Heterogeneous Behavior of the Canine Arterial and Venous Wall

Importance of the Endothelium

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SUMMARY. Experiments were designed to determine the contribution of endothelial cells to the heterogeneous behavior of the arterial and venous wall. Rings of canine femoral, pulmonary, saphenous, and splenic arteries and veins, with and without endothelium, were mounted for isometric tension recording in Krebs-Ringer bicarbonate solution. Endothelium-dependent inhibitory responses to acetylcholine, adenosine triphosphate, bovine thrombin, and arachidonic acid were prominent in the arteries. In the veins, only transient endothelium-dependent relaxations to these substances were observed. Removal of the endothelium decreased the augmentation of the response to norepinephrine caused by anoxia in both arteries and veins. In the veins, arachidonic acid and thrombin caused endothelium-dependent increases in tension during contractions evoked by norepinephrine. The endothelium-independent inhibitory effects of isoproterenol and adenosine and the excitatory effects of acetylcholine and ATP were more pronounced in the veins than in the arteries. These experiments demonstrate that in the arterial and venous wall the endothelial cells can contribute to both inhibitory and excitatory responses of the smooth muscle cells of the media. Inhibitory endothelial responses prevail in the arteries, and excitatory ones in the veins. (Circ Res 51: 439-447, 1982)

BLOOD VESSELS of different anatomical origin can respond in a dissimilar manner to the same pharmacological or physiological stimulus; this can also be the case in the same vascular bed for the pre- and postcapillary vessels (see Furchgott, 1955; Bohr, 1965; Somlyo and Somlyo, 1970; Shepherd and Vanhoutte, 1975; Vanhoutte, 1978, 1980). Studies comparing the reactivity of isolated arteries with and without endothelium have demonstrated that the endothelial cells play an obligatory role in the relaxing effect of acetylcholine, ATP and thrombin (Furchgott and Zawadzki, 1980; De Mey and Vanhoutte, 1981a, 1981b; Furchgott et al., 1981; Chand and Altura, 1981; De Mey et al., 1982). Information is lacking concerning the role of endothelial cells in the response of smooth muscle cells of the venous wall. Therefore, the present experiments were designed to compare the importance of the endothelium in the responsiveness of different arteries and veins.

Methods

The experiments were performed on ring preparations of femoral, intrapulmonary, saphenous, and splenic arteries and veins taken from mongrel dogs of both sexes (24-35 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv). After excision, the preparations were placed in cold Krebs-Ringer bicarbonate solution (composition mEq: NaCl, 118.3; KCl, 4.7; CaCl₂, 2.5; MgSO₄·7H₂O, 1.2; KH₂PO₄, 1.2; NaHCO₃, 25.0; CaEDTA, 0.026; glucose, 11.1) and carefully cleaned with special care being taken not to touch their luminal surface (control rings). Some rings were placed on filter paper, the bent tips of a watchmaker’s forceps were inserted into their lumen, and the endothelial cell layer was removed by gently rolling the preparations back and forth over the filter paper for 15 seconds.

Isometric Force Recording

Each ring was attached to an isometric force transducer (Statham UC3) and suspended in an organ chamber filled with 50 ml of Krebs-Ringer bicarbonate solution (37°C) which was aerated with 95% O₂-5% CO₂. In certain experiments, the organ chamber solution was made anoxic by aerating it with 95% N₂-5% CO₂. Earlier work has shown that changing from the gas mixture containing 95% O₂-5% CO₂ to the one containing 95% N₂-5% CO₂ produced a rapid decrease in bath P0₂ from 640 to below 1 mm Hg without a significant change in pH (Vanhoutte, 1976; De Mey and Vanhoutte, 1980). Two rectangular platinum electrodes were placed parallel to the preparations and used to stimulate the adrenergic nerve endings in the blood vessel wall. Square wave (9 V, 2 msec) electrical impulses were provided by a direct current supply and switching transistor (MBLE BD 139) triggered by a stimulator (Janssen Scientific Instruments SUi) (Vanhoutte et al., 1967, 1979).

Before the experiments were begun, the rings were placed at the optimal point of their length-tension relationship using a standard electrical stimulation (15 Hz, 10 sec) or a standard concentration of norepinephrine (5 X 10⁻⁷ M; Table 1). The preparations were then allowed to equilibrate at their optimal length for 45 minutes.

Histology

To ascertain that the mechanical rubbing applied to the blood vessel rings had successfully removed the endothe-
<table>
<thead>
<tr>
<th>Blood vessel</th>
<th>Endothelium</th>
<th>Basal force at optimal length</th>
<th>Noradrenaline $\text{ED}_{50}$</th>
<th>Noradrenaline $\text{ED}_{10}$</th>
<th>Electrical stimulation $\text{ED}_{50}$</th>
<th>Maximal response</th>
<th>Acetylcholine $\text{ED}_{50}$</th>
<th>Maximal response</th>
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<td>0.61 ± 0.22</td>
<td>17.8 ± 1.7</td>
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<td>1.42 ± 0.31</td>
<td>3.1 ± 0.5</td>
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<td>1.10 ± 0.42</td>
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* Data shown as means ± SEM for blood vessels from different dogs; paired rings from each vessel, with and without endothelium, were studied in parallel. The values of basal force are for 36 rings in each group; for the other data, six vessels were studied in each group. The optimal length was determined using 5 $\times 10^{-7}$ M noradrenaline (7 min, every 20 min) and in all other blood vessels using 25 Hz electrical stimulation (10 sec, every 5 min). Expressed as percent of the maximal response to noradrenaline. In the pulmonary vein, maximal responses were obtained with 3 $\times 10^{-4}$ M, and in the other preparations with 10 $^{-3}$ M noradrenaline. Difference between preparations with and without endothelium is statistically significant ($P < 0.05$).

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**Figure 1.** Transverse sections of two preparations of the same canine saphenous vein (X225). Upper: intima left as intact as possible. Lower: after mechanical rubbing of the intimal layers. Left: when the preparation is transilluminated with white light, the silver nitrate grains show up as black dots. Right: when the same preparation is illuminated with green light, the silver complexes reflect the light. Note that in the presence of the endothelial layer (E), the silver nitrate is strictly localized at the endothelial-luminal border (A and B), which, after mechanical rubbing of the intimal layer (C and D), diffuses toward the media (M).
7.5% sucrose. The vessels were examined under light and electron microscopy following further fixation with osmium tetroxide and a polychromatic staining (Van Reempts and Borgers, 1975). In the arteries and veins with endothelium, "en face" light microscopic examination revealed a mosaic pattern of silver lines, which are considered to represent the borders of adjacent endothelial cells (Poole et al., 1958; Caplan et al., 1974); the study of transverse sections revealed that the silver nitrate had accumulated at the intimal border of the blood vessel wall (Fig. 1). Electron microscopy confirmed the presence of endothelial cells in the control rings (Fig. 2). By contrast, in arteries and veins where the intimal layers had been rubbed mechanically, no mosaic pattern was seen on "en face" light microscopy; the transverse sections revealed diffusion of the silver nitrate grains into the deeper layers of the blood vessel wall (Fig. 1). The absence of endothelial cells and medial diffusion of silver nitrate was confirmed by electron microscopy (Fig. 2). No

**Figure 2.** Electron micrograph (X7500) of the luminal part of two preparations of the same canine pulmonary vein. Upper: endothelium left as intact as possible. Note that the silver nitrate grains do not penetrate beyond the endothelial cells (E). Lower: after mechanical rubbing of the endothelium, no endothelial cells are seen, and the silver nitrate grains permeate into the media. SM = smooth muscle cells.
structural damage was obvious in other layers of the blood vessel wall.

Drugs

The drugs used were acetylcholine chloride (Sigma), adenosine (Sigma), adenosine triphosphate (ATP; Sigma), arachidonic acid (Sigma), isoproterenol hydrochloride (Aldrich Europe), L-norepinephrine bitartrate (Fluka), phentolamine mesylate (Ciba), and thrombin (bovine thrombin, Roche). Stock solutions were prepared in distilled water, from the stock solutions, 100 µl were added to the organ chamber solution. All concentrations are expressed as final molar concentrations (M).

Statistical Analysis

Each experimental group consisted of six preparations taken from six dogs. Rings with and without endothelium were prepared from the same segment of blood vessel. The data are shown as means ± SEM. The data were analyzed by use of analysis of variance. Differences were considered significant at P < 0.05. Only significant differences will be mentioned.

When relaxations were studied, the compounds were added during sustained contractions evoked by the ED30 (De Mey and Vanhoutte, 1980) of norepinephrine, determined individually in each blood vessel (Table 1). The relaxations are expressed as percent depression of these contractions.

Results

Norepinephrine

All blood vessels contracted when exposed to increasing concentrations (10⁻⁸ to 10⁻⁴ M) of norepinephrine. To judge from the ED50 values, the apparent sensitivity to norepinephrine decreased in the following order: pulmonary vein, splenic vein, femoral vein, femoral artery, saphenous vein, pulmonary artery, splenic artery, saphenous artery. Removal of the endothelium did not affect the sensitivity of the arteries and veins to norepinephrine but reduced the maximal contractile response to the catecholamine in all blood vessels studied, except the pulmonary vein (Table 1).

Electrical Stimulation

Electrical stimulation caused frequency-dependent contractions of all blood vessels studied (Table 1). When expressed relative to the maximal response to exogeneous norepinephrine, the largest contractile responses were obtained in the saphenous artery and vein and in the splenic vein, followed, in descending order, by the pulmonary artery, the splenic artery, the pulmonary artery, the pulmonary vein, the femoral vein, and the femoral artery. Removal of the endothelium did not affect the responsiveness of the arteries and veins to electrical stimulation (Table 1). In all preparations, phentolamine (3 × 10⁻⁶ M) abolished the contractions evoked by 8 Hz electrical stimulation.

Isoproterenol

The effect of increasing concentrations of isoproterenol was investigated during contractions of the arteries and veins caused by norepinephrine. In the veins, but not in the arteries, isoproterenol caused comparable, concentration-dependent relaxations; in all blood vessels, higher concentrations (10⁻³ to 10⁻⁴ M) caused increases in tension (Fig. 3). Removal of the endothelium did not affect the responsiveness of the arteries and veins to isoproterenol.

Adenosine

Arteries and veins, made to contract with norepinephrine, relaxed in a concentration-dependent manner when exposed to adenosine (10⁻⁶ to 10⁻³ M); the veins were more sensitive than the arteries (Fig. 3). Removal of the endothelium did not affect the responsiveness of the arteries and veins to adenosine.

Adenosine Triphosphate

In the four arteries, ATP (10⁻⁶ to 10⁻⁴ M) caused comparable, concentration-dependent relaxations during contractions induced by norepinephrine; such relaxations were observed in endothelium-denuded arteries only with concentrations of ATP higher than...
De Mey and Vanhoucke/Vascular Heterogeneity and Endothelium

FIGURE 4. Effect of increasing concentrations of adenosine triphosphate (ATP) on the contractile responses of femoral (○), pulmonary (□), saphenous (△) and splenic (▲) arteries (left) and veins (right) of the same six dogs to norepinephrine (ED₃₀; Table 1). Rings with (full lines) and without (dotted lines) endothelium were studied in parallel, the experimental protocol is identical to that used in Figure 1. Data expressed as percent depression of the response to norepinephrine, and shown as means. For the sake of clarity, the SEM are omitted. Full symbols indicate that the difference between preparations with and without endothelium is statistically significant. In veins with endothelium, ATP caused a biphasic response, with an initial relaxation followed by contractions; the initial relaxatory response to ATP is shown. Relaxations were not observed in the veins without endothelium. The steady state further increases in tension caused by ATP were comparable in the preparations with (data not shown) and without endothelium (data shown).

3 × 10⁻⁵ M (Fig. 4). In all veins, concentrations of ATP higher than 10⁻⁶ M caused further increases in tension during norepinephrine-induced contractions; these further increases in tension were not affected by the removal of the endothelium (Fig. 4). In the veins with endothelium, the increases in tension caused by ATP were preceded by transient relaxations.

Acetylcholine

Acetylcholine (3 × 10⁻⁷ to 10⁻⁴ M) did not significantly affect basal tension in isolated arteries, with and without endothelium. All veins contracted when exposed to acetylcholine; removal of the endothelium did not affect the contractile responses of the femoral, saphenous, and splenic veins, but augmented those of the pulmonary veins (Table 1; Fig. 5).

In the four arteries, made to contract with norepinephrine (ED₃₀; Table 1), acetylcholine caused comparable, concentration-dependent (3 × 10⁻⁹ to 10⁻⁶ M) relaxations, which were not observed in preparations without endothelium (Fig. 6). In the saphenous and femoral veins, 3 × 10⁻⁸ to 3 × 10⁻⁷ M acetylcholine, given during norepinephrine-induced (ED₃₀; Table 1) contractions, caused transient relaxations, which were not seen in deendothelialized preparations. In the splenic vein, both with and without endothelium, 10⁻⁸ to 3 × 10⁻⁷ M acetylcholine caused relaxations (Fig. 7). The same concentrations of acetylcholine did not affect contractile responses of the

FIGURE 5. Direct effect of increasing concentrations of acetylcholine in pulmonary veins with (— — —) and without (— — —) endothelium. Data expressed as percent of the maximal response to norepinephrine (see Table 1), and shown as means ± SEM. Full symbols indicate that the difference between preparations with and without endothelium is statistically significant.

FIGURE 6. Effect of increasing concentrations of acetylcholine on the contractile responses of femoral (○), pulmonary (□), saphenous (△), and splenic (▲) arteries of the same dogs to norepinephrine (ED₃₀; Table 1). Rings with (full lines) and without (dotted lines) endothelium were studied in parallel. The experimental protocol is identical to that used in Figure 1. Data expressed as percent depression of the response to norepinephrine, and shown as means. For the sake of clarity, the SEM are omitted. Full symbols indicate that the difference between preparations with and without endothelium is statistically significant.
FIGURE 7. Effect of increasing concentrations of acetylcholine on the contractile responses of femoral (□, left), saphenous (△, middle), and splenic (◇ right) veins of the same dogs to norepinephrine (ED\(_{30}\); Table 1). Rings with (full lines) and without (dotted lines) endothelium were studied in parallel. The experimental protocol is identical to that used in Figure 1. Data expressed as percent depression of the response to norepinephrine, and shown as means. For the sake of clarity, the SEM are omitted. Full symbols indicate that the difference between preparations with and without endothelium is statistically significant.

pulmonary veins, with and without endothelium, to norepinephrine. Higher concentrations of acetylcholine (10\(^{-6}\) to 10\(^{-4}\) M) caused increases in tension during contractions induced by norepinephrine in the veins, but not in the arteries; these increases were not affected by the removal of the endothelium (Fig. 6 and 7).

Thrombin

In the four arteries made to contract with norepinephrine, bovine thrombin (0.01 to 1 U/ml) caused dose-dependent relaxations which were absent in deendothelialized preparations (Fig. 8). In the four veins, contracted with norepinephrine, thrombin caused slow further increases in tension which stabilized within 10 minutes and were significantly larger in the presence of endothelium (Fig. 8); in the saphenous and splenic veins, the further increases in tension were preceded by transient, endothelium-dependent relaxations.

Arachidonic Acid

Arachidonic acid (10\(^{-7}\) to 3 \times 10\(^{-5}\) M) caused comparable, concentration-dependent relaxations in the four arteries contracted with norepinephrine. Removal of the endothelium shifted the dose-response curve for the fatty acid to the right in the femoral, pulmonary, and saphenous arteries and decreased its maximal effect in the femoral and saphenous artery; in the splenic artery without endothelium, arachidonic acid caused further increases in tension (Fig. 9). In the pulmonary vein, 3 \times 10\(^{-7}\) to 10\(^{-6}\) M arachidonic acid caused an endothelium-dependent decrease in tension during the contractile responses to norepinephrine; this was followed, at higher concentrations, by an increase in tension. In the other veins, the only significant effect of arachidonic acid was to augment the contractile response to norepinephrine. In all veins, the increases in tension caused by arachidonic acid were smaller in the absence of endothelium (Fig. 9).

Anoxia

Anoxia, induced for 10 minutes during contractile responses to norepinephrine, caused a further increase in tension in femoral, pulmonary, saphenous,

FIGURE 8. Effect of increasing concentrations of bovine thrombin on the contractile response of femoral (□), pulmonary (○), saphenous (△), and splenic (◇) arteries (left) and veins (right) of the same dogs to norepinephrine (ED\(_{30}\); Table 1). Rings with (full lines) and without (dotted lines) endothelium were studied in parallel. The experimental protocol is identical to that used in Figure 1. Data expressed as percent depression of the response to norepinephrine, and shown as means. For the sake of clarity, the SEM are omitted. Full symbols indicate that the difference between preparations with and without endothelium is statistically significant.

FIGURE 9. Effect of increasing concentrations of arachidonic acid on the contractile responses of femoral (□), pulmonary (○), saphenous (△), and splenic (◇) arteries (left) and veins (right) of the same dogs to norepinephrine (ED\(_{30}\); Table 1). Rings with (full lines) and without (dotted lines) endothelium were studied in parallel. The experimental protocol is identical to that used in Figure 1. Data expressed as percent depression of the response to norepinephrine, and shown as means. For the sake of clarity, the SEM are omitted, and when symbols overlap, only one is shown. Full symbols indicate that the difference between preparations with and without endothelium is statistically significant.
FIGURE 10. Effect of anoxia (10 min) on the contractile responses to norepinephrine (ED₉₀ Table 1) in canine arteries (upper) and veins (lower). Rings with (full bars) and without (open bars) endothelium were studied in parallel. Changes in tension are expressed as percent of the contractile response to norepinephrine, and shown as means ± sem. * the difference between rings with and without endothelium is statistically significant.

Discussion

The present experiments confirm that the endothelium plays an obligatory role in the relaxations of isolated arteries induced by acetylcholine (Furchgott and Zawadzki, 1980; De Mey and Vanhoutte, 1981a; Furchgott et al., 1981; De Mey et al., 1982). The ability of the endothelial cells to cause relaxation of the smooth muscle cells of the media, when exposed to acetylcholine, is comparable in the three systemic and pulmonary arteries. Greater differences in sensitivity occur for the endothelium-dependent relaxations of isolated arteries caused by ATP (De Mey and Vanhoutte, 1981a) and thrombin (De Mey et al., 1982). The experiments with arachidonic acid confirm that, in isolated arteries, the fatty acid can cause endothelium-dependent relaxations (De Mey et al., 1982), which are remarkably similar in the different canine arteries tested. Thus, despite minor regional differences in apparent sensitivity, the arterial wall appears relatively homogeneous in its ability to exhibit endothelium-dependent relaxations of the smooth muscle cells of the media. This certainly is not the case for the veins studied. Only in the femoral and saphenous vein could moderate endothelium-dependent relaxations be observed during norepinephrine contraction. In the pulmonary vein, endothelium-mediated inhibitory responses were suggested only by the augmented responsiveness to acetylcholine after removal of the intima, whereas, in the splenic vein, no such evidence could be obtained. Obviously, in the veins, the evaluation of endothelium-dependent inhibitory responses to acetylcholine is complicated by the marked regional differences in venous smooth muscle responsiveness to the direct stimulating properties of the cholinergic transmitter (see Shepherd and Vanhoutte, 1975; Vanhoutte, 1978). ATP, thrombin, and arachidonic acid caused only transient relaxations in certain isolated veins. One logical explanation for the transient character of these relaxations is that other actions of the vasoactive substances mask the inhibitory endothelium-dependent effect that they have; the data with isoproterenol and adenosine demonstrate that it cannot be attributed to a relative inability of the venous smooth muscle cells to relax.

The contact of norepinephrine with endothelial cells may cause the smooth muscle to contract before the amine has permeated the vascular wall (Bevan and Duckles, 1975; Pascual and Bevan, 1980). Earlier work in the femoral artery of the dog indicated that the intima may contribute to the contractile responses to high K⁺ and anoxia (De Mey and Vanhoutte, 1981a, 1981b). The present study demonstrates that endothelial cells can facilitate the contraction of the vascular smooth muscle cells of the media. This conclusion is based on the observations that endothelium removal: (1) reduces the maximal contractile response to norepinephrine. This reduction is not likely to be due to adverse effects of the procedure to remove the endothelium on the viability of the preparations. Indeed, the shape of the concentration-response curve was not affected and the maximal response to the catecholamine was only moderately, or not significantly, reduced in some blood vessels. Furthermore, the contractile response to several other interventions was enhanced by the procedure, (2) reduces the increase in tension caused by thrombin in the veins, (3) reduces, or abolishes, the contractions of the veins caused by arachidonic acid, and (4) reduces, or reverses, the increases in tension caused by acute anoxia during norepinephrine-induced contraction.

The predominance of inhibitory endothelial responses in arteries and of excitatory ones in the veins cannot be explained solely by differences in synthesis of prostacyclin, since the endothelium-dependent effects of acetylcholine, ATP, thrombin, and anoxia on the canine femoral artery are not antagonized by inhibitors of cyclooxygenase (e.g., Furchgott et al.,...
1981; De Mey et al., 1982; De Mey and Vanhoutte, 1981b) and since prostacyclin, if anything, depresses the contractile response of the canine saphenous vein to norepinephrine (Herman et al., 1978). Likewise, both inhibitory and excitatory endothelial-dependent responses were observed in blood vessels independently of their embryological origin (Tsuru et al., 1976; Bevan, 1979), their responsiveness to sympathetic nerve stimulation (Vanhoutte, 1978; Vanhoutte, 1979; Abel et al., 1980; Vanhoutte et al., 1981) or their chronic exposure to arterial or venous blood (Vanhoutte, 1978). However, endothelium-dependent augmentation of norepinephrine-induced contractions with thrombin and arachidonic acid are seen only in those blood vessels studied which are located in the low pressure side of the circulation and are not exposed to pulsatile blood flow.

To judge from the ED50 of norepinephrine, the apparent sensitivity to the catecholamine varied among the different arteries and veins reflecting the true heterogeneity in vascular postjunctural adrenergic responsiveness (see Bevan et al., 1980; Vanhoutte et al., 1981). An important difference between deendothelialized arterial and venous preparations was the absence of contractions induced by acetylcholine, ATP, and thrombin in the arteries. In the case of acetylcholine, the present study confirms that the responsiveness of venous smooth muscle cells to the excitatory effect of acetylcholine varies depending on their anatomical origin (see Shepherd and Vanhoutte, 1975; Vanhoutte 1977, 1978). In the splenic vein contracted with norepinephrine, acetylcholine causes a relaxation which depends neither on prejunctional inhibition of norepinephrine release (Vanhoutte, 1977; Vanhoutte et al., 1981) nor on generation of inhibitory signals by the endothelial cells (Furchgott and Zawadzki, 1980; De Mey and Vanhoutte, 1981a). This inhibitory effect occurs at lower concentrations of acetylcholine than those required to activate the smooth muscle cells, and is muscarinic in nature since it is abolished by atropine (unpublished observations). The present study thus implies that, at least in certain vascular beds, the cholinergic transmitter may cause dilation by virtue of a presumably direct inhibitory effect on the vascular smooth muscle cells, in addition to any prejunctional or endothelium-mediated effects it may have (see Vanhoutte, 1977, 1978, 1981; Furchgott et al., 1981 Vanhoutte et al., 1981). Canine veins relax when exposed to isoproterenol (e.g., Vanhoutte and Shepherd, 1970, 1973). These relaxations do not depend on the presence of functional endothelial cells (Furchgott et al., 1981) and are not observed in canine arteries, as already noted in earlier work, at least the splenic artery (e.g., Vanhoutte and Shepherd, 1970; Van Hee and Vanhoutte, 1978). Other qualitative differences in responsiveness, during exposure to norepinephrine, between the arteries and veins were the presence of an inhibitory effect of arachidonic acid in certain deendothelialized arteries, and the absence of contraction caused by anoxia in certain deendothelialized veins (Vanhoutte, 1976). The most striking quantitative difference between arterial and venous preparations was the greater response of the veins to the endothelium-independent inhibitory effect of adenosine (De Mey and Vanhoutte, 1981a; Furchgott et al., 1981). Whether or not the heterogeneity of the smooth muscle cells contributes to the differences in sensitivity to endothelium-mediated phenomena will remain unknown until the exact nature of the link between the endothelial and smooth muscle cells is determined.

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Circulation Research/Vol. 51, No. 4, October 1982
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INDEX TERMS: Acetylcholine • Adenosine triphosphate • Arachidonic acid • Arterial smooth muscle • Endothelium-dependent contractions • Endothelium-dependent relaxation • Thrombin and venous smooth muscle
Heterogeneous behavior of the canine arterial and venous wall. Importance of the endothelium.
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Circ Res. 1982;51:439-447
doi: 10.1161/01.RES.51.4.439

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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