Flow Regulates Vasodilator Responses to Acetylcholine in the Isolated Canine Mesenteric Arterial Bed

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Raising the flow rate of the perfusate from 10 to 20 ml/min significantly suppressed vasodilator responses to acetylcholine, but not to sodium nitroprusside, in the isolated canine mesenteric arterial bed. Both acetylcholine and sodium nitroprusside augmented guanosine 3',5'-cyclic monophosphate (cyclic GMP) levels in the effluent from the vascular bed preparation, though cyclic GMP responses to these agents were not affected by raising the flow rate. These data suggest that the suppression, via raising the flow rate, of vasodilator responses to acetylcholine is not due to impaired function of the nitric oxide (NO)-cyclic GMP pathway in the mesenteric arterial bed.

Key words: flow; canine mesenteric arterial bed; acetylcholine; sodium nitroprusside; perfusion pressure; cyclic GMP

It is well-known that a number of vasoactive agents stimulate endothelial cells to release various substances that relax (EDRF/NO), hyperpolarize (EDHF), or contract (EDCF) vascular smooth muscle, and thereby indirectly alter the tone of blood vessels. Since physical factors, which are continuously generated by the blood stream passing through the blood vessel lumen in vivo, have been demonstrated to regulate functions of both the vascular endothelium and smooth muscle, it is reasonable to hypothesize that changes in hemodynamic conditions influence the actions of vasoactive agents.

In the present study, to investigate the physical effects of blood flow on endothelium-dependent and independent vasodilators, we compared acetylcholine (ACh) and sodium nitroprusside (SNP)-induced vasodilator responses under different flow conditions in a newly established experimental model, designed for examining the functions of resistance arteries, an isolated canine mesenteric arterial bed preparation.

MATERIALS AND METHODS

Experimental Protocol In the first series of experiments, we observed depressor responses to 3 µM ACh and to 1 µM SNP obtained under different flow rates; i.e., 10 and 20 ml/min. To maintain vascular tone, mesenteric arteries were precontracted with 2 µM phenylephrine. This concentration of phenylephrine elicited approximately 80% of the maximum elevation in perfusion pressure.

In the second series of experiments, we measured guanosine 3',5'-cyclic monophosphate (cyclic GMP) content in the effluent from the vascular bed preparation (cyclic GMP output) to estimate the amount of nitric oxide (NO) needed to exert an effect on vascular smooth muscle cell tone. Periods for sampling of the effluent were 60 and 30 s for flow rates of 10 and 20 ml/min, respectively, to obtain the same volume (10 ml), irrespective of the rates.

Isolation and Perfusion of Canine Mesenteric Arterial Bed Healthy mongrel dogs of either sex, weighing 10–18 kg, were anesthetized with sodium pentobarbital (30 mg/kg, i.v.) and exsanguinated from 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 the blood remaining in the lumina of blood vessels. The composition of KHS was as follows (mm): NaCl, 119; KCl, 4.8; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25; and dextrose, 10 (pH 7.4 at 37°C). KHS was oxygenated with 95% O₂ and 5% CO₂. The jejunum was cut into 5–8 pieces, approximately 5 cm in length, with the adhering mesentery containing a major branch of the mesenteric artery perfusing the selected intestinal area. A polyethylene catheter was inserted into the branch and, then, the jejunum was separated by careful incision along the border between the mesentery and the intestinal wall. The mesenteric arterial bed preparations thus obtained were kept in ice-cold KHS until perfusion with oxygenated KHS was started on a Buchner funnel inside a chamber, which was kept at 37°C and 100% humidity. Each preparation was allowed to stabilize for at least 40 min on the funnel at a basal flow rate of 10 ml/min KHS at 37°C. To simplify physical influences on the vessel wall, pulsatile variation in the flow generated by a roller pump (model PA-21 series, Cole-Parmer Instrument, Chicago, IL, U.S.A.) was damped with an air-filled compliance chamber. Perfusion pressure was monitored via a pressure transducer (model TDN-R, Gould, Oxnard, CA, U.S.A.) connected to a vertical branch of the perfusion catheter and recorded on a polygraph system (model RM-6300, Nihon Kohden, Tokyo, Japan). During the stabilization period, each preparation was exposed to 40 mM KCl and to a 30 µg bolus of phenylephrine. The total experimental period, including the stabilization period, for each preparation was limited to 60 min to avoid the development of edema. Indomethacin (5 µM) was included in the KHS to block endogenous prostaglandins.

Quantification of Cyclic GMP Samples were boiled for 5 min immediately after collection and stored at -20°C 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, U.S.A.). Cyclic GMP adsorbed
on the resin was eluted from the cartridge 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 the measurement of cyclic GMP by radioimmunoassay (Yamasaki cyclic GMP assay kit®, Yamasa Shoyu, Choshi, Japan). Preliminary experiments confirmed that this procedure recovers 90% of the cyclic GMP added as an internal standard.

**Drugs** The following drugs were used: acetylcholine chloride (Ovisol®, Daiichi Pharmaceutical, Tokyo, Japan), L-phenylephrine hydrochloride, indomethacin, SNP (Sigma Chemical, St. Louis, MO, U.S.A.) and N^G^-nitro-L-arginine (Aldrich Chemical, Milwaukee, WI, U.S.A.).

**Statistical Analysis** Results, expressed as means ± S.E.M., were analyzed by Fisher's protected least significance test after performing one-factor ANOVA. *p < 0.05* was considered statistically significant.

**RESULTS AND DISCUSSION**

Perfusion pressures obtained before and after 2 μM phenylephrine at the basal flow rate of 10 ml/min were 17.6 ± 1.9 mmHg and 242.2 ± 25.8 mmHg, respectively (n = 10). In mesenteric arterial beds thus constricted with phenylephrine, 3 μM ACh and 1 μM SNP lowered perfusion pressure to a similar extent (Fig. 1). The depressor response to ACh was abolished with 0.1 μM atropine and inhibited with 100 μM N^G^-nitro-L-arginine, an NO synthase inhibitor, by about 50%, indicating that it had been induced by the stimulation of muscarinic cholinergic receptors and that approximately half the response may have been due to NO formation. On the other hand, SNP has been considered to relax vascular smooth muscle via the spontaneous release of NO under physiological conditions. Thus, as to the vasodilator mechanism, ACh and SNP share the same active molecule, NO. Elevation of the flow rate from 10 to 20 ml/min slightly raised the perfusion pressures measured in both the absence and presence of 2 μM phenylephrine, to 30.0 ± 5.2 and 289.9 ± 11.2 mmHg, respectively (n = 10), while attenuating depressor responses to ACh by approximately 60% (p < 0.05). However, SNP-induced decreases in the perfusion pressure were not significantly affected. These observations suggest that, even at a high flow rate, 1) there are no apparent changes in the contractile status of vascular smooth muscle, and that 2) all components required to relax blood vessels via activation of the NO-cyclic GMP system are well-preserved and fully functional in vascular smooth muscle cells.

To obtain additional information on the mechanism underlying these phenomena, we measured the amount of cyclic GMP in the effluent from the mesenteric arterial bed preparation, i.e., the cyclic GMP output, as an index of abuminally released NO from the endo-

![Diagram of experimental set-up](image-url)
Fig. 3. Effects of Raising the Flow Rate on ACh (3 μM; A) and SNP (10 μM; B)-Induced Increases in Cyclic GMP Output from the Isolated Canine Mesenteric Arterial Bed.

In both panels, open and closed columns represent the data obtained at flow rates of 10 and 20 ml/min, respectively. The flow rate was raised 5 min before switching to the buffer containing ACh or SNP. Each column with a vertical bar indicates the mean ± S.E.M. of 8 (ACh) and 5 (SNP) experiments. * and ** indicate p < 0.05 and p < 0.01, respectively, vs. corresponding 0-time controls.

The mechanism underlying these phenomena is unclear at present. Two major hypotheses, as follows, remain to be examined in future studies: Flow rate elevations 1) inhibit ACh-induced formation of vasodilator substance(s) other than NO, or 2) enhance the ACh-induced production of vasoconstrictor substance(s), etc. In conclusion, it merits emphasis that flow rate elevations attenuate vasodilator responses to ACh via a mechanism other than impairment of the NO-cyclic GMP pathway.

REFERENCES AND NOTES

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