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Hypocapnic constriction in rabbit basilar artery in vitro: triggering by N^G-monomethyl-L-arginine monoacetate and dependence on endothelin-1 and alkalosis

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Abstract

This study tested whether hypocapnic constriction of the rabbit basilar artery in vitro can be triggered by a nitric oxide (NO) synthase inhibitor, and whether the resulting constriction is (1) due to the alkaline pH associated with hypocapnia, and (2) endothelin-1 mediated. Hypocapnic (25 mM $NaHCO_3$; pH 7.76; pCO_2 14.2) or isocapnic alkaline solution (50 mM $NaHCO_3$; pH 7.73; pCO_2 35.0) rarely altered basal tension. N^G-monomethyl-L-arginine monoacetate (L-NMMA; 0.1 mM) challenge in hypocapnic or isocapnic alkaline solution resulted in near maximal tension that was maintained for 2–2.5 h even following L-NMMA washout. L-NMMA challenge in normal solution (25 mM $NaHCO_3$; pH 7.42; pCO_2 36.9) also induced near maximal tension, although the tension was maintained for only 25 min (mean). Ac-D-Bhg-L-Leu-Asp-L-Ile-L-Ile-L-Trp (PD145065), homopiperidinyl-CO-Leu-D-Trp(CHO)-D-Trp (BQ610), and *N-cis*-2,6-dimethyl-piperidinocarbonyl L-γ-MeLeu-D-Trp ($COOCH_3$)-Nle (BQ788; 1–3 μM), endothelin ET_A/ET_B, endothelin ET_A, and endothelin ET_B receptor antagonists, respectively, completely relaxed the tension that resulted from L-NMMA challenge in hypocapnic or isocapnic alkaline solution. These results demonstrate that constriction due to hypocapnia in vitro can be triggered by an NO synthase inhibitor and is endothelin-1 mediated. Additionally, alkaline pH in the absence of decreased pCO_2 is sufficient to elicit the constriction.

Keywords: Hypocapnic constriction; Endothelin-1; Basilar artery; Nitric oxide (NO)

1. Introduction

Hypocapnic constriction of the cerebral vasculature represents a major pathway for cerebral blood flow regulation (for a review see Traystman (1997)). While the mechanism underlying the constriction remains to be clarified, it is generally considered that the constriction results from the direct effects of hypocapnia and/or elevated pH on the smooth muscle cells (Kontos et al., 1977a,b; Dacey and Duling 1982; Harder and Madden, 1985; Smeda et al., 1987; West et al., 1992; Apkon and Boron, 1995; Mirro et al., 1993; Smith et al., 1998).

In apparent contrast, we recently reported that, in rabbit basilar artery rings in vitro: (1) hypocapnia and elevated pH were not sufficient to elicit constriction, as hypocapnic alkaline solution induced constriction only following initial challenge with 40 mM KCl; and (2) the resultant constriction was not due to the direct effect of hypocapnia and elevated pH on the smooth muscle cells, as the constriction was endothelin-1 dependent (Zuccarello et al., 2000).

To further investigate the mechanism underlying the ability of KCl to trigger hypocapnic constriction in vitro, we considered whether other agents that induce membrane depolarization or endothelin-1 release also trigger the constriction. We previously demonstrated that a nitric oxide

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(NO) synthase inhibitor depolarized rabbit basilar artery smooth muscle in vitro (Zuccarello et al., 1993), and NO synthase inhibitors were reported to release endothelin-1 from cultured vascular endothelial cells (Kourembanas et al., 1993; Mitsutomi et al., 1999; Boulanger and Luscher, 1990). Thus, the present study tested whether the NO synthase inhibitor, N^G-monomethyl-L-arginine monoacetate (L-NMMA), elicited endothelin-1-mediated hypocapnic constriction in the rabbit basilar artery in vitro.

2. Materials and methods

2.1. Tissue preparation

New Zealand white male rabbits (35; weight, 2.8–3.2 kg) were anesthetized with ketamine HCl (30 mg/kg i.m.), xylazine (6 mg/kg i.m.), and pentobarbital Na^+ (35 mg/kg i.v.). Following exsanguination, the brain was removed and placed in ice-cold Krebs Ringer bicarbonate solution containing (mM): 119 NaCl, 4.7 KCl, 1.18 KH_2PO_4, 1.17 $MgSO_4$, 25 $NaHCO_3$, 11 glucose, 0.026 EDTA, and 2.5 $CaCl_2$. The basilar artery was then removed and cut into ring segments (2 mm) with care taken to preserve the endothelium intact. Each ring segment was placed in an organ bath containing 15-ml normal (maximal bubbling with 94% O_2/6% CO_2; pH 7.42 ± 0.01, pCO_2 36.9 ± 0.3, pO_2 643.0 ± 6.7; $n = 21$; Zuccarello et al. (2000)), hypocapnic (decreased bubbling with 94% O_2/6% CO_2; pH 7.76 ± 0.01, pCO_2 14.2 ± 0.2, pO_2 290.6 ± 6.5; $n = 28$; Zuccarello et al. (2000)), or isocapnic alkaline Krebs Ringer bicarbonate solution (50 mM $NaHCO_3$ (equimolar NaCl substitution) pH 7.73 ± 0.01; pCO_2 35.0 ± 0.4, pO_2 619.4 ± 4.8; $n = 12$; means ± SE), all prepared with 1 μM indomethacin. Although the pO_2 of the hypocapnic solution (291 mm Hg) was less than that of the normocapnic solution, this level of pO_2 is still relatively high and would not be expected to alter constriction.

Isometric tension was recorded by placing two tungsten wires (33-μm diameter) through the vessel lumen, with one wire attached to a microdrive and the other wire to a force displacement transducer. Vessel segments were placed at optimal resting tension (0.5 g-force) and were allowed to equilibrate for 30–40 min prior to agent addition.

2.2. Protocol

Rings exposed to normal, hypocapnic alkaline, or isocapnic alkaline solution were challenged for 20 min with 0.1 mM L-NMMA, a non-selective NO synthase inhibitor (Gross et al., 1990). If significant tension did not develop,

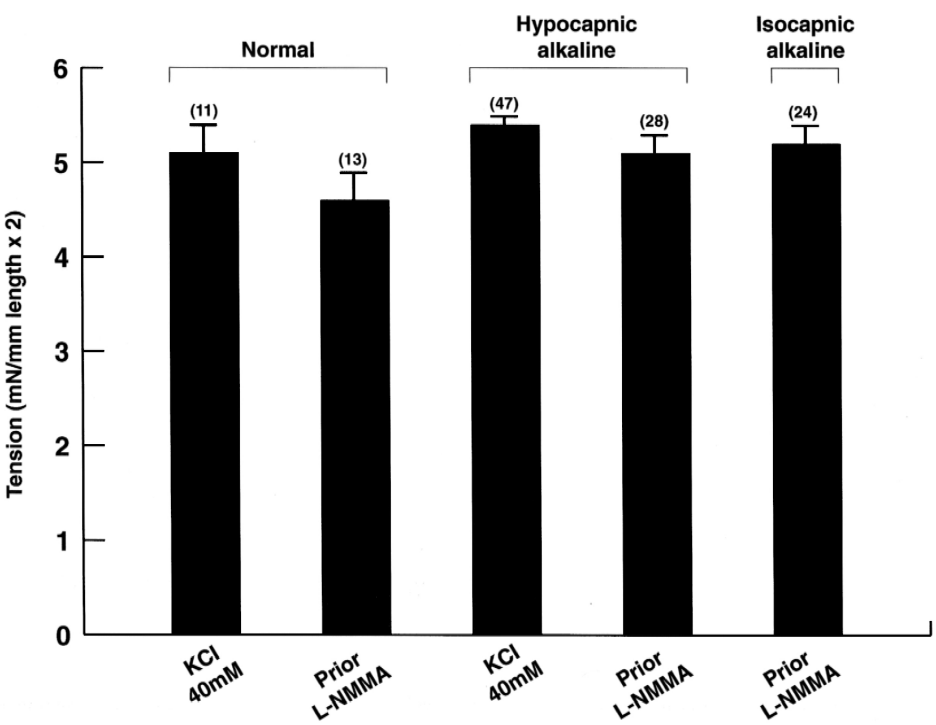

Fig. 1. L-NMMA-induced constriction in normal, hypocapnic alkaline and isocapnic alkaline solution. Rabbit basilar artery rings were incubated in normal, hypocapnic alkaline and isocapnic alkaline solution, challenged with 0.1 mM L-NMMA, and then washed. KCl (40 mM) was added prior to L-NMMA in some tissues incubated in normal and hypocapnic alkaline solution (KCl data of tissues incubated in hypocapnic alkaline solution from Zuccarello et al. (2000)). ''Prior L-NMMA'' indicates that rings were exposed to L-NMMA followed by L-NMMA washout. Note that the duration of plateau tension elicited by L-NMMA in normal and hypocapnic/isocapnic alkaline solution was 10–60 min (mean 25 min) and 120–150 min, respectively (see Section 3). Tension was calculated as mN/mm length × 2. Values shown are means ± SE; '*n*' is indicated in parenthesis.

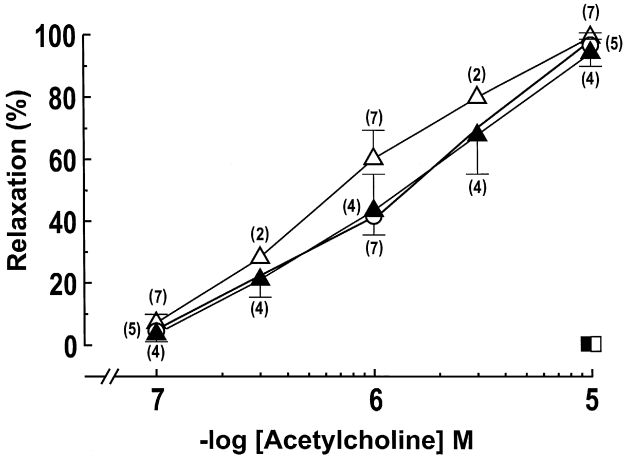

Fig. 2. Acetylcholine relaxation of the L-NMMA-induced constriction in hypocapnic alkaline and isocapnic alkaline solution with and without L-NMMA washout. Tension was induced in rabbit basilar artery by (a) L-NMMA challenge of rings incubated in hypocapnic alkaline (closed square) or isocapnic alkaline solution (open square) followed, in some cases, by wash with hypocapnic alkaline (open triangle) or isocapnic alkaline solution (closed triangle), or (b) as described in Zuccarello et al. (2000), by repeated KCl challenge of rings in hypocapnic alkaline solution followed by wash with hypocapnic alkaline solution (open circle). Results are expressed as percent relaxation of the tension. Values shown are means ± SE; '*n*' is indicated in parenthesis.

L-NMMA was washed out with the appropriate solution and immediately rechallenged with L-NMMA. The L-NMMA challenge/washout sequence was repeated for the number of times indicated. Following attainment of plateau constriction, the L-NMMA was again washed out with the appropriate solution or allowed to remain. Rings were then challenged with endothelin receptor antagonist, papaverine, elevated pCO_2, or acetylcholine.

2.3. Statistical analysis

Statistical significance between two means was determined using Student's paired *t*-test. Significance was accepted at the 0.05 level of probability. Values are expressed as means ± SE '*n*' represents the number of tissues.

2.4. Materials

Reagent sources were as follows. Peptides International for homopiperidinyl-CO-Leu-D-Trp(CHO)-D-Trp (BQ610) and *N-cis*-2,6-dimethyl-piperidinocarbonyl L-γ-MeLeu-D-Trp ($COOCH_3$)-Nle (BQ788), Sigma for indomethacin and papaverine HCl, Calbiochem for L-NMMA, and Parke-Davis Pharmaceutical for Ac-D-Bhg-L-Leu-Asp-L-Ile-L-Ile-L-Trp (PD145065; gift).

3. Results

3.1. L-NMMA and normal solution

Following a 20-min challenge with 0.1 mM L-NMMA, only one tissue constricted to the magnitude induced by 40

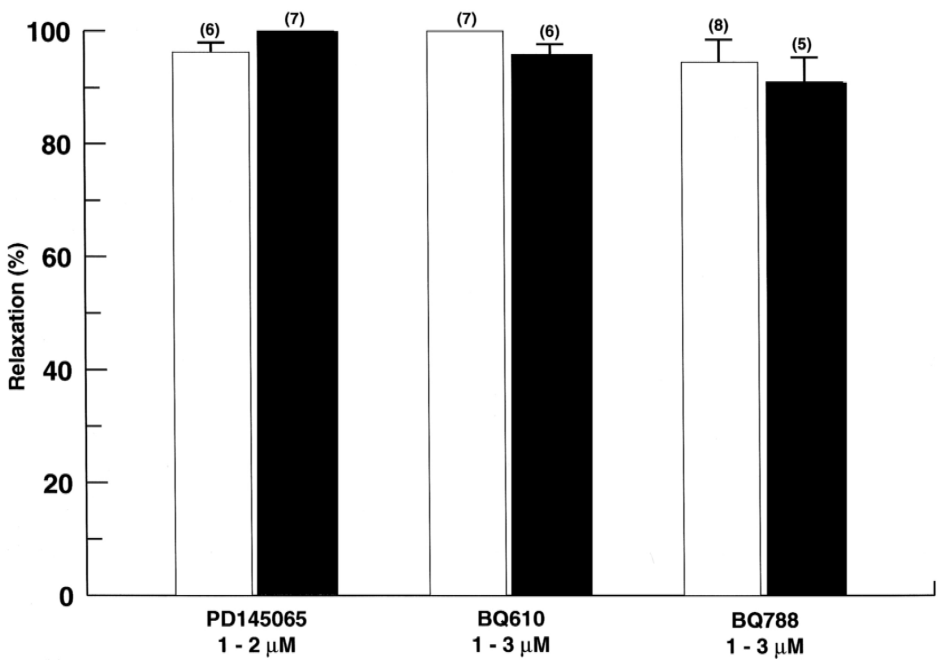

Fig. 3. Endothelin receptor antagonist relaxation of the L-NMMA-induced constriction in hypocapnic alkaline and isocapnic alkaline solution with L-NMMA washout. Rabbit basilar artery rings incubated in hypocapnic alkaline (open bar) and isocapnic alkaline solution (closed bar) were challenged with 0.1 mM L-NMMA, and then washed. Constricted tissues were then challenged with 1–2 μM PD145065, 1–3 μM BQ610, or 1–3 μM BQ788. Results are expressed as percent relaxation of the tension. Values shown are means ± SE; '*n*' is indicated in parenthesis.

mM KCl in normal and hypocapnic alkaline solution (Fig. 1). Five, five, and two tissues required two, three, and four L-NMMA challenges, respectively, to develop this magnitude of constriction, while one tissue did not constrict even following five L-NMMA challenges. The magnitude of constriction in these 13 tissues (sum of one, five, five, and two L-NMMA constricted tissues) was 4.6 ± 0.3 mN/mm length $\times 2$ (means $\pm$ SE; Fig. 1). The constriction was maintained in one tissue for 10 min, in three tissues for 20 min, in one tissue for 30 min, and in another tissue for 60 min even in the continued presence of L-NMMA.

3.2. L-NMMA and hypocapnic alkaline solution

Hypocapnic alkaline solution constricted only four of 51 tissues (Zuccarello et al., 2000). Exposure of rings to 0.1 mM L-NMMA for 20 min in hypocapnic solution constricted 18 of 31 tissues to the magnitude induced by 40 mM KCl in normal and hypocapnic alkaline solution (Fig. 1). Extending the L-NMMA challenge from 20 to 40–50 min did not result in an increased number of tissues developing significant constriction.

A second 0.1 mM L-NMMA challenge constricted an additional 11 tissues to the magnitude of 40 mM KCl (Fig. 1). The magnitude of constriction triggered by L-NMMA in hypocapnic alkaline solution in these 29 tissues (sum of 18 and 11 L-NMMA constricted tissues) was 5.1 ± 0.2 mN/mm length $\times 2$ (means $\pm$ SE; Fig. 1). The plateau constriction was maintained for 2–2.5 h, and repeated wash with hypocapnic alkaline solution did not decrease the tension.

We then considered whether the maintained constriction observed following L-NMMA washout with hypocapnic alkaline solution was dependent on residual NO synthase inhibition. Thus, we tested whether NO synthase activity was restored following L-NMMA washout with hypocapnic alkaline solution. L-NMMA abolished acetylcholine relaxation of constricted hypocapnic tissues (Fig. 2). Following L-NMMA washout of these tissues with hypocapnic alkaline solution, acetylcholine relaxation was restored to the level observed in tissues in which hypocapnic tension was triggered by KCl (Fig. 2).

3.3. L-NMMA and isocapnic alkaline solution

To investigate whether hypocapnia and/or alkalinity was necessary for L-NMMA to trigger the prolonged constriction observed in hypocapnic alkaline solution, tissues were exposed to isocapnic alkaline solution followed by L-NMMA. Isocapnic alkaline solution in the absence of L-NMMA did not result in constriction. Exposure of rings to 0.1 mM L-NMMA for 20 min in isocapnic solution

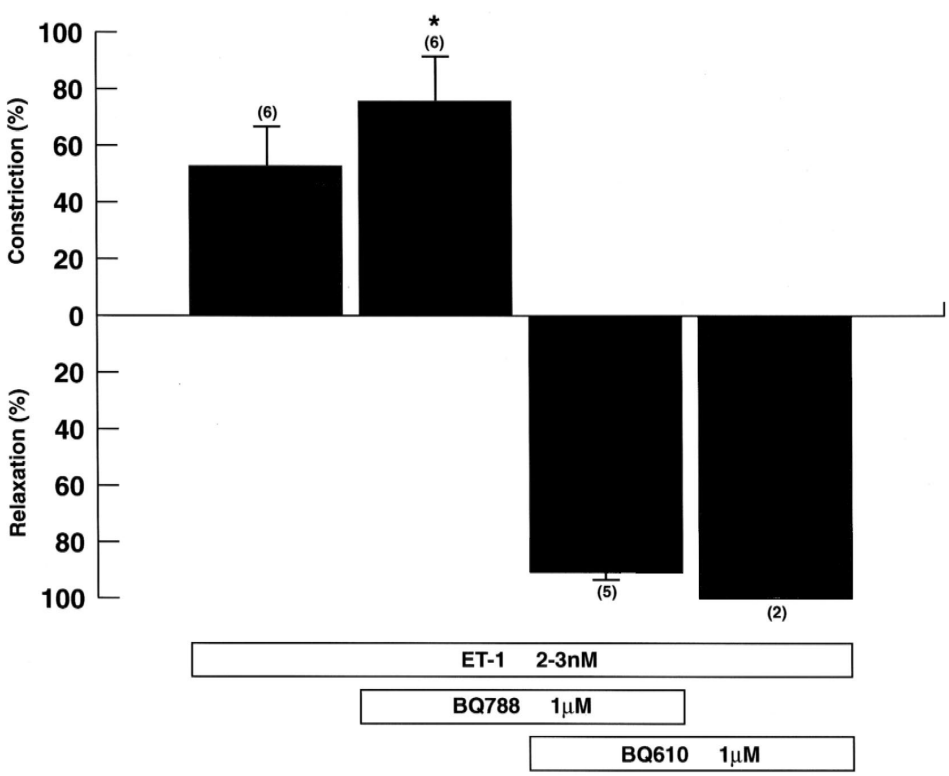

Fig. 4. Effect of BQ788 and BQ610 on constriction due to exogenous endothelin-1. Rabbit basilar artery rings incubated in normal solution were constricted with 2–3 nM endothelin-1, followed by either 1 μM BQ610, or 1 μM BQ788 and then 1 μM BQ610. Constriction is expressed as percent of the constriction due to 40 mM KCl, and relaxation is expressed as percent relaxation of the tension. Values shown are means $\pm$ SE; '*n*' is indicated in parenthesis. * Significantly greater than the constriction in the absence of endothelin receptor antagonist.

constricted 19 of 26 tissues to the magnitude induced by 40 mM KCl in normal and hypocapnic alkaline solution (Fig. 1). Extending the initial L-NMMA exposure period from 20 to 40–50 min did not result in an increased number of tissues developing tension.

Second and third 0.1 mM L-NMMA challenges constricted an additional four and one tissues, respectively, to the magnitude of 40 mM KCl (Fig. 1). The magnitude of constriction triggered by L-NMMA in isocapnic alkaline solution in these 24 tissues (sum of 19, four, and one L-NMMA constricted tissues) was 5.2 ± 0.2 mN/mm length $\times 2$ (means $\pm$ SE; Fig. 1). Plateau tension was maintained for 2–2.5 h, and repeated wash with isocapnic alkaline solution did not decrease the tension.

Again, we investigated whether the maintained constriction observed following L-NMMA washout in isocapnic alkaline solution was still dependent on residual NO synthase inhibition. L-NMMA abolished acetylcholine relaxation of constricted isocapnic alkaline tissues (Fig. 2). Following L-NMMA washout of these tissues with isocapnic alkaline solution, acetylcholine relaxation was restored to the level observed in tissues in which hypocapnic tension was triggered by L-NMMA or KCl (Fig. 2).

3.4. Endothelin ET receptor antagonists, increased pCO_2, and papaverine

PD145065, BQ610, and BQ788 (1 μM; endothelin ET_A/ET_B, endothelin ET_A, and endothelin ET_B receptor antagonists, respectively), relaxed the tension that resulted from initial L-NMMA challenge of tissues incubated in hypocapnic alkaline solution by $89.6 \pm 7.1\%$ (6), $64.2 \pm 17.0\%$ (7), and $49.7 \pm 15.6\%$ (8), respectively, and in isocapnic alkaline solution by $90.9 \pm 5.9\%$ (7), $60.7 \pm 15.2\%$ (6), and $58.8 \pm 15.8\%$ (5), respectively (means $\pm$ SE; *n*). Increasing the endothelin receptor antagonist concentrations to 2–3 μM completely relaxed the constriction (Fig. 3). The endothelin receptor antagonists did not alter basal tone.

In normal solution, the 2–3 nM endothelin-1 constriction was also completely relaxed by 1 μM BQ610 (Fig. 4). In contrast, while BQ788 relaxed the constriction triggered by L-NMMA in hypocapnic and isocapnic solution (Fig. 3), 1 μM BQ788 enhanced the constriction due to endothelin-1 in normal solution (Fig. 4).

Increased pCO_2, with resultant normalization of the pH, abolished the constriction due to prior L-NMMA challenge in hypocapnic alkaline solution ($93.8 \pm 3.6\%$; means $\pm$ SE; $n = 4$). Papaverine (30 μM) also completely relaxed the constriction due to prior L-NMMA challenge in isocapnic alkaline solution (100%; $n = 3$).

Attempts to determine the possible relaxant effects of endothelin receptor antagonists on the tension triggered by L-NMMA in normal solution were inconclusive due to the variable and transient plateau constriction (see Section 3.1).

4. Discussion

The major finding of this study is that the NO synthase inhibitor, L-NMMA, triggers endothelin-1 mediated hypocapnic constriction of the rabbit basilar artery in vitro. While the mechanism whereby L-NMMA triggers hypocapnic constriction is not known, we propose that the constriction results from membrane depolarization following NO synthase inhibition (Zuccarello et al., 1993) and initial release of endothelin-1 (Kourembanas et al., 1993; Mitsutomi et al., 1999; Boulanger and Luscher, 1990). The cellular source of the endothelin-1 might be the endothelium and/or smooth muscle, as endothelin-1 can originate from both cell types (Kanse et al., 1991; Kasuya et al., 1993).

Consistent with this proposal is our previous finding that repeated challenge with 40 mM KCl, a concentration that induces a similar magnitude of membrane depolarization as NO synthase inhibition in rabbit basilar artery in vitro (Zuccarello et al., 1993; membrane potential results with KCl unpublished), also induced hypocapnic constriction (Zuccarello et al., 2000). Interestingly, prolonged exposure to KCl did not substitute for repeated challenges with KCl, suggesting that membrane depolarization was not sufficient to trigger hypocapnic constriction (Zuccarello et al., 2000). We speculate that the repeated KCl challenges also resulted in the initial release of endothelin-1.

The present finding that L-NMMA was required to trigger hypocapnic constriction is in contrast with previous studies demonstrating that hypocapnia/alkalinization was sufficient to elicit constriction in the cerebral vasculature (Kontos et al., 1977a,b; Dacey and Duling 1982; Harder and Madden, 1985; Smeda et al., 1987; West et al., 1992; Apkon and Boron, 1995; Mirro et al., 1993). While the reason for this contrast is not known, it is likely related in part to the different membrane potentials of the smooth muscle cells in the different preparations. That is, intravascular pressure depolarizes smooth muscle cells (Harder, 1984; Dunn et al., 1994) and in all but two of the previous studies demonstrating hypocapnia/alkalinization constriction (Harder and Madden 1985; West et al., 1992), the preparations were either in situ or pressurized in vitro (Kontos et al., 1977a,b; Dacey and Duling 1982; Smeda et al., 1987; Apkon and Boron, 1995; Mirro et al., 1993). In addition, pressure has been demonstrated to increase endothelin-1 release from cultured endothelial cells (Hishikawa et al., 1995).

With respect to the two studies in which hypocapnia/alkalinization constricted cerebral vasculature preparations in the absence of intravascular pressure (Harder and Madden 1985; West et al., 1992), one of these studies employed enzymatically isolated smooth muscle cells from the guinea pig basilar artery (West et al., 1992). Perhaps hypocapnia constricted the cells in the absence of pressure because the isolation procedure altered the sensitivity, and

possibly mechanism, for hypocapnic constriction. In this regard, the isolation procedure may have depolarized the smooth muscle cell membrane, as elevated extracellular Ca^{2+} constricted the isolated cells (West et al., 1992). The other report demonstrated hypocapnic constriction of the cat middle cerebral artery using an endothelium intact in vitro ring preparation similar to the present study (Harder and Madden, 1985). Perhaps the degree of depolarization necessary to allow hypocapnic constriction of vessels placed in vitro in the absence of intravascular pressure depends on the vessel.

With respect to the mechanism(s) of endothelin-1 release, while the initial release of endothelin-1 likely occurs as a result of L-NMMA blockade of NO formation (above), subsequent endothelin-1 release can not be due to L-NMMA disinhibition of endothelin-1 release, as the constriction was maintained even when NO synthase activity was no longer inhibited, i.e., following L-NMMA washout. The mechanism responsible for the endothelin-1 release that underlies the maintained constriction may be a positive feedback loop in which endothelin-1 induces further endothelin-1 release following endothelin ET_B receptor activation, since (1) the endothelin ET_B receptor antagonist, BQ788, relaxed the constriction elicited in hypocapnic alkaline solution, but not that due to exogenous endothelin-1. In fact, BQ788 enhanced the constriction due to exogenous endothelin-1, presumably as the result of blockade of endothelin-1-induced endothelin ET_B receptor mediated relaxation (Zuccarello et al., 1998). Thus, BQ788 relaxation of the constriction elicited in hypocapnic alkaline solution was not due to blockade of endothelin ET_B receptor-mediated constriction; (2) previous studies demonstrated that endothelin-1 can induce further endothelin-1 release and, moreover, the release is endothelin ET_B receptor mediated (Yokokawa et al., 1991; Saijonmaa et al., 1992; Fujitani et al., 1992; Iwasaki et al., 1995); (3) we recently demonstrated that exogenous endothelin-1 can trigger hypocapnic constriction dependent on endogenous endothelin-1 release (Zuccarello et al., 2000).

As L-NMMA triggered endothelin-1 dependent constriction in isocapnic alkaline solution, this positive feedback mechanism for endothelin-1 release is presumably dependent on alkalinization rather than decreased pCO_2. Thus, the inability of L-NMMA to trigger prolonged constriction in normal solution may stem from alkalinity somehow facilitating this positive feedback mechanism. Clearly, it remains for further study whether hypocapnia induces a similar alkaline-dependent endothelin-1-mediated constriction in vivo.

Acknowledgements

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References

Apkon, M., Boron, W.F., 1995. Extracellular and intracellular alkalinization and the constriction of rat cerebral arterioles. J. Physiol. (London) 484, 743–753.

Boulanger, C., Luscher, T.F., 1990. Release of endothelin from the porcine aorta. Inhibition by endothelium-derived nitric oxide. J. Clin. Invest. 85, 587–590.

Dacey, R.G. Jr., Duling, B.R., 1982. A study of rat intracerebral arterioles: methods, morphology, and reactivity. Am. J. Physiol. 243, H598–H606.

Dunn, W.R., Wellman, G.C., Bevan, J.A., 1994. Enhanced resistance artery sensitivity to agonists under isobaric compared with isometric conditions. Am. J. Physiol. 266, H147–H155.

Fujitani, Y., Oda, K., Takimoto, M., Inui, T., Okada, T., Urade, Y., 1992. Autocrine receptors for endothelins in the primary culture of endothelial cells of human umbilical vein. FEBS Lett. 298, 79–83.

Gross, S.S., Stuehr, D.J., Aisaka, K., Jaffe, E.A., Levi, R., Griffith, O.W., 1990. Macrophage and endothelial cell nitric oxide synthesis: cell-type selective inhibition by N^G-aminoarginine, N^G-nitroarginine and N^G-methylarginine. Biochem. Biophys. Res. Commun. 170, 96–103.

Harder, D.R., 1984. Pressure-dependent membrane depolarization in cat middle cerebral artery. Circ. Res. 55, 197–202.

Harder, D.R., Madden, A., 1985. Cellular mechanism of force development in cat middle cerebral artery by reduced PCO_2. Pfluegers Arch. 403, 402–404.

Hishikawa, K., Nakaki, T., Marumo, T., Suzuki, H., Kato, R., Saruta, T., 1995. Pressure enhances endothelin-1 release from cultured human endothelial cells. Hypertension 25, 449–452.

Iwasaki, S., Homma, T., Matsuda, Y., Kon, V., 1995. Endothelin receptor subtype B mediates autoinduction of endothelin-1 in rat mesangial cells. J. Biol. Chem. 270, 6997–7003.

Kanse, S.M., Takahashi, K., Warren, J.B., Perera, T., Porta, M., Ghatei, M., Bloom, S.R., 1991. Production of endothelin by vascular smooth muscle cells. J. Cardiovasc. Pharmacol. 17, S113–S116.

Kasuya, H., Weir, B.K.A., White, D.M., Stefansson, K., 1993. Mechanism of oxyhemoglobin-induced release of endothelin-1 from cultured vascular endothelial cells and smooth-muscle cells. J. Neurosurg. 79, 892–898.

Kontos, H.A., Raper, A.J., Patterson, J.L., 1977a. Analysis of local pH, PCO_2 and bicarbonate on pial vessels. Stroke 8, 358–360.

Kontos, H.A., Wei, E.P., Raper, A.J., Patterson, J.L. Jr., 1997b. Local mechanism of CO_2 action on pial arterioles of the cat. Stroke 8, 226–229.

Kourembanas, S., McQuillan, L.P., Leung, G.K., Faller, D.V., 1993. Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia. J. Clin. Invest. 92, 99–104.

Mirro, R., Pharris, L.J., Armstead, W.M., Shibata, M., Leffler, C.W., 1993. Effects of indomethacin on newborn pig arteriolar responses to PCO_2. J. Appl. Physiol. 75, 1300–1305.

Mitsutomi, N., Akashi, C., Odagiri, J., Matsumura, Y., 1999. Effects of endogenous nitric oxide on endothelin-1 production in cultured vascular endothelial cells. Eur. J. Pharmacol. 364, 65–73.

Saijonmaa, O., Nyman, T., Fyhrquist, F., 1992. Endothelin-1 stimulates its own synthesis in human endothelial cells. Biochem. Biophys. Res. Commun. 188, 286–291.

Smeda, J.S., Lombard, J.H., Madden, J.A., Harder, D.R., 1987. The effect of alkaline pH and transmural pressure on arterial constriction and membrane potential of hypertensive cerebral arterioles. Pfluegers Arch. 408, 239–242.

Smith, G.L., Austin, C., Crichton, C., Wray, S., 1998. A review of the actions and control of intracellular pH in vascular smooth muscle. Cardiovasc. Res. 38, 316–331.

Traystman, R.J., 1997. Regulation of cerebral blood flow by carbon dioxide. In: Welch, K.M.A., Caplan, L.R., Reis, D.J., Srsjo, B.K., Weir, B. (Eds.), Primer on Cerebrovascular Diseases. Academic Press, San Diego, CA, pp. 55–58.

West, G.A., Leppla, D.C., Simard, J.M., 1992. Effects of external pH on ionic currents in smooth muscle cells from the basilar artery of the guinea pig. Circ. Res. 71, 201–209.

Yokokawa, K., Kohno, M., Yasunari, K., Murakawa, K., Takeda, T., 1991. Endothelin-3 regulates endothelin-1 production in cultured human endothelial cells. Hypertension 18, 304–315.

Zuccarello, M., Boccaletti, R., Romano, A., 1998. Endothelin B receptor antagonists attenuate subarachnoid hemorrhage-induced cerebral vasospasm. Stroke 29, 1924–1929.

Zuccarello, M., Bonasso, C.L., Sperelakis, N., Rapoport, R.M., 1993. Role of membrane potential in vasospasm after subarachnoid hemorrhage. In: Findlay, J.M. (Ed.), Cerebral Vasospasm. Elsevier, Amsterdam, The Netherlands, pp. 229–233.

Zuccarello, M., Lee, B.H., Rapoport, R.M., 2000. Endothelin-1 mediates hypocapnic constriction of the rabbit basilar artery in vitro. J. Pharm. Pharmacol. 52, 225–226.