. Each experimental protocol was made after equilibration for 30 min. In the first protocol, the effects of indomethacin (a non-selective COX inhibitor, 10^{-6} and 10^{-5} M), SC-560 (a selective COX-1 inhibitor, 10^{-7} and 10^{-6} M) [21, 22], or NS-398 (a selective COX-2 inhibitor, 10^{-6} and 10^{-5} M) [21, 23] on changes in the diameter of the gingival arterioles were studied at 3-min intervals for 30 min.

In the second protocol, the effects of SC-560 (10^{-6} M) or SC-560 (10^{-6} M) plus AA (a precursor of prostanoid, 10^{-5} M) on changes in the diameter of the gingival arterioles were evaluated at 3-min intervals for 30 min.

In the third protocol, the effects SC-560 (10^{-6} M) or NS-398 (10^{-5} M) on the AA (10^{-7} M to 10^{-4} M)-mediated changes in the diameter of the gingival arterioles were studied. Each dose of AA was superfused onto the gingival tissue until maximum response was obtained.

In the final protocol, the dose-dependent effects of beraprost-Na (BPS, a stable PGI₂ analogue, 10^{-8} to 10^{-5} M) [24] or PGE₂ (10^{-8} to 10^{-5} M) on the mechanical activity of the gingival arterioles were evaluated. Each

dose of prostanoids was superfused onto the gingival tissue until maximum response was obtained. Superfusion with all the drugs used in the present study onto the gingival tissue did not affect the mean arterial pressures of the rats during the experiments.

Immunohistochemical study of smooth muscle α -actin in rat gingival tissues. To evaluate the distribution of smooth muscle layers in rat gingival arterioles and venules, direct immunofluorescence staining for antismooth muscle α -actin antibody was performed on whole-mount preparations of the rat gingiva. Small tissues of the gingiva were excised immediately. The tissues were quickly fixed in acetone for 10 min, rinsed with phosphate-buffered saline (PBS), and postfixed with cold (4°C) 4% paraformaldehyde in PBS for 15 min. After blocking the tissues with 1% bovine serum albumin in PBS for 1 h at room temperature, they were incubated with the FITC-conjugated antismooth muscle α -actin antibody (diluted to 1:500, Sigma, MO, USA) for overnight at 4°C. Thereafter, the parts were washed three times with PBS for 10 min each time, spread on cover glass, and then mounted using aqueous mounting media (Aqua/Poly Mount, Polysciences Inc., PA). Preparations were studied under fluorescence microscopy (TMD, Nikon, Japan) and then photographed.

Immunohistochemical studies of COX and PG synthase in rat gingival tissues. In the present study, we have investigated the localization of COX (COX-1 and COX-2) and PG synthase (PGI synthase [PGIS] and PGE synthase [PGES]) expression in gingival tissues by using immunohistochemical studies. Fresh gingival tissues removed from rats were embedded in an OCT compound (Sakura Fine-technical Co., Japan) and frozen at -80°C . The frozen sections sliced at 5 μm thickness were fixed with 20% buffered formalin for 5 min, then treated with 0.3% H₂O₂ in absolute methanol for 30 min. After being washed with Tris buffered solution (TBS), the tissue sections were incubated with anti-COX-1 antibody (1:200; Santa Cruze, USA), anti-COX-2 antibody (1:200; Santa Cruze, USA), anti-PGIS antibody (1:10; Cayman, USA) or anti-PGES antibody (1:200; Cayman, USA) for 60 min at room temperature. After being washed with TBS, they were incubated with horseradish peroxidase-conjugated rabbit antigoat IgG (Dako A/S, Denmark) for 60 min at room temperature. The peroxidase reaction was developed with a diaminobenzine/H₂O₂ solution and counterstaining was performed with hematoxylin. In the control experiment, primary antibodies were omitted from the staining procedure.

Fig. 2. Effects of superfusion with indomethacin (INDO, 10^{-6} M; closed triangles and 10^{-5} M; closed squares; A, $n = 5$), SC-560 (10^{-7} M; closed triangles and 10^{-6} M; closed squares, B, $n = 5$) or NS-398 (10^{-6} M; closed triangles and 10^{-5} M; closed squares, C, $n = 5$) on the % changes in

the diameter of arterioles in rat gingiva. * In each panel, indicate a significant difference ($p < 0.05$) from the absence of COX inhibitors (distilled water [D.W.], open circles). † in figure B indicate a significant difference ($p < 0.05$) from the presence of 10^{-7} SC-560 (closed triangle).

Drugs. Indomethacin, AA (Sigma, USA), SC-560, NS-398, PGE₂ (Cayman, USA), and BPS (Toray Industries Inc., Japan) were used in the present study. Indomethacin and arachidonic acid were dissolved with ethanol, and SC-560, NS-398, and PGE₂ were dissolved with DMSO as stock solutions. Each stock solution and the other drugs were directly diluted with distilled water. The concentration of ethanol and DMSO used in the present study did not affect the diameter of gingival arterioles. Concentrations of the drugs were expressed as final concentration in the superfusion solution. All drugs were prepared on the day of the experiment.

Data analyses. Changes in the diameter of the gingival arterioles were normalized by the diameter obtained before drug superfusion and expressed as a percentage of the diameter. The data are presented as mean $\pm$ standard error of mean. Each rat was used for each experimental protocol, and n indicates the number of preparations. Significant differences ($p < 0.05$) were determined through repeated-measurement ANOVA, followed by a Student-Newman-Keuls post hoc test and paired Student's t -test, as appropriate.

RESULTS

Immunohistochemical studies for localization of smooth muscle α -actin in gingival tissues

Figure 1E shows a representative fluorescent image of the localization of smooth muscle α -actin in rat gingival tissue. Smooth muscle α -actin was clearly observed in the wall of the gingival arteriole (white arrowheads), but not in the venule (yellow arrowhead).

Effects of COX inhibitors on mechanical activity of rat gingival arterioles

Figure 2A demonstrates summarized data for the effects of distilled water (open circles), 10^{-6} M (closed triangles) or 10^{-5} M (closed squares) indomethacin on changes in diameter of the gingival arterioles. The % changes in the diameter of the arterioles decreased time-dependently after the superfusion with indomethacin, but superfusion with distilled water (open circles) did not affect the % changes in diameter. Thus the % changes in diameter of the gingival arterioles at 30 min after the superfusion with distilled water, 10^{-6} M, and 10^{-5} M indomethacin were $99.8 \pm 0.0\%$ ($n = 5$), $82.0 \pm 3.6\%$ ($p < 0.05$ from the distilled water, $n = 5$), and $74.0 \pm 4.9\%$ ($p < 0.05$ from the distilled water, $n = 5$), respectively.

Figure 2B shows summarized data for the effects of distilled water (open circles), 10^{-7} M (closed triangles) or 10^{-6} M (closed squares) SC-560 on changes in diameter of the gingival arterioles. The % changes in the diameter of the arterioles decreased time- and dose-dependently after the superfusion with SC-560, but

Table 1. Localization of COX-1, COX-2, PGIS, and PGES of arteriolar endothelial cells (EC), arteriolar smooth muscle cells (SM), venular EC, and mast cells in rat gingival tissues. + and – indicate the positive and negative expression of each enzyme, respectively.

Cells	COX-1	COX-2	PGIS	PGES
Arteriolar EC	+	+	–	–
Arteriolar SM	–	–	–	+
Venular EC	–	–	–	–
Mast cells	+	+	+	+

Fig. 3. Effects of superfusion with 10^{-6} M SC-560 alone (closed triangles) and 10^{-6} SC-560 M plus 10^{-5} M arachidonic acid (closed squares) on the % changes in the diameter of arterioles in rat gingiva ($n = 5$). * and † indicate significant differences ($p < 0.05$) from the absence of SC-560 (distilled water [D.W.], open circles) and presence of SC-560 plus arachidonic acid (closed squares), respectively.

superfusion with distilled water (open circles) did not affect the % changes in diameter. Thus the % changes in diameter of the gingival arterioles at 30 min after the superfusion with distilled water, 10^{-7} M, and 10^{-6} M SC-560 were $100.0 \pm 0.1\%$ ($n = 5$), $91.3 \pm 1.9\%$ ($p < 0.05$ from the distilled water, $n = 5$), and $77.4 \pm 2.8\%$ ($p < 0.05$ from the distilled water or 10^{-7} M SC-560, $n = 5$), respectively.

Figure 2C shows summarized data for the effects of distilled water (open circles), 10^{-6} M (closed triangles) and 10^{-5} M (closed squares) NS-398 on changes in diameter of the gingival arterioles. The % changes in diameter of the arterioles did not alter after the superfusion with distilled water or NS-398. Thus the % changes in the diameter of the gingival arterioles at 30 min after the superfusion with distilled water, 10^{-6} M, and 10^{-5} M NS-398 were $100.1 \pm 0.1\%$ ($n = 5$), $100.0 \pm 0.1\%$ (not significantly different from the distilled water, $n = 5$), and $100.1 \pm 0.1\%$ (not significantly different from the distilled water, $n = 5$), respectively.

Effects of AA on SC-560-mediated vascular responses of rat gingival arterioles

Figure 3 shows summarized data for the effects of 10^{-6} M SC-560 plus 10^{-5} M AA (closed squares) on the 10^{-6} M SC-560-mediated constriction (closed triangles) of the gingival arterioles. Additional treatment with 10^{-5} M AA completely reversed the SC-560-mediated vasoconstriction. Thus the % changes in the

Fig. 4. Effects of SC-560 (A; 10^{-6} M, $n = 5$) or NS-398 (B; 10^{-5} M, $n = 4$) on the arachidonic acid (AA; 10^{-7} M to 10^{-4} M)-induced changes in the diameter of the gingival arterioles. * indicates a significant difference from the absence of SC-560 (A, open circles) or NS-398 (B, open circles).

diameter of the gingival arterioles at 30 min after the superfusion with distilled water, 10^{-6} M SC-560 alone, and 10^{-6} M SC-560 plus 10^{-5} M AA were $100.1 \pm 0.1\%$ ($n = 5$), $76.2 \pm 1.4\%$ ($p < 0.05$ from the distilled water or 10^{-6} M SC-560 plus 10^{-5} M AA, $n = 5$), and $100.5 \pm 1.2\%$ (not significantly different from the distilled water, $n = 5$), respectively.

Effects of SC-560 or NS-398 on AA-mediated vascular responses of rat gingival arterioles

Figure 4 shows summarized data for the effects of 10^{-6} M SC-560 (A) or 10^{-5} M NS-398 (B) on the AA (10^{-7} M to 10^{-4} M)-mediated vascular responses of gingival arterioles. In the absence of SC-560 or NS-398, AA caused a dose-dependent dilation of the gingival arterioles (open circles in each panel). The AA (10^{-7} M to 10^{-5} M)-induced % changes in the diameter of the gingival arterioles in the presence of SC-560 were less than 100%, because SC-560 itself significantly constricted the arterioles (Fig. 4A; closed circles), being similar to those observed in Fig. 2B (closed squares) and Fig. 3 (closed triangles). SC-560 completely reduced the AA-induced dilation of the gingival arterioles. Thus the 10^{-4} M AA-mediated increases in the % changes in diameter of the gingival arterioles in the absence and presence of SC-560 were $119.5 \pm 2.7\%$ (Fig. 4A, open circles, $n = 5$) and $101.9 \pm 1.8\%$ (Fig. 4A, closed circles, $p < 0.05$ from the absence of SC-560, $n = 5$), respectively. NS-398 also significantly reduced the AA-induced dilations of the gingival arterioles. Thus the 10^{-4} M AA-mediated increases in the % changes in diameter of the gingival arterioles in the absence and presence of NS-398 were $126.5 \pm 1.9\%$ (Fig. 4B, open circles, $n = 4$) and $107.1 \pm 1.2\%$ (Fig.

Fig. 5. Dose-dependent dilator effects of BPS (10^{-8} M to 10^{-5} M, open circles, $n = 5$) and PGE₂ (10^{-8} M to 10^{-5} M, closed circles, $n = 5$) on the % changes in the diameter of rat gingival arteriole. * indicates a significant difference from the BPS.

4B, closed circles, $p < 0.05$ from the absence of NS-398, $n = 4$), respectively.

Effects of BPS or PGE₂ on mechanical activity of rat gingival arterioles

Figure 5 shows summarized data for the dose-dependent effects of BPS (10^{-8} to 10^{-5} M, open circles) or PGE₂ (10^{-8} to 10^{-5} M, closed circles) on the % changes in the diameter of the gingival arterioles. BPS and

PGE₂ caused dose-dependent dilations of the gingival arterioles. Thus the 10^{-5} M BPS- and 10^{-5} M PGE₂-mediated increases in the % changes in diameter of the arterioles were $200.0 \pm 5.7\%$ ($n = 5$) and $138.6 \pm 11.9\%$ ($n = 5$), respectively. The BPS-induced increases in the % changes in diameter were significantly larger than those obtained with PGE₂ ($p < 0.05$ from BPS).

Immunohistochemical studies for localization of COX and PG synthase expression in gingival tissues

Figure 6 shows representative microphotographs obtained with immunohistochemical studies of rat gingival tissue for the localization of COX-1, COX-2, and its negative control. Table 1 also demonstrates immunohistochemical localization of COX-1 and COX-2 in arteriolar endothelial cells, arteriolar smooth muscle cells, venular endothelial cells, and mast cells in rat gingival tissues. The expressions of COX-1 and COX-2 have been strongly positive in the arteriolar endothelium (a) and mast cells (at the lower right-hand corner) of the gingival tissue. On the other hand, arteriolar smooth muscle cells and venular endothelial cells did not exhibit COX-1 and COX-2.

Figure 7 shows representative microphotographs obtained with immunohistochemical studies of rat gingival tissue for the localization of PGIS and PGES (its negative control has not shown, because it is the same as those in Fig. 6). Table 1 also demonstrates immunohistochemical localization of PGIS and PGES in arteriolar endothelial cells, arteriolar smooth muscle cells, venular endothelial cells, and mast cells in rat gingival tissues. The expressions of PGES have been strongly positive in the arteriolar smooth muscle cells and mast cells of the gingival tissue, but arteriolar endothelial cells and venular endothelial cells did not ex-

Fig. 6. Representative pictures of immunohistochemical studies for expression of COX-1 (left panel), COX-2 (center panel) and the negative control (right panel) of rat gingival tissues. "a" and "v" in each figure show arteri-

ole and venule in the rat gingival tissues, respectively. At the lower right-hand corner of the left panel, a COX-1 positive mast cell is also shown (Bar; 10 μ m). The bar in the right panel indicates 100 μ m.

Fig. 7. Representative pictures of immunohistochemical studies for expression of PGIS (left panel) and PGES (right panel) of rat gingival tissues. "a" and "v" in each figure show the arteriole and venule in the rat gingival tissues, respectively. The bar indicates 100 μ m.

hibit PGES. Only mast cells exhibited PGIS. There is no expression of PGIS in arteriolar endothelial cells, arteriolar smooth muscle cells, or venular endothelial cells.

DISCUSSION

Measurement of gingival microcirculation *in vivo*. It is well known that gingival microcirculation plays a crucial role in maintaining health care of the oral cavity [1, 3, 25]. Recently, it has become clear that the dysfunction of gingival microcirculation in humans contributes to periodontitis [12]. In the present study an intravital microscope enabled us to continuously measure changes in the diameter of rat gingival microvessels *in vivo*. We fixed the lower jaw and head of the rat to the warm plate with strings. The procedure also enabled us to keep a stable field for measuring the rat gingival microcirculation under anesthetized conditions with spontaneous breathing.

We used distilled water (37°C) for solutions with or without the drugs in the present study. Continuous superfusion of distilled water for ~120 min onto the gingiva produced no significant change in the diameter of the arterioles and no significant swelling of the gingival tissues in the present study. Physiological salt solutions have been widely used for investigating microcirculation, including mesentery, cheek pouch, or brain as a superfusion solution. In our preliminary study, it became clear that the superfusion of physiological salt solution (Krebs-bicarbonate solution) on the rat gingiva itself significantly constricted the arterioles and sometimes terminated blood flow in gin-

gival microcirculation. Thus distilled water, a natural solution, was adopted for using the superfusion solution of gingival microcirculation in the present study. We could not show the mechanisms why superfusion with physiological salt solution caused a marked constriction of the gingival arterioles. In the oral cavity, saliva is the physiological solution as well as water. The rate of saliva secretion is strongly controlled by the autonomic nervous system, and electrolytes in saliva affect the flow rate of microcirculation [26]. Thus further investigation will be needed to quantitatively evaluate the effects of saliva, including Na^+ , K^+ , Cl^- , and HCO_3^- , on the regulation of the mechanical activity of gingival microvessels.

Roles of COX in the regulation of gingival microcirculation. COX is a limiting enzyme to produce endoperoxide (PGH_2) via the AA-PGG₂ pathway. PGH_2 subsequently becomes bioactive PGs including PGE_2 , $\text{PGF}_{2\alpha}$, PGI_2 , and THA_2 through each specific synthase. Thus PGs play key roles in the regulation of microcirculation as vasoactive substances and platelet-aggregation regulators in physiological conditions [8–11]. PG-mediated responses are frequently significant pathophysiologically, including in inflammation, cancers and circulatory disorders. It is also known that PGE_2 -mediated inflammatory response via COX-2 activity is the major pathway for the occurrence of periodontitis [13–18].

In the present study, we showed that the superfusion of indomethacin (a nonselective COX inhibitor) or SC-560 (a selective COX-1 inhibitor), but not NS-398 (a selective COX-2 inhibitor), onto rat gingiva significantly constricted the gingival arterioles.

Moreover, the superfusion of AA plus SC-560 onto rat gingiva completely reversed the SC-560-mediated constriction of the arterioles. We used the ten times concentration of AA (10^{-5} M) to reverse the SC-560 (10^{-6} M)-mediated constriction of arterioles in the gingival tissues, based on the substrate-enzyme relationship. The superfusion of SC-560 onto mice cranial windows significantly reduced cerebral blood flow as well as bradykinin- or A23187-induced increases in blood flow [22]. Moreover, COX-1 knockout mice did not produce the COX-1-mediated cerebral vascular responses [22]. These effects of SC-560 on cerebral blood flow in mice strongly support the evidence that COX-1 significantly contributes to the regulation of cerebral circulation. In contrast, indomethacin or NS-398 constricts fetal lamb ductus arteriosus by reducing the production of vasodilator PGs [23]. Thus the findings obtained in the present studies suggest the first information that the basal release of endogenous vasodilator PGs may contribute to the regulation of changes in the diameter of gingival arterioles via the AA-COX-1 pathway *in vivo*.

In the present study, we also evaluated roles of the exogenous application of AA in the regulation of rat gingival arteriolar activity. The superfusion of AA onto rat gingiva significantly and dose-dependently dilated the gingival arterioles. SC-560 completely reduced the 10^{-4} M AA-mediated dilation and still maintained constriction of the arterioles in the presence of lower concentration AA. It is interesting that NS-398 also significantly inhibited the AA-induced dilation of the arterioles. Thus these results suggest that in the absence of exogenous AA in the oral cavity, COX-1 plays a major role in the regulation of gingival arteriolar activity, and in the presence of an excess amount of AA, the COX-2 pathway may coincidentally participate in accordance with the COX-1 cellular signaling mechanisms.

The concentration of NS-398 (10^{-6} M) was enough to inhibit COX-2 in an *in vitro* study [23]. We used the two-times concentration of NS-398 to evaluate the effects of the inhibitor on the basal diameter of the arterioles and to study the effects of inhibitor on the AA-induced dilation of the gingival arterioles *in vivo*. We could not identify whether topical superfusion with NS-398 completely reduces COX-2 activity *in vivo*. Thus further investigation will be needed to evaluate the NS-398-mediated reduction of COX-2 activity in the gingival arterioles by intravenous or intraperitoneal injection.

In epidemiological investigations, it is well known that an intake of unsaturated fatty acids, including ara-

chidonic acid, linoleic acid, and α -linolenic acid, significantly reduce the occurrence of coronary heart disease, especially in Alaskan natives [27] because they effectively utilize food as a source of PGs that inhibit platelet aggregation. Thus the present study will give new insight into clinical benefits that enough supply of AA in food may contribute, in part, to the improvement of gingival microcirculation with the involvement of vasodilation and inhibition of platelet aggregation. However, to investigate the roles of excess AA in food for the regulation of platelet aggregation in gingival microcirculation will also be needed in the future.

It is generally accepted that small arteries and arterioles in various vascular beds have myogenic tone, which significantly contributes to the regulation of local blood flow in tissues and organs [28]. In the present study, the superfusion of PGE₂ and BPS (a stable PGI₂ analogue) onto rat gingiva significantly increased the diameter of the gingival arterioles in a dose-dependent manner. The BPS-mediated dilation of the arterioles was significantly larger than that obtained with PGE₂. 10^{-5} M BPS increased the diameter of the gingival arterioles ~200%. These findings are quite compatible with previous studies because PGE₂ and PGI₂ cause significant dilations of arterioles in skeletal muscles [29] and brain [2, 30] *in vivo*. The present study also gives the first information that exogenous PGE₂ or PGI₂ as well as AA significantly dilate the gingival arterioles maintaining myogenic tone *in vivo*.

Vasodilator PGs, mainly PGI₂, have been released from vascular endothelial cells in response to vasoactive substances [9, 31], chemical [32] and physical factors [33, 34] in the cardiovascular system. Increase in blood flow or stimulation of shear stress against vascular endothelium is quite important factor in activating COX in physiological conditions [35, 36]. In the present study, the superfusion of indomethacin or SC-560 onto the gingiva itself caused a marked constriction in the arterioles in the presence of blood flow. Thus the physical factors involved in the gingival arteriolar blood flow may be a key regulatory factor in controlling COX-mediated activity of gingival arterioles. Further investigation, however, will be needed in the future to evaluate the mechanisms of flow-induced activation of COX in the gingival arterioles.

Localization of COX and PG synthase expression in gingival microcirculation. In the cardiovascular system, the expression of COX-1 has been observed in endothelium [8–11]. The localization of COX-1 expression in rat gingival microcirculation, however, remains unclear. Immunohistochemical studies in the present experiments show that the ex-

pression of COX-1 in the gingival tissues is confirmed to be strongly positive in the endothelium of arterioles and mast cells. On the other hand, venular endothelium did not exhibit an expression of COX-1. These findings suggest that COX-1 in the arteriolar endothelium may contribute to the first step for the production of PG in the gingiva.

The present study also shows there was a strong expression of COX-2 in the arteriolar endothelium and slightly in the mast cells of gingival tissues, but the expression of COX-2 was not evident in the endothelium of venules. The localization of COX-2 in gingival arterioles is quite compatible to the present functional study that NS-398 significantly inhibited the AA-induced dilation of arterioles. Gingival tissues induce the expression of COX-2 through stimulation of interleukins [17, 18] or lipopolysaccharides [16] in pathophysiological conditions. Thus further investigation will be needed to evaluate the physiological role of COX-2 in the regulation of gingival functions.

Several investigators have reported that mast-cell-derived vasoactive substances affect the nearby activity of arterioles [37]. Thus it is possible that the activity of gingival arterioles may be indirectly controlled by COX-1 and COX-2 in the mast cells of gingival tissues. However, further investigation will be needed to evaluate the relationships between the activity of arterioles and the expression of COX-1 and COX-2 of mast cells in gingival tissues.

We also studied localization of PGIS and PGES in gingival tissues. PGIS existed only in mast cells. Arteriolar smooth muscle cells and mast cells expressed PGES. These results were quite similar to human vascular smooth muscle cells but not endothelial cell express PGES [38]. Thus we propose suggested mechanisms in the PG-mediated regulation of rat gingival arterioles by the endothelial cells and smooth muscle cells in the present study. In the absence of AA in the oral cavity, flow or shear stress activates COX-1 in the endothelial cells, and subsequently COX-1 produces PGH_2 . On the other hand, in the presence of an excess amount of AA in the oral cavity, COX-2 cascade in the endothelial cells collaborates with the COX-1 pathway to produce PGH_2 . PGES in the smooth muscle cells synthase PGE_2 from the endothelial-derived PGH_2 via COX-1 and/or COX-2. Finally, the smooth muscle-derived PGE_2 may contribute to the regulation of the arteriolar tone *in vivo* gingival microcirculation.

The authors thank Toray Japan for their generous donation of beraprost-sodium. This study was supported in part by Grants-in-Aid for Scientific Research (15700325) from the Japanese Ministry of Education, Science, Sports, and Culture.

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