Marked Heterogeneity of Endothelin-Mediated Contractility and Contraction Dynamics in Mouse Renal and Femoral Arteries

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Although endothelin (ET)-1 is one of the strongest known vasoconstrictors in most species, we and others have previously found that it is only weakly effective in the mouse aorta. The aim of this study was to further investigate vasoactive effects of ET-1 in vascular beds generally known to be particularly sensitive to ET-1, such as the renal artery. Experiments were performed to determine the vasoconstrictor responses in the thoracic aorta, and in the carotid, femoral, and renal arteries. Isolated vascular rings of C57BL/6 adult male mice (35–40 weeks of age) were exposed to ET-1 (0.01–300 n*M*) in the presence of the nitric oxide synthase inhibitor L-NAME (0.3 m*M*) to exclude effects of nitric oxide. Vessels from different vascular beds demonstrated distinct patterns in potency of the contractions to ET-1 and the dynamics of the responses. The maximal contraction to ET-1 was strong and significantly greater in the femoral (105 ± 7% KCl) and renal artery (62 ± 7% KCl) than in the carotid artery or the aorta ($P < 0.05$). The dynamics of the contractile response to ET-1 varied between the different vessels: the renal artery showed a rapid vasoconstriction, followed by a near complete loss of tension, whereas in the aorta, carotid, and femoral artery, vasoconstriction was more sustained. In conclusion, the data demonstrate that mouse femoral and renal arteries exhibit strong contractions in response to ET-1 compared with aorta and carotid artery, and that contractile dynamics differ markedly between arterial vascular beds. These findings may be important for studying the effects of endothelin in mouse models of human disease.
Exp Biol Med 231:777–781, 2006

Key words: anatomic; vascular; heterogeneity; femoral; mouse

Introduction

Endothelin (ET)-1 is the strongest known vasoconstrictor of a wide range of blood vessels in most species and contributes to pathophysiologic conditions such as atherosclerotic lesion development (1). The C57BL/6 mouse serves as the genetic background for most transgenic mice used to study animal models of human disease. However, mouse vascular physiology has not been extensively studied, and a study comparing vasoconstrictor agonists in mouse aorta suggested that the ET system may not function in the same way in mice as it does in other species (2). Although ET-1 effects a sustained and strong contraction in the rabbit, rat, and human vasculature (3, 4), the mouse vasculature appears to be less sensitive to ET-1. Vascular effects of ET-1 are mediated by activation of ET_A and ET_B receptors. The ET_A receptor is located on vascular smooth muscle cells and mediates potent and long-lasting vasoconstriction and also cell proliferation (5) and contributes to atherosclerosis (1). In contrast, stimulation of the ET_B receptor largely results in endothelial cell–mediated vasodilatation via release of nitric oxide (NO) and prostacyclin and only rarely causes vasoconstriction (1, 6, 7). To delineate the vasoconstrictor role of the non-selective receptor agonist ET-1 in the vascular system of the mouse, the present study was conducted to investigate the functional responses to ET-1 in different vascular beds during acute NO synthase inhibition.

Materials and Methods

Animals. Experiments were performed using male C57BL/6 mice (35–40 weeks of age, mean body weight 32 ± 3 g, Charles River, Sulzfeld, Germany). Animals were housed in the institutional animal facilities and received standard rodent chow and tap water *ad libitum*. The study was approved by the local authorities for animal research (Komission für Tierversuche des Kantons Zürich, Switzerland).

Organ Chamber Studies. Experiments were performed as previously described (8). Briefly, animals were anesthetized (ketamine, xylazine, and acepromazine) and

Supported by the Swiss National Foundation (SCORE 3200–058426.99, 3232–058421.99, and 3200–108258/1), and the Hanne Liebermann Stiftung, Zürich.

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Received August 22, 2005.
Accepted November 17, 2005.

1535-3702/06/2316-0777$15.00

Table 1. Endothelin-Mediated Responses in Different Vascular Beds[a]

	Femoral artery	Renal artery	Carotid artery	Aorta
E_{max} (% KCl)	104.9 ± 7.4	61.9 ± 7.4[b]	46.0 ± 6.7[b,c]	10.6 ± 1.4[b,c,d]
pD_2	8.1 ± 0.1	8.4 ± 0.1[b]	8.5 ± 0.1[b,c]	8.5 ± 0.1[b]
AUC	180 ± 17	126 ± 16[b]	89 ± 12[b,c]	21 ± 3[b,c,d]

[a] Maximal responses (E_{max}) expressed as % constrictor response to KCl (100 m*M*); pD_2 values (–log *M* of EC_{50}) and the area under the curve (AUC, a measure of overall contractility) are given in arbitrary units, femoral artery, $n = 21$; renal artery, $n = 15$; carotid artery, $n = 19$; aorta, $n = 19$. Data are means ± SEM.
[b] $P < 0.05$ versus femoral artery.
[c] $P < 0.05$ versus renal artery.
[d] $P < 0.05$ versus carotid artery.

sacrificed by exsanguination. The thoracic aorta and the carotid, renal, and femoral arteries were immediately isolated, cleaned of perivascular tissue, and cut into rings 3 mm in length; renal artery: 1.5–2 mm. Vascular rings were mounted onto fine tungsten wires (100 μm for aorta and carotid artery; 50 μm for the femoral artery), placed into organ chambers containing Krebs-Ringer Solution (118.6 NaCl, 4.7 KCl, 2.5 $MgSO_4$, 25.1 $NaHCO_3$, 1.2 KH_2PO_4, 1.6 $CaCl_2$, 10.1 glucose, and 0.026 $EDTA_{Na2Ca}$ [in m*M*]) and connected to force transducers (Hugo Sachs Elektronic Harvard Apparatus GmbH, March-Hugstetten, Germany) for recording of isometric tension (Rikadenki, Freiburg, Germany). The renal artery was mounted on two 40-μm tungsten wires in a Mulvany myograph (DMT, Aarhus, Denmark) (9).

Experimental Protocols. After an equilibration period of 20 minutes, resting tension was gradually increased over 60 minutes to 1.75 g in the aortic, 1.25 g in the carotid, and 1 g in the femoral segments; passive tension in renal arteries was normalized via passive stretching to 90% of the unconstricted *in vivo* internal circumference at a transmural pressure of 100 mm Hg (10). The integrity of vascular smooth muscle cell function was confirmed by challenging the rings three times with 100 m*M* KCl for maximal contraction (aorta, 1 ± 0.07 g; carotid artery, 0.2 ± 0.02 g; femoral artery, 0.2 ± 0.02 g; renal artery, 0.8 ± 0.06 g). Rings were preincubated with the NO synthase inhibitor L-nitro-arginine methyl ester (0.3 m*M*) for 30 minutes to block basal NO synthesis. Thereafter, rings were exposed to cumulative concentrations of ET-1 (0.01–300 n*M*).

Drugs. Endothelin-1 and N^G-L-nitro-arginine methyl ester were purchased from Alexis Biochemicals, Lausen, Switzerland. All other chemicals were from Sigma Chemicals Co. (Buchs, Switzerland). Drugs were dissolved in purified water (MiliQ, Volketswil, Switzerland) and diluted with Krebs solution to the concentration needed before each experiment. Concentrations are expressed as final molar concentration in the organ chamber.

Calculation and Statistical Analysis. Data are expressed as mean ± SEM, and *n* equals the number of animals used. Contraction to ET-1 is expressed as the percentage of contraction to potassium chloride (KCl, 100 m*M*). EC_{50} values (as negative logarithm: pD_2), and area under the curve was calculated by nonlinear regression analysis (Sigmaplot, Version 9). Rate of loss of tension was calculated by taking the percentage of maximum contraction of ET-1 as a function of time (5 and 20 minutes). A *P* value < 0.05 was considered significant.

Results

Contractile Responses to ET-1. In all vessels investigated ET-1 produced a concentration-dependent vasoconstriction. The different vascular beds demonstrated distinct patterns in potency of ET-1–mediated contractions (Table 1). Contractions were stronger in the femoral artery than in all other vessels investigated ($P < 0.0001$, Fig. 1). Responses in the renal artery were also significantly stronger compared with the carotid artery ($P < 0.05$, Fig. 1C) and the aorta ($P < 0.0001$, Fig. 1D). Contractions were less potent in the aorta than in the carotid artery ($P < 0.005$, Fig. 1C and 1D), renal, and femoral artery ($P < 0.0001$, Fig. 1A and 1B). Maximal contraction, area under the curve and pD_2 values of responses are given in Table 1.

Contraction Dynamics of ET-1–Mediated Responses. Endothelin-1 contractile dynamics varied across the different vascular beds. Endothelin-1 induced a rapid vasoconstriction in all vessels; however, tension maintenance was markedly different between arteries. The aorta, carotid, and femoral arteries demonstrated a slow loss of tension over time, whereas the renal artery showed a near complete and rapid loss of tension (Fig. 2). Within the first 5 minutes after reaching the maximal contraction, the loss of tension was greatest in the renal artery (58 ± 4% of ET-1_{max}), whereas contractions in the aorta (69 ± 4% of ET-1_{max}), carotid (76 ± 4% of ET-1_{max}, $P < 0.005$ vs. renal) and femoral artery (83 ± 4% of ET-1_{max}, $P < 0.0001$ vs. renal) were more sustained (Fig. 2).

Twenty minutes after the maximum ET-1–induced contraction (ET-1_{max}), tension in the renal artery had decreased by 80 ± 3%, whereas the decrease was 61 ± 4% in the femoral artery ($P < 0.005$ vs. renal), 72 ± 4% in the carotid artery, and 77 ± 4% in the aorta (Fig. 3). The loss of tension, calculated as a function of time 5 minutes

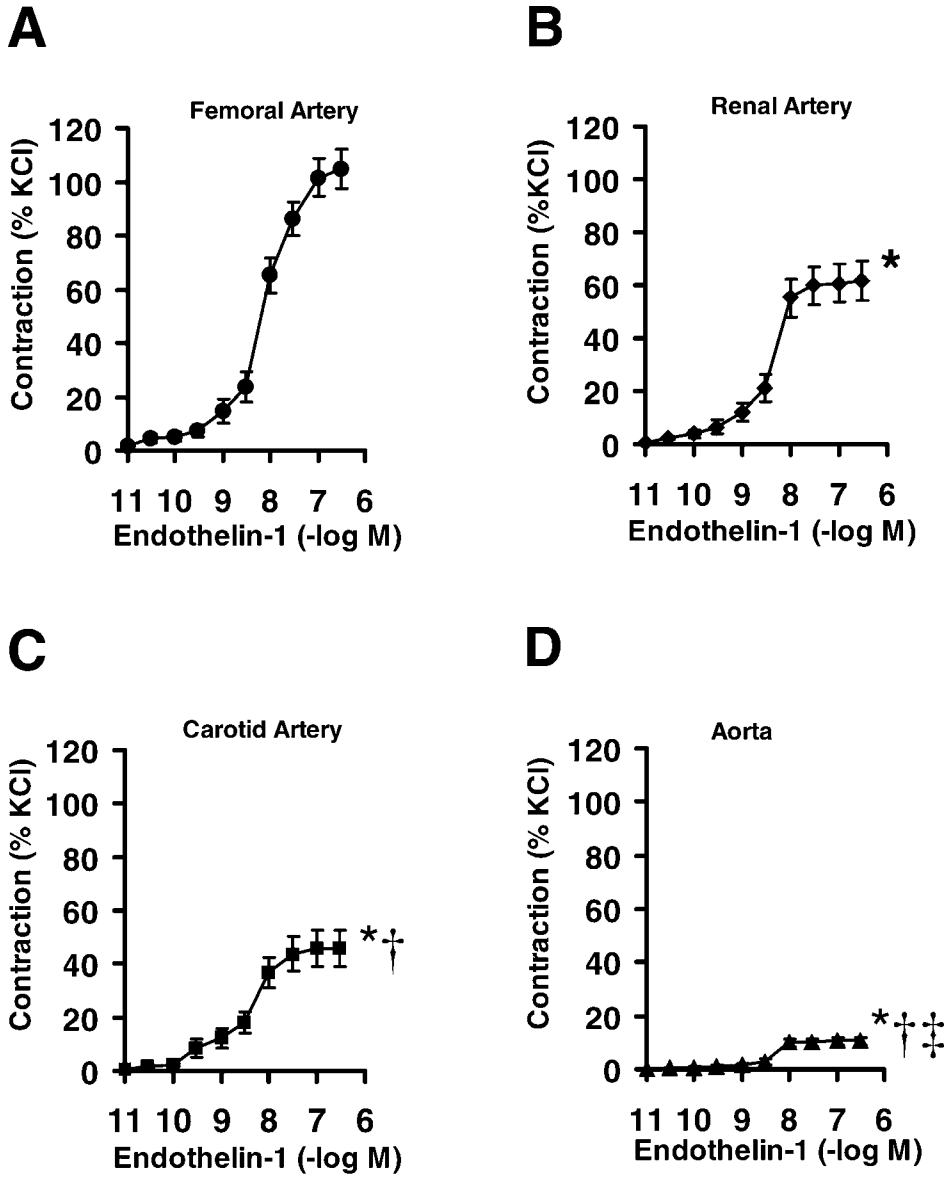

Figure 1. Concentration-response curves to ET-1 in the presence of L-nitro-arginine methyl ester in the femoral artery (A), renal artery (B), carotid artery (C), and aorta (D) of healthy C57BL/6 mice. Contractions are expressed as a percentage of 100 m*M* KCl. *$P < 0.05$ vs. femoral artery. †$P < 0.05$ vs. renal artery. ‡$P < 0.05$ vs. carotid artery.

after reaching maximum contraction, was 8 ± 1%/min (renal artery), 5 ± 1%/min (carotid artery), and 3 ± 1% (femoral artery), both $P < 0.005$ versus renal artery.

Discussion

In the current study, we investigated the effect of ET-1 on different vascular beds in healthy laboratory mice. The main new finding of this study is that different vessels in the C57BL/6 mouse show significantly different contraction responses to ET-1. This study is also the first to demonstrate strong contractility to ET-1 in mouse femoral and renal arteries and different contraction dynamics between vascular beds.

It has previously been reported that ET-1 is only weakly effective as a constrictor in the mouse aorta (3, 8); we have previously confirmed and extended these findings demonstrating that the carotid artery of the mouse shows a greater vasoconstrictor response to ET-1 than the aorta (8). In the present study, examining other vascular beds, we now report that vessels distant from the heart (renal and femoral artery) show a much stronger response to ET-1 than arteries close to the heart (aorta and carotid artery). To our knowledge, the findings reported here indicate that the femoral artery displays the strongest contraction to ET-1 in the mouse seen to date. This difference in the vascular contractile response may be related to differences in

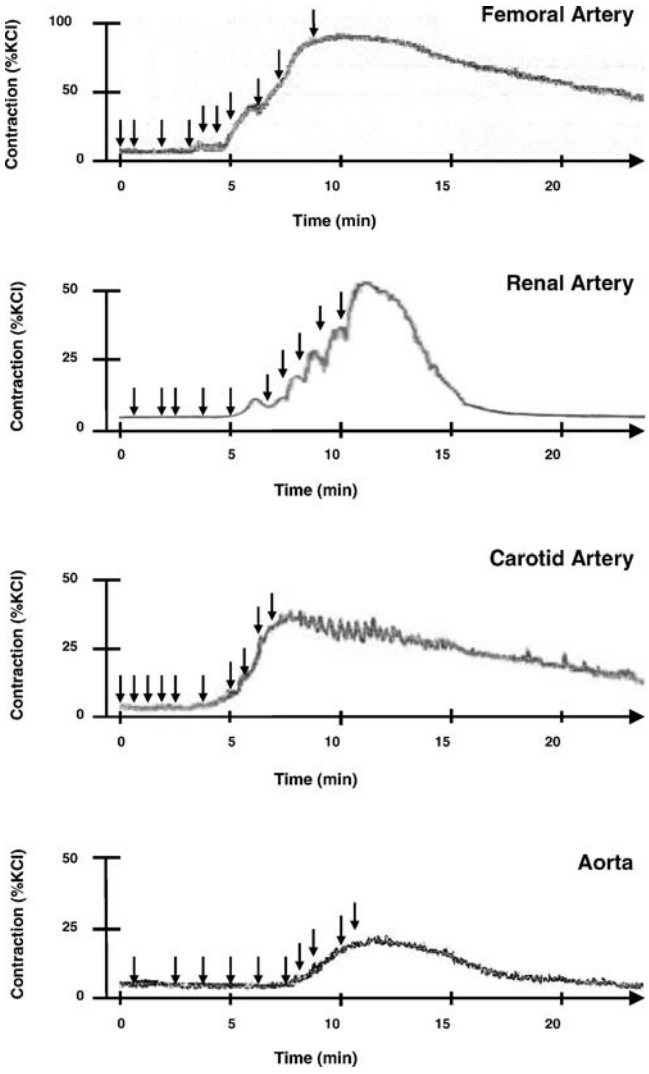

Figure 2. Original recordings of representative tracings of responses to ET-1 (0.01–300 n*M*) in femoral artery (top panel), renal artery (second panel), carotid artery (third panel) and aorta (fourth panel) of healthy C57BL/6 mice, ↓ indicates addition of increasing ET-1 concentrations. The scale on *y* axes indicates contraction related to KCl 100 m*M*.

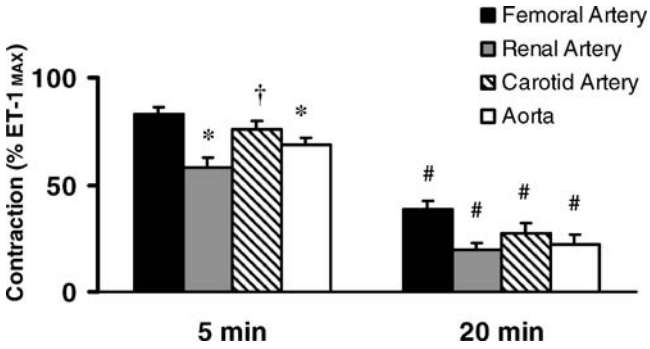

Figure 3. Tension loss after maximal contraction to ET-1 (ET-1_{max}) after 5 minutes (left panels) and 20 minutes (right panels) in the femoral, renal, and carotid arteries, and aorta, given as percent of ET-1_{max}. *$P < 0.05$ vs. femoral artery, †$P < 0.005$ vs. renal artery, #$P < 0.05$ vs. 5 minutes in the same vessel.

compliance between the vessels. Arteries of the elastic type such as the aorta and carotid artery showed weaker responses than the femoral and renal artery, which show features of "muscular" type arteries. The different responsiveness to ET-1 may also be affected by intracellular Ca^{2+} concentrations of different vascular beds, which mediates the contraction potential (11) and, thus, overall contractility. Also, that the anatomy of the muscle cell layer (tunica muscularis) differs between conducting vessels (elastic type) and regulating vessels (muscular type) can affect their response to ET-1 (12). Specifically, arteries with long and thin cell architecture such as the femoral and renal arteries have been found to exhibit different contraction dynamics than those with shorter but thicker cell architecture such as the aorta and carotid arteries (13). Moreover, it is possible that the ability of muscular arteries to contract is facilitated by the organization of the structural components of the arterial wall and a lower elastic tissue volume (12).

Another new finding of this study was the observation that in different mouse arteries a decrease in tension occurred during ET-1–induced contractions that varied between the vessels investigated. Although both the femoral and renal artery showed a highly sensitive response to ET-1, these vessels demonstrated markedly different contraction dynamics. In the femoral artery, tension was sustained over the first 5 minutes, whereas the renal artery showed a rapid and nearly complete loss of tension. We subsequently challenged the same vessels with 100 m*M* KCl, confirming that the vascular smooth muscle was not damaged, and obtained strong contraction responses. Again, as with contraction potency, contraction dynamics could be due to different compliances of the vessels, differences in intracellular Ca^{2+} signaling (11), or different anatomic architecture of the vascular smooth muscle cells. Additionally, the loss of tension may be, in part, due to an active vasodilation effected by stimulation of the ET_B receptor. This receptor is commonly known to produce vasodilation through activation of NO; however, ET_B-receptor activation may stimulate prostacyclin release under certain conditions (14–16). Although NO production was inhibited by L-nitroarginine methyl ester in these experiments, ET_B-receptor stimulation may have induced prostacyclin release, thereby producing a dilator effect, and possibly contributing to the observed differences (1, 17). Finally, distribution of ET_A and ET_B receptors may vary between vascular beds (6, 18); therefore, differences in the density of contractile ET_A and dilator ET_B receptors may play a role in the differences seen between the vessels examined in this study.

In conclusion, these data demonstrate that the vascular response to ET-1 in healthy C57BL/6 mice show a unique reactivity that is markedly different to that normally seen in other species, including man, rat, or pig (4). Future studies will help to address whether different disease states affect

the vascular reactivity to ET in mouse models of human disease.

This work is to meet the requirements for the M.D. Thesis of Corinne Widmer at the Medical Faculty of the University of Zürich. For her work, Corinne Widmer received the Young Investigator Award at the 9th International Conference on Endothelin, Park City, Utah in September 2005. The authors gratefully acknowledge the support of Wilhelm Vetter.

1. Luscher TF, Barton M. Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs. Circulation 102:2434–2440, 2000.
2. Russell A, Watts S. Vascular reactivity of isolated thoracic aorta of the C57BL/6J mouse. J Pharmacol Exp Ther 294:598–604, 2000.
3. Wiley KE, Davenport AP. Endothelin receptor pharmacology and function in the mouse: comparison with rat and man. J Cardiovasc Pharmacol 44(Suppl 1):S4–S6, 2004.
4. Zubkov AY, Rollins KS, Zhang JH. KCl activates mitogen-activated protein kinase in rabbit bailar artery. Biochem Biophys Res Commun 293:660–664, 2002.
5. Traupe T, D'Uscio LV, Muenter K, Morawietz H, Vetter W, Barton M. Effects of obesity on endothelium-dependent reactivity during acute nitric oxide synthase inhibition: modulatory role of endothelin. Clin Sci (Lond) 103(Suppl 48):13S–15S, 2002.
6. Zhou Y, Dirksen WP, Zweier JL, Periasamy M. Endothelin-1-induced responses in isolated mouse vessels: the expression and function of receptor types. Am J Physiol Heart Circ Physiol 287:H573–H578, 2004.
7. Lamping K, Faraci F. Enhanced vasoconstrictor responses in eNOS deficient mice. Nitric Oxide 8:207–213, 2003.
8. Traupe T, Lang M, Goettsch W, Munter K, Morawietz H, Vetter W, Barton M. Obesity increases prostanoid-mediated vasoconstriction and vascular thromboxane receptor gene expression. J Hypertens 20:2239–2245, 2002.
9. Mulvany MJ, Halpern W. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. Circ Res 41:19–26, 1977.
10. DeLean A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves. Am J Physiol 235:E97–E102, 1978.
11. Keatiuge WR. Ionic requirements for arterial action potential. J Physiol (London) 194:169–182, 1968.
12. Levicky V, Dolezel S. Elastic tissue and smooth muscle volume in elastic and muscular type arteries in the dog. Physiol Bohemoslov 29: 351–360, 1980.
13. McGrath JC, Deighan C, Briones AM, Shafaroudi MM, McBride M, Adler J, Arribas SM, Vila E, Daly CJ. New aspects of vascular remodelling: the involvement of all vascular cell types. Exp Physiol 90: 469–475, 2005.
14. Dusting GJ, Chapple DJ, Hughes R, Moncada S, Vane JR. Prostacyclin (PGI_2) induces coronary vasodilatation in anaesthetised dogs. Cardiovasc Res 12:720–730, 1978.
15. Hirata Y, Emori T, Eguchi S, Kanno K, Imai T, Ohta K, Marumo F. Endothelin receptor subtype B mediates synthesis of nitric oxide by cultured bovine endothelial cells. J Clin Invest 91:1367–1373, 1993.
16. Rebsamen MC, Capoccia R, Vallotton MB, Lang U. Role of cyclooxygenase 2, p38 and p42/44 MAPK in the secretion of prostacyclin induced by epidermal growth factor, endothelin-1 and angiotensin II in rat ventricular cardiomyocytes. J Mol Cell Cardiol 35: 81–89, 2003.
17. Filep JG, Battistini B, Cote YP, Beaudoin AR, Sirois P. Endothelin-1 induces prostacyclin release from bovine aortic endothelial cells. Biochem Biophys Res Commun 177:171–176, 1991.
18. Soma S, Takahashi H, Muramatsu M, Oka M, Fukuchi Y. Localization and distribution of endothelin receptor subtypes in pulmonary vasculature of normal and hypoxia-exposed rats. Am J Respir Cell Mol Biol 20:620–630, 1999.