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Involvement of barium-sensitive K^+ channels in endothelium-dependent vasodilation produced by hypercapnia in rat mesenteric vascular beds

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1 We examined the vasodilatory effect of hypercapnia in the rat isolated mesenteric vascular bed. The preparation was perfused constantly (5 ml min^{-1} with oxygenated Krebs-Ringer solution, and the perfusion pressure was measured. In order to keep the extracellular pH (pHe) constant (around 7.35) against a change in CO_2, adequate amounts of $NaHCO_3$ were added to Krebs-Ringer solution.

2 In the endothelium intact preparations, an increase in CO_2 from 2.5% to 10% in increments of 2.5% decreased the 10 μM phenylephrine (PE)-produced increase in the perfusion pressure in a concentration-dependent manner. Denudation of the endothelium by CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-l-propanesulphonate) (5 mg l^{-1}, 90 s perfusion) abolished the vasodilatory effect of hypercapnia.

3 An increase in CO_2 from 5% to 10% reduced the increases in the perfusion pressure produced by 10 μM PE and 400 nM U-46619 by 48% and 44%, respectively. N^G-monomethyl-L-arginine (100 μM) and indomethacin (10 μM) did not affect the vasodilatory effect of hypercapnia, whereas the vasodilatory response of the preparation to hypercapnia disappeared when the preparation was contracted by 60 mM K^+ instead of PE or U-46619.

4 The vasodilatory effect of hypercapnia observed in the PE- or U-46619-precontracted preparation was affected by neither tetraethylammonium (1 mM), apamin (500 μM), glibenclamide (10 μM), nor 4-aminopyridine (1.5 mM). On the other hand, pretreatment with Ba^{2+} at a concentration of 0.3 mM abolished the hypercapnia-produced vasodilation.

5 An increase in the concentration of K^+ in Krebs-Ringer solution from 4.5 mM to 12.5 mM in increments of 2 mM reduced the PE-produced increase in the perfusion pressure in a concentration-dependent manner. Pretreatment of the preparations with not only Ba^{2+} (0.3 mM) but also CHAPS abolished the vasodilatory effect of K^+.

6 The results suggest that an increase in CO_2 produces vasodilation by an endothelium-dependent mechanism in the rat mesenteric vascular bed. The membrane hyperpolarization of the endothelial cell by an activation of the inward rectifier K^+ channel seems to be the mechanism underlying the hypercapnia-produced vasodilation. Neither nitric oxide nor prostaglandins are involved in this response.

Keywords: Carbon dioxide; endothelium; hyperpolarization; barium; mesenteric vascular bed; inward rectifier K^+ channel

Introduction

The arterial CO_2 tension ($PaCO_2$) plays a role in regulating a variety of circulations such as cerebral, coronary and visceral circulations (Sokoloff, 1960; Daugherty *et al*., 1967; Cullen & Eager, 1974; Hughes *et al*., 1979). Although an increase in PCO_2 generally induces vasodilation except the pulmonary vasculature (Hyde *et al*., 1964), the mechanism underlying the vasodilatory effect of PCO_2 seems to be complex and remains to be clarified.

It is well known that a change in the extra- and/or intracellular pH of vascular smooth muscle produces marked effects on the vascular tone (Wray, 1988; Austin & Wray, 1993; Tian *et al*., 1995). Inasmuch as the change in PCO_2 can influence both extracellular pH (pHe) and intracellular pH (pHi), it is plausible that the vasoactive effect of PCO_2 derives from the secondary change in pHe and/or pHi. However, it has also been shown that PCO_2 modulates vascular tone in some isolated blood vessels by a mechanism that is independent of a change in pH (Kontos *et al*., 1968; Nielsen *et al*., 1991).

Recent studies have demonstrated that the vascular endothelium plays a role in the vasoactive effects of PCO_2 in the cerebral and coronary circulations. Inhibition of nitric oxide synthase (NOS) attenuates the increase in the cerebral blood flow and coronary blood flow elicited by hypercapnia (Fabricius & Lauritzen, 1994; Gurevicius *et al*., 1995). Hsu *et al*. (1995) also demonstrated that hypercapnia stimulates synthesis of vasodilatory prostanoid(s) in cerebral vascular endothelial cells but not in vascular smooth muscle.

The aims of the present study were to establish the following: (1) does an increase in PCO_2 with a concomitant increase in bicarbonate at constant pH decrease the phenylephrine-increased perfusion pressure in the rat mesenteric vascular bed? (2) if so, is such an effect endothelium-dependent? (3) if the mechanism is endothelium-dependent, which are the factors involved?

Methods

All procedures with animals were in accordance with the guidelines of the Animal Care Committee of Yokohama City University School of Medicine.

Mesenteric vascular beds with aortae and digestive tracts were isolated *en bloc* very rapidly from freshly exanguinated male Wistar rats weighing 200–250 g, that were anaesthetized with diethyl ether and injected heparin (500 units per kg) *via*

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tail veins. The preparation was placed in a dissection dish filled with oxygenated Krebs-Ringer solution of the following composition (mM): NaCl 119.0, KCl 4.5; $CaCl_2$ 2.5; $MgCl_2$ 0.5; NaH_2PO_4 1.2; $NaHCO_3$ 25 and glucose 11. The vascular bed was carefully separated from the digestive tract and the mesenteric artery was cannulated through the abdominal aorta. The vascular bed was subsequently transferred to a warmed chamber and perfused with Krebs-Ringer solution (maintained at 37°C and aerated with 95% O_2 and 5% CO_2) at a constant flow rate of 5 ml min^{-1} by a peristaltic pump (model RP-NF3; Furue Science Co., Tokyo, Japan). Changes in the perfusion pressure were recorded by a pressure transducer (model T4812AD; Gould Inc., Oxnard, CA, U.S.A.) connected to a recorder (model RTA-1200; Nihon Kohden, Tokyo, Japan). The vascular bed was equilibrated for 45–60 min before starting experiments. All preparations were tested for the presence of a functional endothelium by means of confirming the vasodilatory effect of 1 μM acetylcholine (ACh) on 10 μM phenylephrine (PE)-precontracted preparations. When required, the endothelium was destroyed by perfusion of the preparation for 90 s with a 0.5% (w/v) solution of CHAPS (3-[(3-cholamidopropyl)-dimethylammonio]-l-propanesulphonate). Functional destruction of the endothelium was confirmed by showing that ACh no longer reduced the perfusion pressure increased by PE.

Vasodilatory effect of an increase in CO_2

The vascular tone was increased by an infusion of 10 μM PE-containing Krebs-Ringer solution aerated with 95% O_2 and 5% CO_2. The change in the perfusion pressure reached a steady level within 20 min, then the solution was changed to one containing PE (10 μM) and aerated with 90% O_2 and 10% CO_2. The PCO_2, PO_2, and pH of the buffer solutions were intermittently measured (model 280 Blood Gas System; Ciba Corning Diagnostics Corp., Medfield, MA, U.S.A.). In some experiments, in order to examine the concentration-dependent effect of CO_2, the vascular tone was increased by an infusion of the 10 μM PE-containing solution aerated with 97.5% O_2 and 2.5% CO_2 initially, then CO_2 was increased stepwise to 5.0%, 7.5% and 10%. In the cases of the 2.5%, 7.5% and 10% CO_2-aerated solutions, the amount of $NaHCO_3$ added was changed in order to keep the extracellular pH (pHe) constant. The concentration of $NaHCO_3$ was 15 mM in the 2.5% CO_2 solution, 35 mM in the 7.5% CO_2 solution and 44 mM in the 10% CO_2 solution. As shown in Table 1, an increase in CO_2 of the bubbling gas increased the PCO_2 of the solution in a concentration-dependent manner, while the pH of the solution remained constant as a whole although the pH of the 10% CO_2-aerated solution was slightly but significantly lower than that of the 2.5% CO_2-aerated solution. There was no significant difference in pH between the 5% and 10% CO_2-aerated solutions. In some experiments, the osmolarity of the 10% solution was kept equal to that of the 5% solution by means of an equimolar replacement of $NaHCO_3$ with NaCl. Test compounds were added to the solution 10 min before the application of PE, and remained present throughout the rest of the experiments. When indomethacin and 4-aminopyridine (4-AP) were used, the vascular tone was increased by U-46619 at a concentration of 400 nM, because the response of the vascular bed to PE in the presence of indomethacin or 4-AP was considerably small, compared with the response to PE in the absence of the agents (see Results). In some experiments, the vascular tone was increased by 60 mM K^+ instead of PE. The osmolarity was maintained by an equimolar replacement of KCl with NaCl.

Vasodilatory effect of an increase in extracellular potassium

The vascular tone was increased by an infusion of the 10 μM PE-containing Krebs-Ringer solution aerated with 95% O_2 and 5% CO_2. After the vascular tone reached a steady level, KCl was added to the solution in a cumulative manner in increments of 2 mM.

Chemicals

The following compounds were used: acetylcholine chloride, sodium nitroprusside dihydrate, tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP), (Wako Pure Chemical, Tokyo, Japan), SKF525A hydrochloride (Funakoshi, Tokyo, Japan), apamin, (-)phenylephrine hydrochloride, U-46619 (9,11-dideoxy-11α, 9α-epoxymethano-prostaglandin $F_2\alpha$), N^G-monomethyl-L-arginine acetate, indomethacin, glibenclamide, clotrimazole, CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) (Sigma Chemical Co, St. Louis, MO, U.S.A.), 5,8,11,14-eicosatetraynoic acid (ETYA) (Calbiochem, San Diego, CA, U.S.A.). U-46619, indomethacin, clotrimazole and ETYA were dissolved in ethanol, and glibenclamide was dissolved in dimethyl sulphoxide. Further dilutions were made with distilled water; at the concentration used, ethanol and dimethyl sulphoxide had no effect in the present study. TEA, 4-AP and CHAPS were dissolved in the perfusion solution. All other chemicals were dissolved in distilled water.

Statistics

All values are presented as mean ± s.e.mean. Comparisons of variables obtained during the concentration-response curves and comparisons of more than two groups were made by one-way analysis of variance followed by Scheffé's *t*-test or Bonferroni's *t*-test. Analysis by Student's *t*-test was performed for unpaired and paired comparisons. A value of $P < 0.05$ was considered statistically significant.

Results

Effect of an increase in CO_2 on vasoconstrictor-increased perfusion pressure

In the preparations with intact endothelium, acetylcholine (ACh) at a concentration of 1 μM completely relaxed the 10 μM phenylephrine (PE)-contracted rat mesenteric vascular beds as shown in Figure 1A, left panel. After perfusing the preparations with Krebs-Ringer solution containing 0.5% CHAPS for 90 s, the ACh-produced relaxation was almost abolished as shown in Figure 1B, left panel, indicating that functional endothelium was fully removed by CHAPS. On the other hand, the treatment of the preparation with CHAPS did not inhibit the relaxant effect of 1 μM sodium nitroprusside (Figure 1B, left panel). Figure 1A, right panel shows the representative effect of CO_2 on the PE-produced increase in the perfusion pressure. The stepwise increment of CO_2 reduced the perfusion pressure. In sharp contrast, the CHAPS-treated vascular bed did not respond to the increase in CO_2 (Figure 1B, right panel). Figure 2 summarizes the vasodilatory effect of hypercapnia on the mesenteric vascular bed. In the endothelium-intact preparations, CO_2 reduced the perfusion pressure in a concentration-dependent manner. When the preparations were treated with CHAPS, the vasodilatory effect of CO_2 was abolished completely.

We added sodium bicarbonate to the buffer solution to keep the pH of the solution constant (around 7.35) against the increase in CO_2 of the mixed gas (Table 1). Thus the osmolarity of the solution increased as CO_2 increased. Therefore, we examined the vasodilatory effect of CO_2 under conditions where the osmolarity of the solution was kept constant by substituting $NaHCO_3$ for NaCl on an equimolar basis. Figure 3A shows the representative effect of an increase in CO_2 to 10% without correcting the osmolarity. The increase in CO_2 of the mixed gas increased the PCO_2 of the buffer solution from 38 mmHg to 70 mmHg, while no significant difference between the pH of the 5% CO_2-saturated solution and that of the 10% CO_2-saturated solution occurred (Table 1). A sustained decrease in the perfusion pressure, following a transient and small increase in it was observed after changing CO_2 from 5% to 10%, although the transient increase in the perfusion pressure was sometimes faint. The substitution of $NaHCO_3$ for NaCl did not modify the biphasic effect of CO_2. PE at a concentration of 10 μM increased the perfusion pressure from 25.8 ± 0.5 mmHg to 108.7 ± 5.6 mmHg ($n=20$, pooled data). The PE-produced increase in the perfusion pressure was reduced by 48.5 ± 1.8% by increasing CO_2 to 10% from 5% without osmotic correction ($n=12$), and by 40.4 ± 3.7% with correction ($n=8$). There was no statistically significant difference between these two values.

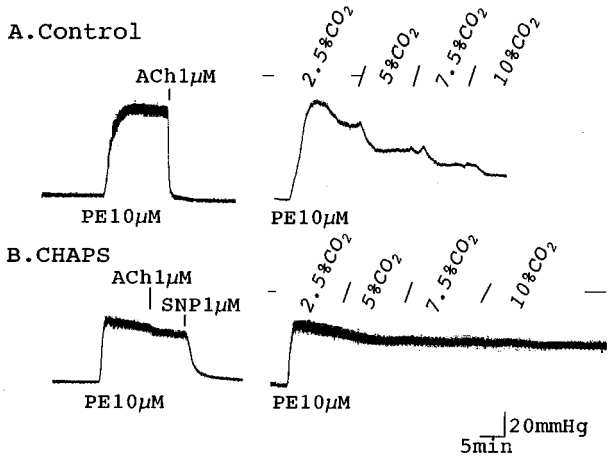

Figure 1 Representative effects of an increase in CO_2 on phenylephrine (PE)-produced increase in perfusion pressure in the rat mesenteric vascular bed. In an endothelium intact preparation (A), acetylcholine (ACh, 1 μM) opposed completely PE (10 μM)-increased perfusion pressure (left panel). A stepwise increase in CO_2 reduced the PE-increased perfusion pressure (right panel). Pretreatment of the preparation with CHAPS abolished the vasodilatory effect of ACh, whereas 1 μM sodium nitroprusside (SNP) still dilated PE-constricted preparation (B, left panel). The CHAPS-treated vascular bed did not respond to the increase in CO_2 (B, right panel).

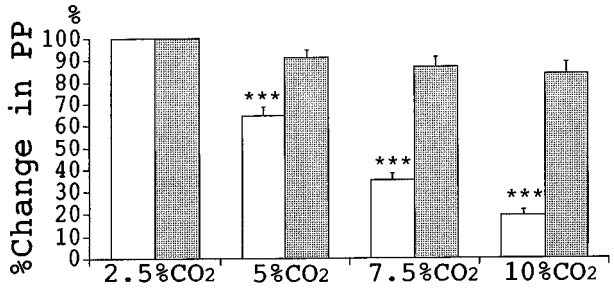

Figure 2 Effects of an increase in CO_2 on phenylephrine (PE, 10 μM)-increased perfusion pressure (PP) in endothelium-intact (open columns, $n=6$) and endothelium-denuded (solid columns, $n=6$) rat mesenteric vascular beds. Data are mean $\pm$ s.e.mean of % increase in perfusion pressure. PE-increased perfusion pressure in 2.5% CO_2-saturated Krebs-Ringer solution was taken as control (100%). ***Significantly different from control ($P<0.001$) by one-way analysis of variance followed by Scheffé's t-test.

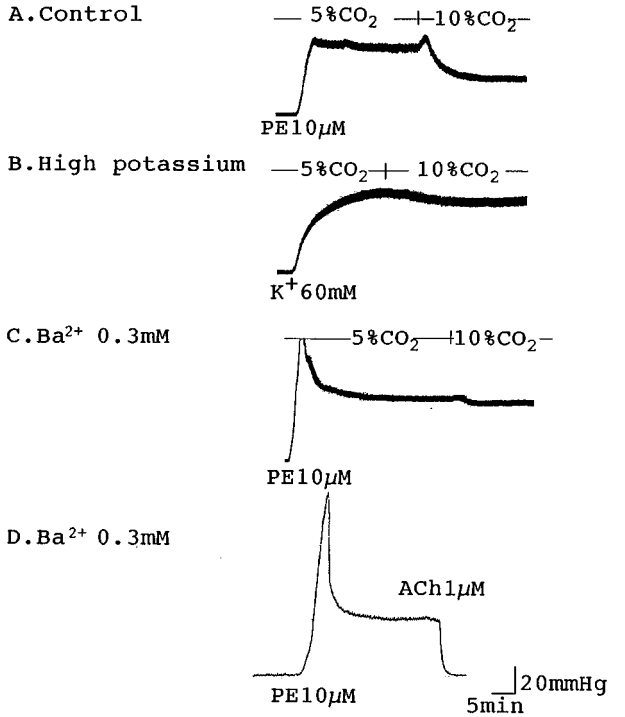

Figure 3 Representative effects of an increase in CO_2 from 5% to 10% (A–C) and acetylcholine (ACh) (D) on the perfusion pressure in the rat mesenteric vascular bed. A: the perfusion pressure was increased by 10 μM phenylephrine (PE). B: the perfusion pressure was increased by 60 mM K^+. C, D: the preparation was pretreated with 0.3 mM $BaCl_2$, then the perfusion pressure was increased by 10 μM phenylephrine (PE).

Table 1 pH, PCO_2 and PO_2 values of Krebs-Ringer solution aerated with various concentrations of carbon dioxide

	2.5% CO_2/97.5% O_2	*5% CO_2/95% O_2*	*7.5% CO_2/92.5% O_2*	*10% CO_2/90% O_2*
pH	7.420 ± 0.012	7.384 ± 0.016	7.371 ± 0.012	7.343 ± 0.007*
PCO_2	23 ± 1	38 ± 1***	52 ± 1***	70 ± 1***
PO_2	576 ± 20	602 ± 16	595 ± 17	604 ± 6

Data are expressed as mean $\pm$ s.e.mean ($n=12$). *$P<0.05$, ***$P<0.001$. Significantly different from corresponding value of 2.5% CO_2/97.5% O_2 by analysis of variance followed by Scheffé's t-test.

Effects of L-NMMA, indomethacin, and high-K^+ on CO_2-produced vasodilation

Figure 4 summarizes the effects of the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) and the cyclooxygenase inhibitor indomethacin on the vasodilatory effect of CO_2. Indomethacin (10 μM) attenuated the vasoconstricting effect of PE considerably. Thus we used U-46619, a stable thromboxane A_2 analogue, as a vasoconstrictor instead of PE. U-46619 at a concentration of 400 nM in the absence and presence of 10 μM indomethacin increased the perfusion pressure by 53.8 ± 5.2 mmHg ($n=6$) and 37.6 ± 2.6 mmHg ($n=8$), respectively. The increase in CO_2 from 5% to 10% also reduced the U-46619-produced increase in the perfusion pressure by $44.1 \pm 6.1\%$ ($n=6$). Neither L-NMMA at a concentration of 100 μM nor indomethacin at a concentration of 10 μM affected the vasodilatory effect of CO_2.

In sharp contrast, the vasodilatory effect of CO_2 was abolished when the vascular bed was contracted by 60 mM K^+ as shown in Figure 3B. The increases in the perfusion pressure by 60 mM K^+ were 56.7 ± 9.4 mmHg in the 5% CO_2-saturated solution and 55.1 ± 8.6 mmHg in the 10% CO_2-saturated solution ($n=7$, NS).

Effects of K^+ channel inhibitors and cytochrome P-450 inhibitors on CO_2-produced vasodilation

Table 2 summarizes the effect of an increase in CO_2 from 5% to 10% in the presence of several K^+ channel inhibitors such as 1 mM TEA, 500 nM apamin, 10 μM glibenclamide, 1.5 mM 4-AP, and 0.3 mM $BaCl_2$. In the case of 4-AP, the vascular beds were contracted by U-46619 (400 nM) instead of PE, because the vasoconstricting effect of PE was attenuated considerably in the presence of 4-AP. Neither TEA, apamin, glibenclamide nor 4-AP inhibited the vasodilation produced by hypercapnia. In contrast, the relaxing response of the vascular bed to the increase in CO_2 disappeared in the presence of 0.3 mM $BaCl_2$ (Figure 3C). As shown in Figure 3D, 0.3 mM $BaCl_2$ did not inhibit the ACh-produced decrease in the perfusion pressure ($n=5$), suggesting that barium specifically suppressed the hypercapnia-produced vasodilation.

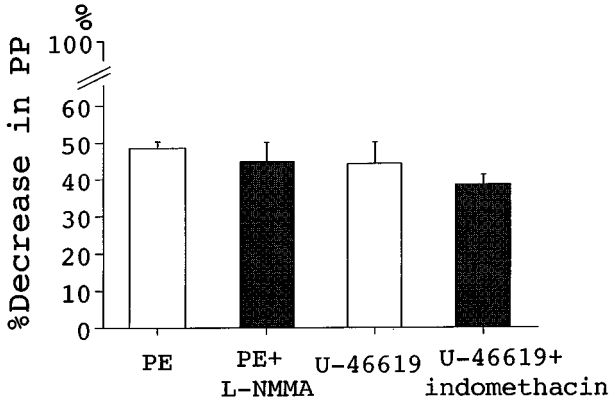

Figure 4 Effects of N^G-monomethyl-L-arginine (L-NMMA, 100 μM) and indomethacin (10 μM) on hypercapnia-produced vasodilation in the rat mesenteric vascular bed. The perfusion pressure (PP) was increased by PE (10 μM) or U-46619 (400 nM). Subsequently, CO_2 was changed from 5% to 10%. L-NMMA or indomethacin was applied to the Krebs-Ringer solution 10 min before the application of PE or U-46619. Each column represents hypercapnia-produced per cent decrease in the perfusion pressure that was increased by PE or U-46619 (mean $\pm$ s.e.mean). Number of preparations were (from left to right) 8, 8, 6, and 8, respectively.

The influence of two cytochrome P-450 inhibitors and of ETYA on the hypercapnia-produced vasodilation are summarized in Table 3. None of these compounds inhibited the hypercapnia-produced decrease in the perfusion pressure.

Effect of an increase in K^+ concentration in Krebs-Ringer solution on PE-produced increase in perfusion pressure

A typical effect of an increase in K^+ concentration on the PE-produced increase in the perfusion pressure is shown in Figure 5A. An increase in the K^+ concentration from 4.5 mM in increments of 2 mM decreased the perfusion pressure. The

Table 2 Effects of K^+ channel inhibitors on hypercapnia-produced vasodilation in phenylephrine- or U46619-contracted mesenteric vascular beds

	n	*Hypercapnia-produced decrease in PP (% of agonist-increased PP)*
PE 10 μM		
Control	12	45.8 ± 1.8
TEA 1 mM	7	35.7 ± 4.1
Apamin 500 nM	7	44.2 ± 5.3
Glibenclamide 10 μM	6	37.7 ± 4.2
$BaCl_2$ 0.3 mM	7	3.6 ± 2.3***
U46619 400 nM		
Control	6	44.1 ± 6.1
4AP 1.5 nM	9	44.0 ± 4.1

PP = perfusion pressure. Values are mean $\pm$ s.e.mean. *n*, number of preprations. PE or U46619-increased PP in 5% CO_2-saturated Krebs-Ringer solution was taken as 100%. ***Significantly different from control value by analysis of variance followed by Bonferroni's *t*-test ($P<0.001$).

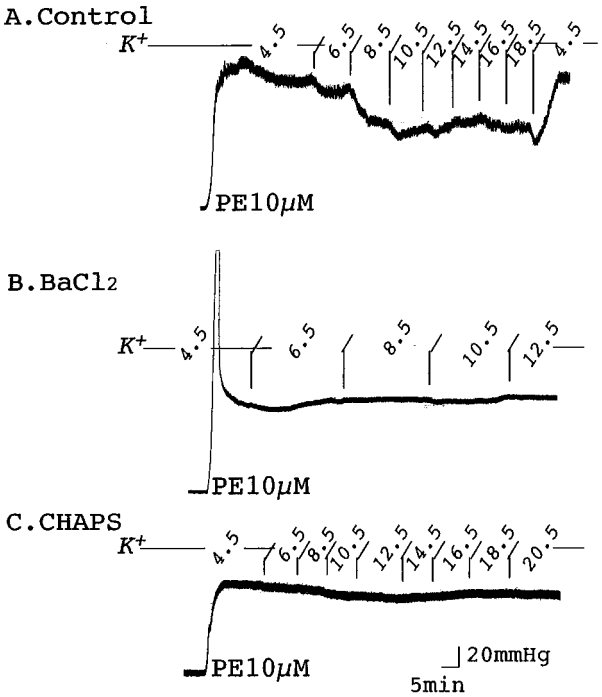

Figure 5 Representative effect of an increase in K^+ concentration of Krebs-Ringer solution on PE (10 μM)-increased perfusion pressure (A), and effects of $BaCl_2$ (0.3 mM, B) and CHAPS (C) on K^+-produced vasodilation.

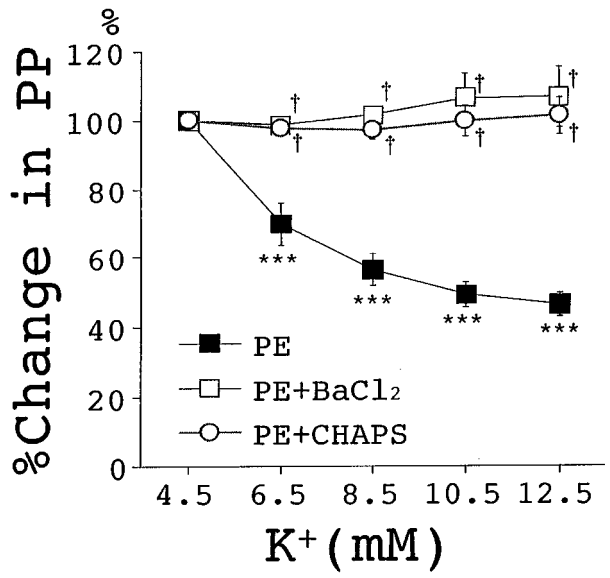

Figure 6 Concentration-response curves for the effect of K^+ on PE (10 μM)-produced increase in the perfusion pressure (PP) without treatment (control response: solid squares), with pretreatment with $BaCl_2$ (0.3 mM, open squares) and CHAPS (open circles). Means $\pm$s.e.m. ($n=8$ for control group and $BaCl_2$-treated group, and $n=6$ for CHAPS-treated group) of per cent increase in the perfusion pressure. The perfusion pressure increased by 10 μM PE in 4.5 mM K^+-containing Krebs-Ringer solution was taken as 100%. ***Significantly different from the initial increase in the perfusion pressure ($P<0.001$) by analysis of variance followed by Scheffé's *t*-test. †Significantly different from corresponding control ($P<0.05$) by analysis of variance followed by Bonferroni's *t*-test.

Table 3 Effects of two inhibitors of cytochrome P-450 and of ETYA on hypercapnia-produced vasodilation in 10 μM phenylephrine-contracted mesenteric vascular beds

	n	*Hypercapnia-produced decrease in PP (% of agonist-increased PP)*
Control	12	45.8±1.8
Clotrimazole 10 μM	7	40.8±3.6
SKF-525A 10 μM	6	56.2±2.7
ETYA 10 μM	8	48.0±2.9

PP = perfusion pressure. Values are mean±s.e.mean. *n*, number of preparations. PE-increased PP in 5% CO_2-saturated Krebs-Ringer solution was taken as 100%.

maximum decrease in the perfusion pressure was obtained in the 12.5 mM K^+ containing solution, and a return of the K^+ concentration to 4.5 mM restored the perfusion pressure to control level. This K^+-produced vasodilation was abolished by pretreatment of the preparation with 0.3 mM $BaCl_2$ (Figure 5B). Pretreatment of the vascular bed with CHAPS also abolished the K^+-produced vasodilation (Figure 5C). As summarized in Figure 6, an increase in the concentration of K^+ reduced the PE-produced increase in the perfusion pressure in a concentration-dependent manner. The basal perfusion pressure was 28.1±1.1 mmHg, and 10 μM PE increased it to 120.6±10.5 mmHg ($n=8$). Subsequent increase in the potassium concentration to 12.5 mM decreased the perfusion pressure to 70.5±4.7 mmHg. Pretreatment with $BaCl_2$ (0.3 mM) or CHAPS abolished the vasodilatory response of the vascular beds to the increased consentration of K^+.

Discussion

The present study demonstrates that an increase in CO_2 causes vasodilation by means of an endothelium-dependent mechanism in the rat mesenteric vascular bed precontracted with PE or U-46619. The vasodilatory effect of hypercapnia was inhibited by neither L-NMMA nor indomethacin. However, the vasodilation produced by an increase in CO_2 disappeared when the vascular bed was precontracted by 60 mM K^+ instead of the vasoconstrictors, indicating that hyperpolarization may be relevant to the vasodilatory effect of the increase in CO_2.

Nielsen *et al.* (1991) and Jensen *et al.* (1993) reported that the steady-state pHi of the smooth muscle in 10% CO_2-saturated solution was comparable to the level in 5% CO_2-saturated solution in rat mesenteric small arteries if pHe was kept constant. In the present study we added adequate amounts of $NaHCO_3$ to Krebs-Ringer solution and kept pHe constant (Table 1). Thus we could expect that the steady-state pHi of the smooth muscle remained constant after increasing CO_2 in the present study. With regard to the addition of $NaHCO_3$, the change in osmolarity in the buffer might regulate contractility of the blood vessel (Lang *et al.*, 1989; Bulow & Johansson, 1994; Lang *et al.*, 1995). However, a reduction of NaCl concentrations in the buffer to keep the osmolarity constant did not modify the vasoactive effect of CO_2. Taken together, it is highly likely that the vasodilatory effect of CO_2 observed in the present study is independent of the change in pHi in the smooth muscle or the change in the osmolarity of the buffer solution.

The importance of the endothelium for the vasodilatory effect of CO_2 has been demonstrated in the cerebral and coronary circulations (Fabricius & Lauritzen, 1994; Gurevicius *et al.*, 1995). Therefore, we investigated the significance of the endothelium on the hypercapnia-produced relaxation in the mesenteric vascular bed. Pretreatment of the preparation with CHAPS abolished not only the ACh-produced vasodilation but also the hypercapnia-produced vasodilation, whereas sodium nitroprusside could still dilate the CHAPS-treated vascular beds. These results demonstrate the involvement of an endothelium-dependent mechanism in the hypercapnia-produced vasodilation in the rat mesenteric vascular bed. It is widely accepted that vascular endothelial cells reduce the vascular tone by at least three mechanisms; production of NO, prostaglandin(s), and hyperpolarization of the vascular smooth muscle (Cocks, 1996). Neither L-NMMA nor indomethacin inhibited the vasodilatory effect of the increase in CO_2 on the agonist-contracted vascular beds, whereas the relaxing effect of CO_2 was not observed when the vascular bed was contracted by high potassium instead of the agonists. These results indicate that NO and prostaglandins are not relevant to the vasodilatory effect of hypercapnia, but hyperpolarization of the smooth muscle cells due to an increase in potassium conductance (Chen & Suzuki, 1989) seems to be the mechanism of the vasodilatory effect of hypercapnia.

There are at least five types of K^+ channels which exist in vascular endothelial or vascular smooth muscle cells (Daut *et al.*, 1994; Murphy & Brayden, 1995). In order to clarify which type of K^+ channel is involved in the vasodilatory effect of the increase in CO_2, we examined the effects of five K^+ channel inhibitors. Inasmuch as each K^+ channel inhibitor does not

necessarily inhibit one type of K^+ channel specifically, the concentration of each inhibitor used is very important to discriminate the various K^+ channels. In the present study, we used 1 mM TEA to inhibit the large conductance Ca^{2+}-activated K^+ channel, 500 nM apamin to inhibit the small conductance Ca^{2+}-activated K^+ channels, 10 μM glibenclamide as an ATP-sensitive K^+ channel inhibitor, 1.5 mM 4-AP as a delayed rectifier K^+ channel inhibitor, and 0.3 mM Ba^{2+} to inhibit the inward rectifier K^+ channel (Nelson & Quayle, 1995). Among these inhibitors, only Ba^{2+} inhibited the relaxing response of the mesenteric vascular bed to the increase in CO_2. Because Ba^{2+} did not inhibit the ACh-produced, endothelium-dependent vasodilation, the inhibitory effect of Ba^{2+} on the hypercapnia-produced vasodilation seems to be specific. These results strongly suggests the involvement of the inward rectifier K^+ channel in the vasodilatory effect of the increase in CO_2.

Epoxyeicosatrienoic acids formed from arachidonic acid by the action of cytochrome P-450 monooxygenase are candidates for the endothelium-derived hyperpolarizing factor (EDHF) (Bauersachs *et al*., 1994; Campbell *et al*., 1996; Chen and Cheung, 1996). Thus we tested two cytochrome P-450 inhibitors (clotrimazole and SKF 525A), and ETYA which inhibits arachidonic acid metabolism *via* cyclooxygenase, lipoxygenase and cytochrome P-450 monooxygenase (Pinto *et al*., 1987; Capdevila *et al*., 1988). The results shown in Table 3 do not support the participation of the cytochrome P-450 products in the hypercapnia-produced vasodilation.

Because both the vascular endothelial cells and the vascular smooth muscle cells could express the inward rectifier potassium channel (Daut *et al*., 1994), our next concern was to clarify which inward rectifier K^+ channel was relevant for the vasodilatory effect of CO_2. McCarron & Halpern. (1990) have reported that the small cerebral arteries in the rat dilate paradoxically when extracellular K^+ is increased in the 5–15 mM range. The potassium-produced vasodilation was inhibited by 50 μM Ba^{2+}. A hypothetical mechanism underlying the vasodilatory effect of K^+ is thought to be the following (Edwards *et al*., 1988; Quayle *et al*., 1993): A slight increase in the extracellular K^+ concentration increases potassium conductance provided by the inward rectifier K^+ channel. Therefore, although an increase in the extracellular potassium concentration makes the equilibrium potential of potassium less negative, the outward K^+ current flowing at potentials near the resting membrane potential of vascular smooth muscle increases substantially, resulting in hyperpolarization and vasodilation. Based on the above observations and interpretation, we expected that a slight increase in the K^+ concentration of the buffer solution could reduce the PE-increased perfusion pressure if the inward rectifier K^+ channel played a role in the rat mesenteric vascular bed. As shown in Figures 5 and 6, the stepwise increment of the extracellular K^+ concentration from 4.5 mM to 12.5 mM reduced the PE-increased perfusion pressure in a concentration-dependent manner, and Ba^+ (0.3 mM) abolished the vasodilatory effect of K^+. The vasodilatory effect of potassium was not observed in the endothelium-removed mesenteric vascular bed, indicating that the inward rectifier potassium channel which responded to the increase in K^+ concentration might exist on the endothelial cells.

The ionic current through the inward rectifier K^+ channel is thought to be the predominant current in the endothelial cell under resting conditions, and thus play an important role in setting the resting membrane potential of the endothelium (Daut *et al*., 1994; Vaca *et al*., 1996). Olesen *et al*. (1988) demonstrated that shear stress activated the inward rectifier K^+ channel in bovine aortic endothelial cells. More recently, Wellman and Bevan (1995) also showed in the rabbit middle cerebral artery that the flow-induced shear stress activated the endothelial cell inward rectifier K^+ channel. The present study further demonstrates the physiological significance of the vascular endothelial cell inward rectifier K^+ channel. In contrast to our result, however, the flow-induced relaxation of the rabbit middle cerebral artery is also suppressed by an inhibition of NO synthase (Wellman and Bevan, 1995). In the present experiment, relaxation of the vascular bed produced by the increase in CO_2 might be due to the direct electrical communication of the endothelial cells and the smooth muscle cells through gap junctions (Davies *et al*., 1988). Further experiments including electrophysiological studies are needed to clarify the precise mechanism of the hypercapnia-produced vasodilation in the rat mesenteric vascular bed.

In summary, an increase in CO_2 dilates the rat mesenteric vascular bed precontracted by PE or U-46619 in an endothelium-dependent manner. The membrane hyperpolarization which is derived from the activation of the endothelial inward rectifier K^+ channel might be involved in the hypercapnia-produced vasodilation.

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