Difference in Dilatation Between Endothelium-Preserved and -Desquamated Segments in the Flow-Loaded Rat Common Carotid Artery

Kohei Tohda, Hirotake Masuda, Koichi Kawamura, and Takeshi Shozawa

To study arterial dilatation in response to increased flow, we observed the bilateral common carotid arteries (CCAs) of 10 16-week-old rats that were maintained for 8 weeks after construction of an arteriovenous (AV) fistula between the left CCA and the jugular vein at a level 20 mm distal from the aortic orifice. The flow in the left CCA increased 11-fold and that of the right CCA increased twofold compared with values before surgery. The left CCA showed complete desquamation of endothelial cells in the distal one third of the segment proximal to the AV fistula. In the left CCA the endothelium-preserved area dilated significantly (the luminal radius was 1.34 times larger than control; \( p<0.001 \), \( n=4 \)) with a significant increase of the cross-sectional area of the media (\( p<0.01 \), \( n=4 \)) and showed high wall shear stress (70±11 dynes/cm\(^2\) near the aortic orifice). In contrast, the endothelial cell-desquamated area did not dilate but did show very high wall shear stress (231±23 dynes/cm\(^2\)) without any intimal smooth muscle cell proliferation. The right CCA dilated significantly (luminal radius was 1.07 times larger than control; \( p<0.001 \), \( n=4 \)) with a wall shear stress of 30 dynes/cm\(^2\) near the brachiocephalic orifice. All CCAs retained their fundamental arterial structure. We conclude that in the rat CCA, arterial dilatation in response to increased flow is a gradual remodeling process related to the presence of endothelial cells that have been influenced by the level of flow increase. (Arteriosclerosis and Thrombosis 1992;12:519–528)

KEY WORDS • endothelial cells • blood flow • shear stress • rats • common carotid artery • arteriovenous fistula • ultrastructure • lumen diameter • adaptive dilatation

A significant effect of blood flow on the growth of vessel caliber was initially pointed out by Thoma in 1893. As a long-term response to flow, Kamiya and Togawa, using the arteriovenous (AV) fistula model in the canine carotid artery, suggested that the increased wall shear stress (WSS) induced the adaptive enlargement of the vessel radius, which acted as a negative feedback to reduce the stress itself. Recently Zarins et al., using the AV fistula model, showed a reduction of WSS to baseline level in the flow-loaded iliac artery of cynomolgus monkeys. As a short-term response to flow, Hull et al. showed that acute dilatation due to increased flow velocity was endothelium dependent after the endothelial surface of the canine femoral artery was rubbed with a cotton ball. On the other hand, Guyton and Hartley and Langille et al. demonstrated that the in vivo reductions in arterial diameter could be induced by a decrease in flow and were endothelium dependent.

Our experiments were designed to induce a blood flow increase in the common carotid artery (CCA) by formation of an AV fistula between the left CCA and the left external jugular vein in rats; we then observed the morphological changes in the bilateral CCAs after 8 weeks. In this model, because the shunted left CCA showed a marked increase of flow (over 10-fold) and the right contralateral CCA showed a constant mild increase of flow (twofold), we could simultaneously observe both markedly and mildly increased effects of flow in the same animal. Furthermore, we found that the endothelium-desquamated segment was always present in the shunted left CCA. Our present report describes the morphological changes of the flow-induced artery dilatation and the abolishment of those changes in the endothelium-desquamated segment.

Methods

Animals

Twenty-two male Sprague-Dawley rats (8 weeks old, 250–300 g) were used. Animals were divided into three groups as follows: 1) Ten animals were chosen for the AV fistula operation in the left CCA and the external jugular vein. Of the 10 rats, four were chosen for histological examination, four for scanning electron microscopy (SEM), and two for transmission electron microscopy (TEM). 2) Ten animals were designated as age-matched nonsurgical control rats. Of these 10 rats, four were chosen for histological examination, four for SEM, and two for TEM. 3) Two animals were designated as sham-operated control rats. Sample size (\( n \)) for
the analysis of CCA dimension (by histological examination) was four surgical and four nonsurgical animals. All animals were maintained for 8 weeks after the operation. To investigate the surgical trauma of the endothelium and to document the timing and extent of endothelial desquamation, which was observed at 8 weeks, 12 animals underwent the operation. Two animals were killed immediately after anastomosis had been completed but before flow had been reinitiated. Two animals were killed 24 hours after surgery, two after 3 days, two after 7 days, two after 2 weeks, and two after 4 weeks. The endothelial surface of the left CCAs was observed by SEM. The protocols for animal experimentation described in this article were previously approved by the Animal Research Committee, Akita University School of Medicine. All subsequent animal experiments adhered to the Guidelines for Animal Experimentation of the university.

Surgical Procedures

The animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.). Through a midline skin incision of the neck, the bilateral CCA and the left external jugular vein were exposed. After the segments of the left CCA and the left external jugular vein were stabilized with microsurgical clamps, we used a stereoscopic microscope (model OMS-60, Topcon, Tokyo) to make 1.5-mm-long longitudinal incisions to open both vessels ~20 mm distal from the aortic orifice. (The distance from the aortic orifice to the AV fistula was divided into three segments (proximal, middle, and distal). RCCA, right common carotid artery.

For TEM the resected left CCA between the aortic orifice and the left external jugular vein were exposed. After the segments of the left CCA and the left external jugular vein were stabilized with microsurgical clamps, we used a stereoscopic microscope (model OMS-60, Topcon, Tokyo) to make 1.5-mm-long longitudinal incisions to open both vessels ~20 mm distal from the aortic orifice. (The distance from the aortic orifice to the AV fistula was divided into three segments (proximal, middle, and distal). RCCA, right common carotid artery.

Tissue Preparation

Bilateral CCAs were fixed by perfusion with 3% glutaraldehyde as follows: 1) cannulation of a catheter into the abdominal aorta; 2) flushing of the whole blood by injection of 75 ml heparinized lactated Ringer’s solution through the catheter and blood drainage via a right renal vein cutdown; 3) injection of 50 ml 3% glutaraldehyde solution in sodium cacodylate buffer (pH 7.4) via the catheter under a constant pressure of 100 mm Hg that was maintained for 3 minutes by adjusting the height of the irrigator; 4) immediately after fixation, resection of the left CCA from the aortic orifice to the AV fistula and the proximal segment of the right CCA of the same length.

For histology, the resected CCAs were further fixed in 10% formaldehyde solution. The segment distal to the AV fistula and the aortic tissue near the aortic orifice were removed from the left CCA, which was then almost 20 mm long. The right CCA was trimmed by removing the brachiocephalic tissue near the brachiocephalic orifice and a segment 20 mm distant from the orifice. The resected arteries appeared almost straight but were slightly distorted. To obtain a precise cross section, straightly embedded specimens in paraffin are required. Therefore, we chose a postfixed human liver block, 4x2x1 cm, as the solid stage on which to fix the arteries. Perpendicular grooves were cut into the liver block to hold the arteries, after which both arterial edges were sutured with 9-0 nylon strings. All cross sections were obtained using the grooved liver tissue block. Complete serial thin sections from the aortic orifice to the AV fistula orifice, each 5.0 µm thick, were made continuously through the resected CCA. Every 10th section was stained with Masson’s elastica stain, and every section adjacent to the 10th was stained with hematoxylin and eosin.

For SEM the resected CCA was postfixed in 3% glutaraldehyde solution in sodium cacodylate buffer (pH 7.4) for 1 hour at 4°C. It was dehydrated through a series of alcohols and critical-point dried. The dried CCA was cut longitudinally to obtain two half-cylinder shapes. Then they were cut in the middle. The sample was finally divided into four segments, placed on a stage for SEM, and sputter-coated with gold–platinum. The endothelial surface was observed with an SEM (model JSM-T200, JEOL Co., Tokyo). Similar portions of the right CCA from animals that underwent surgery and bilateral CCAs from sham-operated and nonsurgical control rats were observed with an SEM.

For TEM the resected left CCA between the aortic orifice and the AV fistula was divided into three segments, namely distal, middle, and proximal (Figure 1). Each segment was further cut cross-sectionally into pieces 1–2 mm long. They were postfixed in 3% glutaraldehyde solution in sodium cacodylate buffer (pH 7.4) for 1 hour at 4°C and then fixed with 1% OsO₄ solution in phosphate buffer for 1 hour at 4°C. They were embedded in Epon, and ultrathin cross sections were made. They were stained with lead citrate and uranyl acetate and were observed with a TEM (model LEM.
2000, Akashi Co., Tokyo). Corresponding portions of the right CCA of the animals that underwent surgery and bilateral CCAs from sham-operated and nonsurgical control rats were also observed with a TEM.

**Measurement of Vessel Diameter**

Each of the 100 serial cross sections stained with Masson's elastica stain was used. Thirty-two sections taken every 0.625 mm (5 μm × 100 × 1.25, where 1.25 is a factor correcting for tissue shrinkage) were obtained, and they were numbered (from 1 to 32) starting from the aortic orifice to the AV fistula orifice (Figure 1). Because sections No. 1 and No. 2 included the aortic orifice and sections No. 31 and No. 32 included the AV fistula, actual values were obtained for 28 sections (from No. 3 to No. 30), which are approximately equally divided among the three segments (proximal, middle, and distal). With a profile projector (model V-16A, Nikon Co., Tokyo), the luminal and medial contours of the enlarged profile of the cross section (×200) were traced on tracing paper. Lumen circumference (Lh, centimeters) and cross-sectional area (CSAh, square centimeters) of the media on the histological section were then obtained using a digital image-analyzer system (Cosmozone 1, Nikon Co., Tokyo). Assumed in situ lumen circumference (L, centimeters), assumed in situ internal radius (r, centimeters), assumed in situ cross-sectional area (CSA, square centimeters), and assumed average medial thickness (MT, centimeters) were then calculated using the following equations after tissue shrinkage had been corrected for, using a factor of 1.25.8

\[
L = 1.25 \times Lh \\
\frac{r}{1} = L / 2 \pi \\
CSA = 1.25^3 \times CSAh \\
MT = CSA / L
\]

These measurements were performed in the bilateral CCAs of the four surgical and four nonsurgical control animals. In each numbered section of each CCA, values for r, MT, and CSA were calculated. To analyze statistical differences, average values of the same numbers of the four left CCAs of surgical animals were compared with those of the four right CCAs of surgical animals and the four left CCAs of nonsurgical animals. The four right CCAs of the surgical animals were compared with those of the right CCAs of the nonsurgical animals. Using the average lumen radii, we computed a three-dimensional projection of the CCA using the Cosmozone 2 system (Nikon Co.).

**Endothelial Cell Density**

With the SEM specimens described above, two areas were chosen in the endothelium-preserved segment of the left CCA of the surgical animals. One area was around the proximal segment near the aortic orifice, and the other was at the middle segment near the border of the endothelial cell—desquamated area. The average endothelial cell number per square millimeter was counted from 10 × 1,000 SEM photomicrographs taken in each area. The same counting was performed at the same levels in the right CCA of the four surgical animals and the bilateral CCAs of the four nonsurgical controls.

**Calculation of Hemodynamic Parameters**

Mean blood flow velocity (U, centimeters per second) was calculated as follows from the blood flow rate (BFR, milliters per minute) and r at the time the animals were killed:

\[
U = \frac{BFR}{(60 \times \pi \times r^2)}
\]

When blood flow is fast enough and the shear rate is high (as in the artery), blood flow is known to perform in a Newtonian fashion and to show a constant viscosity of about 0.03 poise in the dog and other animals.29,10 When the specific gravity of rat whole blood is assumed to be 1.056 g/cm3,11,12 the Reynolds' number (Re) can be calculated as follows:

\[
Re = 1.056 \times 2r \times U / 0.03
\]

In our experiments the maximal Reynolds' number was 220, which was well below the critical value of 2,000, at which value turbulent flow may occur in a smooth straight tube.

The entrance length (EL, centimeters) for the full development of steady laminar flow was calculated using the following equation, where 0.03 is an experimentally determined constant:10

\[
EL = 0.03 \times 2r \times Re
\]

In our experiments maximal entrance length was 0.45 cm in the shunted left CCA, which suggests that the WSS near the entrance zone is larger than the assumed value calculated for steady laminar flow. However, because the entrance lengths of the control CCA and the right CCA were within 0.1 cm, we assumed laminar flow conditions for our calculation of WSS, which was obtained by the following equation and was generally acceptable for these CCAs:

\[
WSS = 0.03 \times 4 \times BFR / (60 \times \pi \times r^2)
\]

**Statistical Analysis**

Our results are expressed as mean±SD. Statistical analysis was performed by Student's \(t\) test. Differences were considered significant if probability values were less than 0.05.

**Results**

**Blood Flow (Table 1)**

In the left CCA of the surgical animals, blood flow rate was significantly increased after anastomosis (nine times greater than before anastomosis) and 8 weeks after operation (11 times greater than before anastomosis). In the right CCA of surgical animals, 8 weeks after operation the blood flow rate was significantly increased (twofold greater than before anastomosis).

**Desquamation of the Endothelium**

Observation of the endothelial lining along its entire length (by serial histological sections) showed that endothelial cells were completely desquamated in the distal segment near the AV fistula of the left CCA in the...
Table 1. Blood Flow Rate

<table>
<thead>
<tr>
<th>Time</th>
<th>Surgical animals (n=10)</th>
<th>Nonsurgical controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left CCA</td>
<td>Right CCA</td>
</tr>
<tr>
<td>Before operation</td>
<td>2.3±0.6</td>
<td>2.6±0.7</td>
</tr>
<tr>
<td>After operation</td>
<td>21.5±2.9*</td>
<td>2.7±0.5</td>
</tr>
<tr>
<td>8 Weeks after operation</td>
<td>26.1±4.3*</td>
<td>5.5±1.4†</td>
</tr>
</tbody>
</table>

CCA, common carotid artery.
*Significantly larger than before operation (p<10^-4) in 10 surgical animals vs. the right CCA (p<0.0001) and the left CCA (p<0.0001) of 10 nonsurgical controls.
†Significantly larger than before operation (p<0.001) vs. immediately after operation (p<0.001) and vs. the right CCA of nonsurgical controls (p<0.01).

for surgical animals (Figure 2). Observation with SEM of the entire luminal surface of the left CCA from the four surgical animals showed that endothelial cells were completely desquamated in the distal segment as well. This desquamation was also confirmed by TEM observations in segments from two surgical animals. From SEM photomicrographs (Figure 3), the border between the desquamated area and the non-desquamated area was clearly defined and slightly oblique to the longitudinal axis of the vessel, which was placed 7.81±0.31 mm (n=4; average value of four SEM-observed data corrected by a tissue shrinkage factor) proximal from the orifice of the AV fistula (near section No. 21 of the serial histological sections). On the other hand, the left CCA of the sham-operated controls showed no endothelial desquamation. In the animals killed immediately after anastomosis but before flow was reinitiated, the endothelial desquamation was located in small limited areas near the suture line and clamped portions, but most of the endothelium was preserved in all segments of the left operated CCA. Similar desquamation of the endothelium in the distal segment occurred in the 8-week experimental group and was already observed 24 hours after operation. Such desquamation was also observed 3 days, 7 days, 2 weeks, and 4 weeks after operation.

Morphology of the Left Common Carotid Artery (Loaded by Markedly Increased Flow)

Endothelial layer. Near the border of desquamation, endothelial cells appeared markedly protruded (Figures 2c, 2d, 3, 4, and 5). They were slender (mostly 4 µm wide) and elongated along the longitudinal axis of the vessel. Microvillus projections were observed at the tips of their protrusions. They had irregularly thickened basement membranes and many well-developed microfilament bundles, mainly on the abluminal side. Endothelial cell density (Table 2) in the middle segment of the left CCA from surgical animals was very high and significantly greater than that of the nonsurgical controls, the proximal segment of the left CCA of surgical animals, and the distal and proximal segments of the right CCA of surgical animals.

On the other hand, in the proximal segment near the aortic orifice of the left CCA from surgical animals,
endothelial cells protruded slightly (Figures 6 and 7). They were wider than the cells near the border but not so wide as those from nonsurgical controls. Their basement membranes were slightly thickened, and microfilament bundles were moderately developed. Endothelial cell density was moderately increased and was significantly greater than that in the right proximal segment of surgical animals and the nonsurgical controls (Table 2).

Artery wall structure. In the endothelium-preserved segment there was no intimal thickening (Figures 2a–2d). The internal elastic lamina was preserved without corrugation, and the media showed no specific histological or ultrastructural changes. In the desquamated area, there was no intimal thickening but rather a smooth surface (Figures 2e, 2f, and 3). With TEM, a few platelets were observed adhering to the luminal surface (Figure 8). A thin layer, consisting of basement membrane–like material, covered the abluminal side of the internal elastic lamina. The internal elastic lamina and media were preserved, and there were no specific histological and ultrastructural changes.

Morphology of the Right Common Carotid Artery (Loaded by the Mildly Increased Flow)

Endothelial cells were mostly flat. Although no morphological changes were detected in the endothelial cells, their density was significantly greater than in the nonsurgical controls (Table 2). There was no intimal thickening. The internal elastic lamina and media were well preserved.

<table>
<thead>
<tr>
<th>Segment</th>
<th>Cell density (cells/mm²)</th>
</tr>
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<tbody>
<tr>
<td>Proximal</td>
<td>3,631 ± 361†</td>
</tr>
<tr>
<td>Middle</td>
<td>7,690 ± 714*</td>
</tr>
<tr>
<td>Distal</td>
<td>Desquamated</td>
</tr>
</tbody>
</table>

CCA, common carotid artery.

†Significantly larger than the left proximal segment (p < 0.0001), the right CCA (proximal segment, 2,856 ± 214 cells/mm² [p < 0.0001], and distal segment, 3,202 ± 312 cells/mm² [p < 0.0001]), and the nonsurgical controls (left proximal segment, 2,449 ± 88 cells/mm² [p < 10⁻³]).
Internal Radius (Figures 9 and 10)

The internal radius of the left CCA near the aortic orifice (section No. 3) of the four surgical animals was $0.628\pm0.019$ mm, which was significantly larger than that of the right CCA near the brachiocephalic orifice ($0.489\pm0.009$ mm; $p<0.001$) and the left CCA near the aortic orifice of the four nonsurgical controls ($0.479\pm0.018$ mm; $p<0.001$). The internal radius was gradually reduced distally, but proximal and middle segments (sections No. 3 to No. 20) were all significantly larger than the right CCA and the left CCA of nonsurgical controls. At the border between the endothelium-desquamated and -nondesquamated areas (section No. 21), the internal radius was $0.406\pm0.018$ mm, which was similar to that of the right CCA ($0.420\pm0.021$ mm) and the left CCA of the nonsurgical controls ($0.393\pm0.011$ mm). It showed a rather steep telescope-like reduction of the lumen diameter around the border and at the end of the reduced portion where the endothelium-desquamated zone started (Figure 10). The right CCA of the surgical animals showed slight dilatation in the middle and distal segments (sections No. 13 to No. 30), which were significantly larger than the right CCA of the nonsurgical controls ($p<0.05-0.01$). Furthermore, the internal radius of the desquamated segment of the left CCA (from sections No. 23 to No. 30) was slightly smaller than that of the right CCA of the surgical animals, although this difference was not statistically significant.

Cross-Sectional Area of the Media (Table 3)

In the nonsurgical controls ($n=4$), the cross-sectional area of the media was gradually reduced distally. In the left CCA of the surgical animals ($n=4$), the cross-sectional area of the proximal segment was significantly greater than that of the right CCA and the left CCA of the nonsurgical controls.

Thickness of the Media

In the left CCA of the surgical animals ($n=4$), medial thickness was $0.0318$ mm in the proximal segment, $0.0269$ mm in the middle segment, and $0.0279$ mm in the distal segment. These values were not significantly different from those from the same levels of the right CCA of surgical animals and the left CCA of nonsurgical animals ($n=4$).
The endothelium-desquamated segment is shown in black; note the tapering near the border.

30). The proportion of the length has been diminished by one almost at the arteriovenous fistula (right side) (section No. the aortic orifice (left side) (section No. 3) to the segment underwent surgery. The contour is for the segment almost at the contour of the left common carotid artery from a rat that

FIGURE 10. Computer-assisted reconstruction of the lumen contour of the left common carotid artery from a rat that underwent surgery. The contour is for the segment almost at the aortic orifice (left side) (section No. 3) to the segment almost at the arteriovenous fistula (right side) (section No. 30). The proportion of the length has been diminished by one fifth. The endothelium-desquamated segment is shown in black; note the tapering near the border.

FIGURE 9. Plot of internal radius of the rat common carotid artery. Closed and open circles are average values for the left and right common carotid arteries, respectively, from four surgical animals. Closed and open triangles are average values for the left and right common carotid arteries, respectively, from four nonsurgical controls. Error bars in the symbols represent standard deviations. Horizontal line shows two parameters (number of section [#] and distance from the aortic orifice [mm]). EC desquamation, segment where endothelial cells are desquamated; AV, arteriovenous.

Hemodynamic Parameters

Mean blood flow velocity (Table 4). In the surgical animals (n=4), blood flow velocity of the left CCA in the proximal segment was slightly greater than that in the right CCA. In the distal segment blood flow velocity was very high. In the right CCA of surgical animals, blood flow velocity was 13–17 cm/sec and was significantly greater than that of the right CCA of the nonsurgical controls (n=4).

Reynolds' number. In the left CCA of the surgical animals (n=4), the Reynolds' number was ~220 in the distal segment where the endothelium was desquamated, slightly reduced to ~170 in the middle segment, and 142 in the proximal segment near the aortic orifice, whereas in the right CCA the Reynolds' number was 44, 42, and 38 in the distal, middle, and proximal segments, respectively. In the bilateral CCAs of the nonsurgical controls (n=4), the Reynolds' number was 15–19.

Entrance length. The entrance length of the left CCA of the surgical animals (n=4) at the aortic orifice was 0.45 cm, whereas the entrance length of the right CCA at the orifice was 0.093 cm. The entrance length of the bilateral CCAs of the nonsurgical controls (n=4) at the orifice was 0.04 cm.

Wall shear stress (Table 4 and Figure 11). In the left CCA of the surgical animals (n=4), WSS was constantly very high, ~260–280 dynes/cm² in the distal segment from sections No. 24 to No. 30. WSS was then sharply reduced from sections No. 23 (260 dynes/cm²) to No. 13 (100 dynes/cm²) because of significant dilatation of the luminal radius. WSS at the border between the desquamated and non-desquamated areas was ~230 dynes/cm². Then it was gradually reduced to ~70 dynes/cm² near the aortic orifice. In the right CCA of the surgical animals (n=4), WSS was somewhat high, ~50 dynes/cm² in the distal segment, and was gradually reduced to 30 dynes/cm² at the proximal segment. In the nonsurgical controls (n=4), WSS of the bilateral CCA was almost fairly constant, between 15 and 28 dynes/cm².

Discussion

The artery is known to adapt to blood flow.1–7,13 A number of in vivo7,12–18 and in vitro20–34 studies have suggested that this flow-induced artery change is endothelium dependent. Langille and O'Donnell6 showed that when flow was decreased, the luminal diameter of the artery was reduced, and they demonstrated that this response was endothelium dependent by showing that the experimentally induced endothelium-desquamated artery loses its flow-dependent luminal diameter reduction. On the other hand, Hull et al4 showed that short-term dilatation induced by the elevated blood flow velocity was endothelium dependent, but for long-term flow-dependent dilatation, it is still unproved that this response is endothelium dependent, probably because it is rather difficult to maintain endothelial desquamation for such a long time. (Fishman et al35 showed that regeneration of the endothelium occurred after 14 days when the rat CCA was denuded by air-stream drying.)

In our present experiments, we found the occurrence of a segment showing distinct constant desquamation of the endothelium in all the rat CCAs that were loaded by markedly increased flow for 8 weeks (11-fold and 4.7-fold increases of flow compared with before operation and the right side of the same animals, respectively), which was accomplished by creating an AV fistula between the left CCA and the external jugular vein. The endothelium-desquamated segment was located in the distal segment of the CCA near the AV fistula. The border between the endothelium-desquamated and the endothelium-preserved areas was at a level 7.81±0.31 mm.
proximal to the AV fistula orifice. In this flow-loaded rat CCA we found that the endothelium-desquamated segment failed to dilate, whereas the endothelium-preserved segment showed lumen dilatation.

Fry showed that in the artery where extremely high WSSs occur (379±85 dynes/cm²), acute erosion of endothelial cells might occur within 1–3 hours. Also, Zand et al., using aortic stenosis in the rat, reported that the desquamation had occurred in 1 hour. In our present experiments, WSS at the border between desquamated and nondesquamated areas was very high (230 dynes/cm²), although WSS was an approximate value calculated by using the luminal radius obtained by histometry (correction factor of 1.25) and assuming laminar flow. We propose that one of the main causes of endothelial desquamation is very high WSS.

In the canine carotid artery, Kamiya and Togawa showed that in the artery where extremely high WSSs occur (379±85 dynes/cm²), acute erosion of endothelial cells might occur within 1–3 hours. Also, Zand et al., using aortic stenosis in the rat, reported that the desquamation had occurred in 1 hour. In our present experiments, WSS at the border between desquamated and nondesquamated areas was very high (230 dynes/cm²), although WSS was an approximate value calculated by using the luminal radius obtained by histometry (correction factor of 1.25) and assuming laminar flow. We propose that one of the main causes of endothelial desquamation is very high WSS.

This value may be smaller than the real value because this segment was within the entrance length.

### Table 3. Cross-Sectional Area of the Media

<table>
<thead>
<tr>
<th>Segment</th>
<th>Surgical animals (n=4)</th>
<th>Nonsurgical controls (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left CCA</td>
<td>Right CCA</td>
</tr>
<tr>
<td></td>
<td>Left CCA</td>
<td>Right CCA</td>
</tr>
<tr>
<td>Proximal (section No. 3)</td>
<td>10.10±0.56*</td>
<td>7.50±0.43</td>
</tr>
<tr>
<td>Proximal (section No. 7)</td>
<td>8.21±0.48*</td>
<td>6.78±0.53</td>
</tr>
<tr>
<td>Middle (section No. 13)</td>
<td>6.52±0.51</td>
<td>6.02±0.70</td>
</tr>
<tr>
<td>Distal (section No. 23)</td>
<td>5.81±0.31</td>
<td>6.03±0.14</td>
</tr>
</tbody>
</table>

Values are mean±SD in 10⁻² square millimeters. CCA, common carotid artery.

Values for the right CCA of nonsurgical controls are almost the same as the left CCA.

*Significantly greater than the middle segment (section No. 13; p<0.0001) and the distal segment (section No. 23; p<0.0001), the right CCA of surgical animals (n=4) (p<0.0001), and the left CCA of nonsurgical controls (n=4) (p<0.01).

### Table 4. Mean Blood Flow Velocity and Wall Shear Stress

<table>
<thead>
<tr>
<th>Segment/parameter</th>
<th>Surgical animals (n=4)</th>
<th>Nonsurgical controls (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left CCA</td>
<td>Right CCA</td>
</tr>
<tr>
<td></td>
<td>Left CCA</td>
<td>Right CCA</td>
</tr>
<tr>
<td>Proximal (section No. 3)</td>
<td>36±9</td>
<td>13±4</td>
</tr>
<tr>
<td></td>
<td>70±11†</td>
<td>39±14</td>
</tr>
<tr>
<td>Proximal (section No. 7)</td>
<td>41±9</td>
<td>15±5</td>
</tr>
<tr>
<td></td>
<td>98±14</td>
<td>42±13</td>
</tr>
<tr>
<td>Middle (section No. 13)</td>
<td>49±9</td>
<td>15±5</td>
</tr>
<tr>
<td></td>
<td>140±10</td>
<td>48±13</td>
</tr>
<tr>
<td>Distal (section No. 23)</td>
<td>87±16</td>
<td>17±5</td>
</tr>
<tr>
<td></td>
<td>263±51</td>
<td>51±14</td>
</tr>
</tbody>
</table>

Values are mean±SD and are in centimeters per second for U and dynes per square centimeter for WSS.

U, mean blood flow velocity; WSS, wall shear stress.

†WSS of the left CCA is significantly larger than that of the right CCA (proximal segment, p<0.01; middle segment, p<0.001; distal segment, p<0.0001) and the left CCA of the nonsurgical controls (n=4) (p<0.0001).

§WSS of the right CCA is significantly larger than that of the right CCA of nonsurgical controls (p<0.05).

This value may be smaller than the real value because this segment was within the entrance length.
ably induced by volume-loaded cardiac hypertrophy. In our preliminary data, the blood flow for the cranial side of the left CCA leading to the anastomosis showed negative flow (~1.0 ml/min), which indicates counterflow from the brain. All head and neck arteries have various collaterals; therefore, when the AV anastomosis produces the low-blood-pressure state, the contralateral CCA and the two vertebral arteries increase their blood flow, linking the collateral circulations via the circle of Willis. For the second reason, volume load due to the AV anastomosis may have induced dilatational hypertrophy. It has been suggested that this hypertrophy induces an increase of cardiac output. We suggest that these functional changes of the heart may influence blood flow in the contralateral CCA. The right CCA of surgical animals showed significantly higher mean blood flow velocity (13–17 cm/sec) than the controls (5–8 cm/sec; \( p < 0.05, n = 4 \)), but the WSS of the former was reduced to about 30 dynes/cm² at the proximal segment, which was almost similar to the normal level, 16–28 dynes/cm², of the CCA of control rats. On the other hand, WSS was still high, ~70 dynes/cm², even in the well-dilated proximal segment of the surgical left CCA. We consider that the rat CCA dilates significantly in response to increased flow and reduces its WSS to the normal baseline level when the flow increase is mild, around twofold. However, in the case of extremely great flow increases, the rat CCA fails to adapt sufficiently and cannot reduce its WSS to the normal baseline level.

Endothelial cells showed distinct changes that are known to be induced by elevated WSS, such as protrusion and increased cell density near the border of desquamation, where a very high WSS was expected, even 8 weeks after operation. However, these changes became indistinct in the proximal segment near the aortic orifice of the same flow-loaded CCA, where elevated WSS was considerably reduced due to luminal dilatation. The appearance of endothelial cells in the mildly flow-increased right CCA, where the level of WSS would be normal, was almost similar to the controls. We suggest that WSS, which is high even 8 weeks after operation, induces distinctive effects in the endothelial layer.

Zarins et al showed a distinct increase in cross-sectional area of the media, which indicated the presence of distinctive structural change. Masuda et al showed a restructuring of the artery wall, indicating intimal thickening, internal elastic lamina disruption, and changing of the medial smooth muscle cell arrangement. In our experiment with the endothelium-preserved area, we could not detect any fundamental structural changes, such as intimal thickening, destruction of the internal elastic lamina, or disturbance of the medial smooth muscle cell arrangement in the arterial wall. However, there was a significant increase in cross-sectional area of the media in the proximal segment near the aortic orifice of the markedly flow-loaded left CCA, where significant dilatation had occurred. This suggests that a remodeling in the media of the markedly flow-loaded rat artery should occur without destroying the fundamental structure. Also, we could not find any significant changes in the right CCAs of surgical animals, where flow had increased mildly. On the other hand, in the endothelium-desquamated segment of the left CCA, we could not detect any fundamental structural changes or intimal thickening, similar to the endothelium-preserved area. Moreover, no changes in cross-sectional area of the media were observed despite the very high WSS. Reidy and Bowyer described the endothelial damage in high-WSS areas of rabbit aortas and presumed those changes to be the one causative factor of atherosclerosis. However, in our observations, the endothelium-desquamated segment did not show any intimal thickening or structural changes, which is rather similar to observations for nonsurgical controls. We recognize that this flow-induced remodeling occurs in the segment where the endothelium is preserved. When blood flow increase is mild (as much as twofold), this remodeling may not be sufficiently adaptive when the blood flow increase is very high.

Remodeling of the arterial wall usually occurs in the human arterial system, for example, the coronary arteries, which dilate their diameters as atherosclerosis progresses, and we frequently find aneurysms in the abdominal aortas of older persons. From a histopathological point of view, because the endothelium is a flat single-cell layer on the luminal surface that is easily lost during preparation of histological section and blood flow occurs in the lumen only in situ, neither has usually been considered to be responsible for such a remodeling.
of the arterial wall. We suggest that this remodeling in response to flow change occurs in the endothelium (which we have shown in our experiments) and may occur in human arterial lesions as well.

References

Difference in dilatation between endothelium-preserved and -desquamated segments in the flow-loaded rat common carotid artery.
K Tohda, H Masuda, K Kawamura and T Shozawa

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