Flow-Dependent Regulation of Nitric Oxide Formation in the Isolated Canine Mesenteric Arterial Bed

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ABSTRACT—Effects of flow rate changes on nitric oxide (NO) formation in vascular endothelial cells were investigated in isolated canine mesenteric arterial bed preparations. Stepwise increases in the flow rate from 8 ml/min to 40 ml/min significantly ($P<0.05$) elevated perfusion pressure in a rate-dependent manner. In the presence of N^G-nitro-L-arginine (L-NNA, 100 μM), perfusion pressures were significantly ($P<0.01$) higher than those observed under control conditions at all flow rates examined. Sodium nitroprusside (SNP) (0.1－10 μM) counteracted the pressor effect of L-NNA in a concentration-dependent manner. Increases in the flow rate from 10 ml/min to 40 ml/min significantly ($P<0.05$) augmented cyclic GMP production in the vascular bed preparation. The flow-induced cyclic GMP response was significantly ($P<0.05$) attenuated by L-NNA (100 μM). These results demonstrate that 1) the amount of NO released from endothelial cells toward vascular smooth muscle cells can be semi-quantified with SNP, and 2) an increase in the flow rate stimulates NO formation in endothelial cells of resistance arteries, which may play an important part in regulating systemic blood pressure.

Keywords: Canine mesenteric artery, Flow, Nitric oxide (NO), N^G-nitro-L-arginine, Perfusion pressure

Nitric oxide (NO) is continuously produced in vascular endothelial cells, from which it diffuses in both luminal and abluminal directions. NO released into the blood stream inhibits platelet aggregation (1), suppresses adhesion of platelets to the endothelium (2) and prevents leukocyte-endothelial interaction (3). On the other hand, NO liberated outside of blood vessels relaxes vascular smooth muscle cells and thereby decreases the tone of blood vessels. Thus, NO plays several important roles in the circulatory system (4).

Endothelial cell NO formation changes in response to various chemical stimuli (4). In addition, physical forces, such as sheer stress, transmural pressure, stretching of the vascular wall, etc., which are continuously generated by blood flow in vivo, also affect the amount of NO released from endothelial cells (5－15). However, to date, probably due to technical difficulties with experimental procedures, little information has become available regarding the physical influence on NO release from the endothelium of resistance arteries.

In the present study, we attempted to answer the following two questions in small arteries or arterioles, which are of practical importance in the regulatory mechanism of systemic blood pressure. 1) Is it possible to determine the amount or concentration of abluminally released NO? 2) How do changes in the flow rate of the fluid passing through the lumina of blood vessels affect NO production in the endothelium? To achieve these aims, we first established a new experimental model of the isolated canine mesenteric arterial bed, and then we observed the flow-pressure relationship and examined the effects of changes in flow rate on cyclic GMP production using this new model. We present herein two new findings on the regulatory mechanism of the cardiovascular system of an experimental animal larger than rodents, the dog, which we have been studying intensively for the past few years (16－18).

MATERIALS AND METHODS

Isolation and perfusion of canine mesenteric arterial bed preparation

Healthy mongrel dogs of either sex, weighing 10－18

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kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and exsanguinated via the common carotid arteries. The mesentery was dissected together with the jejunal portion of the small intestine and perfused with ice-cold Krebs-Henseleit solution (KHS) via a polyethylene cannula introduced into the superior mesenteric artery to wash out blood remaining in the lumina of blood vessels. The composition of KHS was as follows: 119 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$ and 10 mM dextrose (pH 7.4 at 37℃). KHS was oxygenated with 95% O_2 and 5% CO_2. The jejunum was cut into lengths of approximately 5 cm with adhering mesentery that contained a major branch of the mesenteric artery perfusing the selected intestinal area. A polyethylene catheter was inserted into the first branch of the superior mesenteric artery, and then the jejunum was separated by careful incision along the border between the mesentery and intestinal wall. Thus the primary part of resistance of the arterial bed is attributable to the arterioles located adjacent to the border. Usually, no occlusion of any blood vessels was necessary to obtain a perfusion pressure of the desired range. At least five mesenteric arterial bed preparations were obtained from each dog. The preparations were kept in ice-cold KHS until perfusion with oxygenated KHS at 37℃ was started on a Buchner funnel mounted in a chamber, which was kept at 37℃ and 100% humidity (Fig. 1). Each preparation was allowed to stabilize for at least 40 min under the basal flow rate of 10 ml/min. To simplify physical influences on the vessel wall, pulsatile pressure variations in the flow generated by a roller pump (model PA-21 series; Cole-Parmer Instrument, Chicago, IL, USA) were damped with an air-filled compliance chamber (Fig. 1). Perfusion pressure was monitored via a pressure transducer (model TDN-R; Gould, Oxnard, CA, USA) connected to a vertical branch of the catheter for tissue perfusion (Fig. 1) and recorded on a polygraph system (model RM-6300; Nihon Kohden, Tokyo). During the stabilizing period, each preparation was exposed to KHS containing 40 mM KCl and to a 30-μg bolus of phenylephrine. The function of the endothelium present in thus obtained arterial bed preparations was considered to be intact since acetylcholine induced depressor responses in a concentration-dependent manner as follows: under perfusion with KHS containing 4 μM phenylephrine, 0.01, 0.03, 0.1, 0.3 and 1 μg acetylcholine lowered the perfusion pressure by 24±5%, 39±7%, 52±6%, 62±3% and 65±5%, respectively. The total experimental period, including the stabilizing period, for each preparation was limited to 60 min to avoid the development of edema. Indomethacin (5 μM) was included in the KHS to eliminate the influence of endogenous prostaglandins.

Experimental procedure

In the first series of experiments, the KHS flow rate was changed in a stepwise manner; first, the flow rate was slightly decreased to 8 ml/min and then raised to 40 ml/min in increments of 8 ml/min. Each flow rate was maintained for 15 sec to allow the perfusion pressure to

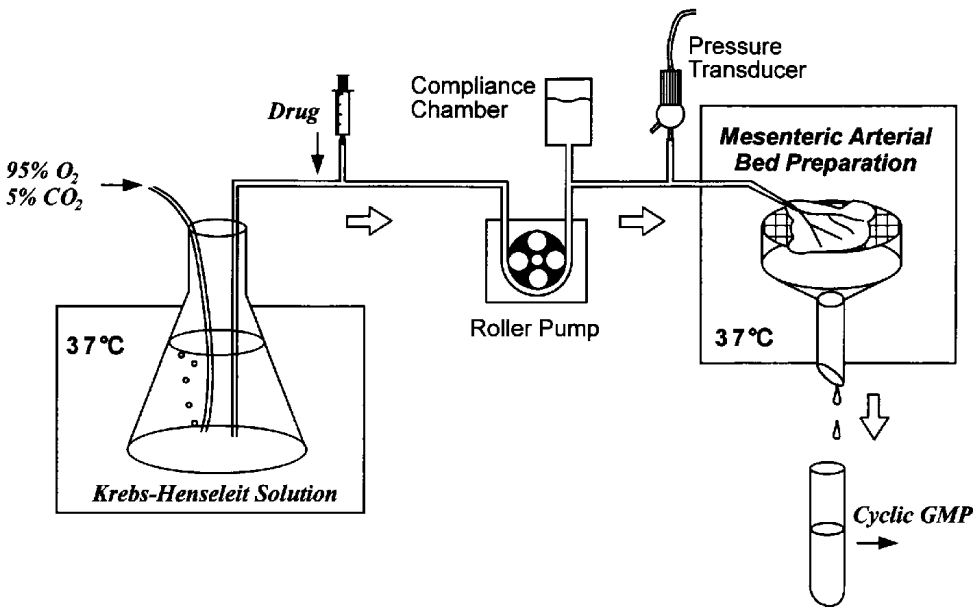

Fig. 1. Schematic representation of the experimental set-up for a measurement of the perfusion pressure of and sampling of the effluent from an isolated canine mesenteric arterial bed.

reach a new steady state. Of the five preparations isolated from the same dog, the first was used to obtain the flow-pressure relationship under control conditions. The second was perfused with KHS that contained N^G-nitro-L-arginine (L-NNA, 100 μM) throughout the experiment; the flow-pressure relationship was observed under conditions of NO synthase inhibition. For the third to fifth preparations, the effects of 0.1 – 10 μM sodium nitroprusside (SNP) on the flow-pressure relationship were examined in the continuous presence 100 μM L-NNA. To avoid edema development due to prolonged perfusion with an artificial fluid, only one flow-pressure relationship was obtained in each preparation.

In the second series of experiments, cyclic GMP in the effluent (cyclic GMP output) was quantified under basal (10 ml/min) and elevated (20 or 40 ml/min) flow conditions. Periods of sampling were 60, 30 and 15 sec for flow rates of 10, 20 and 40 ml/min, respectively, to obtain the same volume (10 ml) at all flow rates.

Measurement of cyclic GMP

The effluent from the preparation was boiled for 5 min immediately after collection and stored at −20℃ until the assay procedure was undertaken. Cyclic GMP was extracted and concentrated before radioimmunoassay as follows: 9 ml of each sample was loaded onto a series of two Waters C-18 reversed-phase Sep-Pak® cartridges (Millipore, Milford, MA, USA). Cyclic GMP was eluted from the cartridges with 3 ml of 1-propanol. After evaporation of the 1-propanol to dryness, each sample was reconstituted with 120 μl of distilled water for measurement of cyclic GMP by radioimmunoassay (Yamasa Cyclic GMP Assay Kit®; Yamasa Shoyu, Choshi). Preliminary experiments confirmed that this method recovers 90% of the cyclic GMP added to the sample as an internal standard.

Drugs

The following drugs were used: acetylcholine chloride (Ovisot®; Daiichi Pharmaceutical, Tokyo), indomethacin, sodium nitroprusside (Sigma Chemical, St. Louis, MO, USA), N^G-nitro-L-arginine (Aldrich Chemical, Milwaukee, WI, USA) and N^G-nitro-D-arginine (Peptide Institute, Minoh).

Statistical analyses

Results are expressed as means ± S.E.M. Preparations isolated from the same dog were studied in parallel, and the data were handled like those obtained by repeated measures. Statistical analyses were performed with the SuperANOVA™ (Abacus Concepts, Berkeley, CA, USA) software on a Macintosh™ computer (Apple Computers, Cupertino, CA, USA): first, ANOVA for repeated measures was applied, and then P values for each data set of two given groups were calculated by applying contrasts. $P<0.05$ was considered statistically significant.

RESULTS

Increasing the flow rate significantly ($P<0.01$) raised the perfusion pressure in a rate-dependent manner irrespective of the presence or absence of L-NNA and SNP (Fig. 2). Treatment of the preparation with 100 μM L-NNA significantly ($P<0.01$) increased the perfusion pressure at all flow rates examined (8 – 40 ml/min). SNP (0.1 – 10 μM) counteracted these changes induced by L-NNA in a concentration-dependent fashion. The flow-pressure curve obtained under the control conditions and that constructed in the presence of 100 μM L-NNA plus 1 μM SNP appeared to intersect at a flow rate of 16 ml/min. At 40 ml/min, the control perfusion pressure was significantly ($P<0.05$) lower and higher than those obtained in the presence of 100 μM L-NNA plus 1 μM SNP and 100 μM L-NNA plus 10 μM SNP, respectively.

Increasing the flow rate from 10 ml/min to 40 ml/min, but not to 20 ml/min, significantly ($P<0.01$) increased

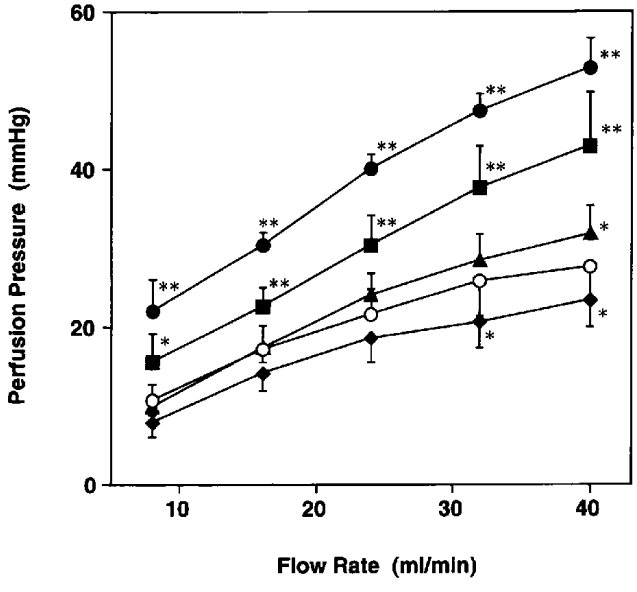

Fig. 2. Flow-pressure relationship obtained in isolated canine mesenteric arterial bed preparations in the absence and presence of N^G-nitro-L-arginine (L-NNA) and sodium nitroprusside (SNP). Symbols used are as follows: ○, control; ●, 100 μM L-NNA; ■, 100 μM L-NNA + 0.1 μM SNP; ▲, 100 μM L-NNA + 1 μM SNP and ◆, 100 μM L-NNA + 10 μM SNP. Preparations were stabilized for 40 min under a constant flow (10 ml/min) of Krebs-Henseleit solution. After the stabilization period, the flow rate was decreased to 8 ml/min and then raised to 40 ml/min, in 8 ml/min increments every 15 sec. Points and vertical bars indicate the mean ± S.E.M. of 6 experiments. * and **: $P<0.05$ and $P<0.01$, respectively, vs corresponding control values.

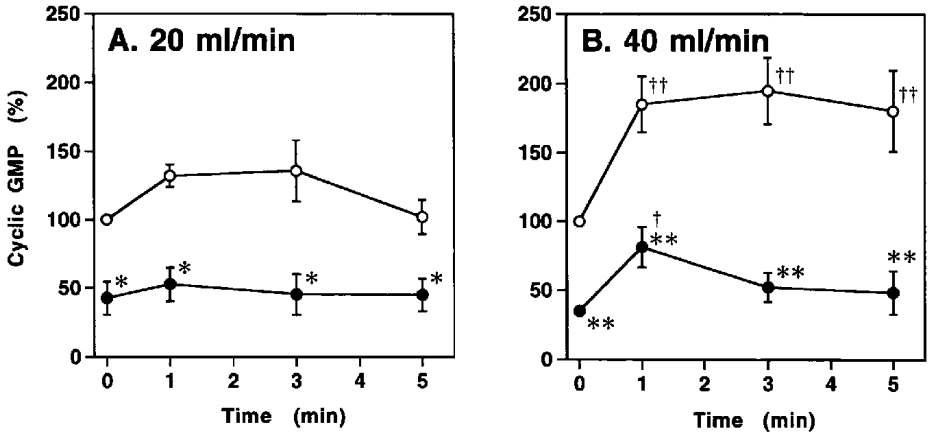

Fig. 3. Flow-induced changes in the cyclic GMP output from the isolated canine mesenteric arterial bed in the absence (open circle) and presence (closed circle) of N^G-nitro-L-arginine (L-NNA, 100 μM). Preparations were stabilized for 40 min under a constant flow (10 ml/min) of Krebs-Henseleit solution. After the stabilization period, the flow rate was increased to 20 ml/min (A) and 40 ml/min (B). Points and vertical bars indicate the mean ± S.E.M. of 5 experiments. * and **: $P<0.05$ and $P<0.01$, respectively, vs corresponding control values. † and ††: $P<0.05$ and $P<0.01$, respectively, vs corresponding values obtained at time 0.

the cyclic GMP output (Fig. 3). The cyclic GMP response reached a plateau level within 1–2 min after raising the flow rate. L-NNA (100 μM) significantly ($P<0.05$ and $P<0.01$ for flow rates of 20 ml/min and 40 ml/min, respectively) lowered the basal cyclic GMP output and significantly ($P<0.01$) inhibited the flow-induced changes in cyclic GMP production. Even in preparations treated with L-NNA, increasing the flow rate to 40 ml/min resulted in significantly ($P<0.05$) augmented cyclic GMP output at 1 min later. L-NNA at 300 μM produced no further inhibition of these responses. D-NNA (300 μM) altered neither basal cyclic GMP levels nor flow-induced cyclic GMP responses (data not shown).

DISCUSSION

First of all, we would like to stress that the present study was carried out using the canine mesenteric arterial bed. The effects of physical stimuli, such as transmural pressure, sheer stress, etc., on endothelium-derived relaxing factor (EDRF)/NO formation in the vascular endothelium have drawn considerable attention over the past decade. However, information regarding the significance of physical factors in the regulatory mechanism of systemic blood pressure is extremely limited. To our knowledge, this is the first demonstration of flow-induced increases in EDRF/NO formation occurring in the vascular regions that may play a physiologically important role in determining systemic blood pressure. Furthermore, in contrast to the majority of previous experiments, which utilized vascular tissues from small animals, such as rodents, this study employed the blood vessels from a relatively large animal species, the dog. Thus, the data presented herein are particularly valuable in considering human cardiovascular physiology.

Our experiments established the flow-pressure relationship in the canine mesenteric arterial bed, under conditions of both functional and malfunctional NO synthase, and yielded several important findings. 1) Increasing the flow rate of the perfusate elevated perfusion pressure in a non-linear manner, reflecting the elastic nature of the blood vessels in the preparation. 2) An NO synthase inhibitor, L-NNA, raised the perfusion pressure by approximately 100% at all flow rates examined, revealing that under control conditions, an appreciable amount of NO is formed in endothelial cells and released toward vascular smooth muscle cells, resulting in dilation of blood vessels even in the absence of a chemical stimulus in the perfusate, KHS. 3) SNP at concentrations of 1–10 μM completely counteracted the pressor effect of L-NNA, indicating that the NO concentration of affecting vascular tone could be semi-quantified with SNP. It is noteworthy that the slopes of the flow-pressure curves obtained under conditions of NO synthase inhibition are steeper than that of the control curve. In other words, it is apparently not possible to compensate for the vasodilator action of NO that is released at various flow rates under control conditions at a single SNP concentration. Thus, the amount of NO varies depending on the flow rate of fluid passing through the blood vessel lumen. Apparently at higher flow rates, more SNP is needed to produce a vasodilator action equal to that induced with NO.

NO released from endothelial cells to the abluminal side dilates blood vessels by activating soluble guanylate

cyclase, which results in accumulation of cyclic GMP, in vascular smooth muscle cells (4, 19). It is known that the amount of cyclic GMP appearing in the extracellular space reflects cyclic GMP levels within the cell (20). Therefore, we adopted the cyclic GMP output as an index of abluminally released NO, which is considered to be one of the major causes of the flow-induced vasodilation observed in the present study. In a cell culture system, NO also increases cyclic GMP levels in endothelial cells to some extent (21, 22). However, since endothelial cell denudation does not apparently decrease SNP-induced formation of cyclic GMP in vascular segments (23), cyclic GMP produced in endothelial cells might be negligible relative to the total cyclic GMP output from vascular tissues.

Raising the flow rate augmented cyclic GMP output, which reached a plateau level within 1–2 min. Since the flow-induced cyclic GMP response was significantly attenuated with L-NNA, a major portion of the increased cyclic GMP production may be attributable to augmented release of NO from the endothelium to the abluminal side. Taken together with the basal cyclic GMP output also decreasing after treatment with L-NNA, it is apparent that blood flowing through the blood vessel lumen contributes substantially to controlling local tone in resistance arteries, within a certain range, via regulation of NO formation in endothelial cells.

L-NNA diminished, but failed to abolish, the flow-induced cyclic GMP response. Since 100 or 300 μM L-NNA is considered to be sufficient to completely inhibit NO synthase activity, this observation suggests mechanisms other than NO, which may contribute to the augmented cyclic GMP output in response to increased flow. Although the mechanism remains to be elucidated, we can speculate that flow also regulates the activity of particulate guanylate cyclase, which is located on the endothelial cell plasma membrane (19, 24).

In conclusion, NO formation in vascular endothelial cells of arterial blood vessels in vivo is controlled by blood via two fundamentally different mechanisms; i.e., chemical substances contained in plasma and physical factors generated by blood flow. Thus, physical stimuli must be taken into account when considering the mechanisms regulating arterial pressure and blood distribution.

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