Euhydric hypercapnia increases vasoreactivity of rat pulmonary arteries via HCO\textsubscript{3}\textsuperscript{-} transport and depolarisation

Marie Vaňková\textsuperscript{1}, Vladimir A. Snetkov, Greg A. Knock, Philip I. Aaronson, Jeremy P.T. Ward*  

Department of Asthma, Allergy and Respiratory Science, GKT School of Medicine,  
Guy’s Hospital Campus, London SE1 1UL, UK  

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Abstract

Objective: To examine whether altered PCO\textsubscript{2} or HCO\textsubscript{3}\textsuperscript{-}/CO\textsubscript{2} at normal pH potentiate agonist-induced vasoconstriction of small pulmonary arteries, and if so to determine the mechanism.

Methods: Small intrapulmonary arteries (IPA) from rats were mounted on a myograph and PGF\textsubscript{2\alpha} (3 \mu M)-induced tension recorded before and 40 min after replacing normal bath solution (5% CO\textsubscript{2}, 24 mM [HCO\textsubscript{3}/CO\textsubscript{2}], pH 7.4) with one containing either normal [HCO\textsubscript{3}/CO\textsubscript{2}] (24 mM) gassed with 10% CO\textsubscript{2} (pH 7.12; hypercapnic acidosis) or high [HCO\textsubscript{3}/CO\textsubscript{2}] (48 mM) gassed with 10% CO\textsubscript{2} (pH 7.4; euhydric hypercapnia).

Results: Hypercapnic acidosis had no significant effect on the response of IPA to PGF\textsubscript{2\alpha}. Euhydric hypercapnia however caused a substantial ~5.5-fold potentiation of the response (n=17, p<0.001) in the majority of preparations, whilst 20% of IPA (11 of 58) developed a slow spontaneous vasoconstriction after ~20 min. No equivalent responses to euhydric hypercapnia were observed in either mesenteric or renal arteries. Both the potentiation of PGF\textsubscript{2\alpha}-induced vasoconstriction and the spontaneous vasoconstriction in IPA were inhibited by the L-type channel blocker diltiazem (10 \mu M). The potentiation was also suppressed by DIDS, an inhibitor of anion transporters, removal of extracellular Na\textsuperscript{+}, and anthracene-9-carboxylic acid (A9C; 200 \mu M), reported to inhibit Ca\textsuperscript{2+}-activated Cl\textsuperscript{-} channels. Inhibition of nitric oxide synthase with L-NAME (100 \mu M) did not prevent potentiation. Depolarisation with 20 mM [K\textsuperscript{+}] mimicked the effect of euhydric hypercapnia in that it also potentiated the response to PGF\textsubscript{2\alpha} (>sixfold, n=6).

Conclusions: Euhydric hypercapnia increases vasoreactivity of IPA, but not mesenteric or renal arteries, via a mechanism involving Na\textsuperscript{+}-dependent HCO\textsubscript{3}\textsuperscript{-} transport, activation of Ca\textsuperscript{2+}-activated Cl\textsuperscript{-} channels, and subsequent depolarisation. These results may have consequences for patients with CO\textsubscript{2}-retaining chronic respiratory disease where plasma [HCO\textsubscript{3}] is raised following renal compensation, and could explain the increased propensity to pulmonary hypertension and increased mortality in such patients.

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1. Introduction

Patients developing hypoxaemia as a result of respiratory disease or critical illness tend to have an increased pulmonary vascular resistance (PVR) due to vasoconstriction of small pulmonary arteries [hypoxic pulmonary vasoconstriction (HPV)]. Respiratory dysfunctions that lead to hypoxaemia are also likely to cause perturbations in CO\textsubscript{2} exchange, leading to hypercapnia and respiratory acidosis. However, in chronic respiratory disease, the respiratory acidosis is corrected primarily by renal compensation and increased plasma [HCO\textsubscript{3}]. These conditions are reported to promote development of pulmonary hypertension and increase mortality in COPD patients [1], suggesting that hypercapnia per se and/or the increase in [HCO\textsubscript{3}] may affect pulmonary vascular reactivity.
For many years, there has been a general acceptance that hypercapnia increases PVR in perfused lungs and in vivo, and this is indeed consistent with a recent study in healthy humans [2]. However, hypercapnia has also been reported to reduce PVR and suppress agonist-induced vasoconstriction and HPV in perfused lungs [3,4], and Brimioulle et al. [5] reported that whereas metabolic acidosis enhanced HPV in intact dogs, an equivalent respiratory acidosis was without effect. Conversely, Gordon et al. [6] have shown in piglets that although an acute hypercapnic acidosis had no immediate effect on PVR, if maintained for 60 min then baseline PVR was increased and HPV was potentiated. This is perhaps consistent with the study of Balanos et al. [2] in humans, where the increase in PVR during hypercapnia developed slowly over several hours.

Other studies have also shown that hypercapnic acidosis suppresses the response to vasoconstrictors in isolated pulmonary arteries [7]. This suppression was attributed to the effects of acidosis, as euhydric hypercapnia (i.e., pH maintained at 7.4) did not cause any significant changes in agonist-induced tension [8,9]. However, in a subsequent paper, the same group reported that euhydric hypercapnia enhanced depolarisation-induced vasoconstriction in an endothelium- and nitric-oxide-dependent manner, and suggested that elevated CO₂ suppressed nitric oxide production [10]. Although this might be consistent with reports of hypercapnia-induced vasoconstriction, it remains unclear why, if hypercapnia does suppress nitric oxide production, a similar potentiation of agonist-induced vasoconstriction was not observed in the same preparation [8,9].

We have therefore reevaluated the effects of hypercapnic acidosis and euhydric hypercapnia in small intrapulmonary arteries (IPA) of the rat, using a protocol designed to detect any delayed effects similar to those described by Gordon et al. [6]. In addition, we examined the role of Na⁺-dependent HCO₃⁻ transport, as it has been suggested that this may play a role in HPV in small pulmonary arteries [11].

2. Methods

Male Wistar rats (200–300 g) were anaesthetized with sodium pentobarbital (55 mg·kg⁻¹·IP) and killed by cervical dislocation as approved by the local Home Office Inspector. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The lungs, intestines and kidneys were excised and placed in cold physiological salt solution (PSS, see below). Small (250–550 μm i.d.) IPA, mesenteric and renal arteries were dissected free of adventitia, mounted in a temperature-controlled myograph at 37 °C (Danish MyoTechnology, Aarhus, Denmark), and gassed continuously with 95% air/5% CO₂ (pH 7.4). After 30 min, arteries were stretched to give an equivalent transmural pressure of 30 mm Hg for pulmonary and 100 mm Hg for systemic arteries as previously described [12]. Preparation viability was assessed by measuring the response to 80 mM K⁺ PSS (KPSS; isotonic replacement of NaCl by KCl), and was deemed suitable for use in experiments only when they produced at least 5 mN tension. Prior to beginning experiments, arteries were equilibrated with three 2-min exposures to KPSS.

2.1. Measurement of intracellular pH in isolated arteries

Following mounting, arteries were incubated with PSS containing 1 μM BCECF/AM for 30 min at room temperature, followed by a further 15 min at 37 °C to facilitate intracellular hydrolytic cleavage of the dye. The preparation was washed with PSS and stimulated repeatedly with high KPSS until a stable tension response was achieved. The myograph was mounted on an inverted microscope (Zeiss Axiointert 200) equipped with an imaging system (Universal Imaging, Downingtown, PA, USA) and illuminated alternately at 490 and 440 nm while measuring the intensities of emitted light via a 550-nm filter using a CCD camera. The ratio was calibrated with nigericin as described by Aalkjaer and Cragoe [13].

2.2. Protocol

Studies on the potentiation of PGF₂α-induced vasoconstriction were performed as follows: After a stable baseline was obtained for >20 min, PGF₂α was added directly to the bath to give a final concentration of 3 μM (~EC₁₀; tension: 8.8±0.5% of the response to KPSS, n=58; change in bath volume <0.1%). After developed tension reached a plateau, the preparation was washed repeatedly with fresh PSS and allowed to return to baseline. Following incubation with any inhibitors for 20 min, the process was repeated. The bath solution was then exchanged for prewarmed and appropriately gassed experimental solution containing inhibitors as required, the gassing of the bath changed if necessary, and the preparation allowed to stabilise for 40 min before addition of the next PGF₂α challenge. In timed controls, there was no significant change in PGF₂α-induced tension at 40 min (n=6).

2.3. Solutions

Control physiological salt solution (PSS; in mM): NaCl 118; NaHCO₃ 24; KCl 4; CaCl₂ 1.8; MgSO₄ 1; NaH₂PO₄ 0.434; glucose 5.56. HEPES-buffered PSS (in mM): NaCl 130; KCl 4; CaCl₂ 1.8; MgCl₂ 1; glucose 5.56; HEPES 10, pH adjusted to 7.4 with NaOH. Low Na⁺ PSS: N-methyl-D-glucamine (NMDG) 118; choline bicarbonate 24; CaCl₂ 1.8; MgSO₄ 1; NaH₂PO₄ 0.434; glucose 5.56; pH adjusted to 7.4 with HCl. High [HCO₃⁻] PSS and 80 mM [K⁺] containing PSS (KPSS) were made by iso-osmotic substitution for NaCl. Unless otherwise stated, all experimental solutions were adjusted to pH 7.4, and were isotonic with normal PSS. All chemicals and drugs were obtained from Sigma-
Aldrich (Poole, Dorset, UK) or Calbiochem (Notts., UK). 4,4'-Disothiocyanato-stilbene-2,2'-di-sulphonic acid (DIDS) and acetazolamide were made up as stock solutions in DMSO; final concentration of DMSO was <0.1%, which had no effect on its own.

2.4. Calculations and statistics

Developed tension is presented as a percentage of the maximum tension obtained to the final 2-min exposure to KPSS during the equilibration procedure, and expressed as % KPSS. Results are shown as mean±S.E.M., and means compared using paired or unpaired Student’s t test as appropriate (SigmaStat, SPSS, Chicago, USA). A difference was deemed significant if P<0.05.

3. Results

3.1. Effect of hypercapnic acidosis on IPA

Changing the aerating gas from 5% CO₂ to 10% or 15% in air without adjusting [HCO₃⁻] (hypercapnic acidosis) had no effect on baseline tension, and did not significantly alter the response to 3 μM PGF₂α (Control: 10.3±1.0% KPSS, n=19; 10% CO₂, pH 7.12: 11.2±1.4% KPSS, n=9; 15% CO₂, pH 6.95: 10.3±1.7% KPSS, n=10).

3.2. Effect of increased PCO₂ and [HCO₃⁻] (euhydric hypercapnia) on IPA

IPA were challenged with 3 μM PGF₂α under control conditions, washed and exposed to euhydric hypercapnia by exchanging the PSS for one containing 48 mM [HCO₃⁻] gassed with 10% CO₂, pH 7.4. There was little or no immediate change in tension (0.9±0.4% KPSS) following transition to euhydri hypercapnia, but thereafter we observed two patterns of response. In the majority of preparations, tension remained stable for at least 40 min, but the tension induced by 3 μM PGF₂α at 40 min was greatly potentiated (Control: 10.3±0.7% KPSS; euhydric hypercapnia: 56.5±7.1% KPSS; n=17; p<0.001; Fig. 1A). This potentiation was reversed by 10 μM diltiazem (Fig. 1A), and was prevented by preincubation with diltiazem (Control, diltiazem: 9.7±1.7% KPSS; euhydric hypercapnia, diltiazem: 9.6±1.6% KPSS; n=5; NS; data summarised in Fig. 2). We examined whether euhydric hypcapnia (CO₂ 2%, [HCO₃⁻] 10 mM, pH 7.4) might conversely suppress PGF₂α-induced tension, but this was without effect (Control: 10.7±1.0% KPSS; Euhydric hypcapnia: 10.8±0.6% KPSS; n=4; NS).

In ~20% of all IPA exposed to euhydric hypcapnia (11 of 58), a relatively slow spontaneous vasoconstriction of variable size developed after a consistent delay of 18.8±1.8 min (Fig. 1B). Application of the L-type Ca²⁺ channel blocker diltiazem (10 μM) caused immediate vasorelaxation by 90.6±13.5% (Fig. 1B), suggesting that the vasoconstriction was due to a slowly developing depolarisation and activation of Ca²⁺ entry via L-type channels. To test whether the potentiation of PGF₂α-induced tension observed above was also related to a slowly developing mechanism, we also examined the effects of euhydric hypercapnia after 20 min. PGF₂α-induced tension was only potentiated by a factor of 1.9±0.3 (n=3) at this time. All further experiments were carried out only on IPA that showed a stable baseline tension for 40 min after transition to euhydric hypercapnia.

In order to determine whether a raised PCO₂ alone might affect the response to PGF₂α, we examined whether 10% CO₂ in the absence of HCO₃⁻ could induce potentiation. HEPES PSS was gassed with 10% CO₂ and pH corrected to 7.4 with NaOH. No potentiation of PGF₂α-induced tension was observed under these conditions (Fig. 3; Control: 9.9±1.4% KPSS; High CO₂, HEPES: 7.4±2.2% KPSS; n=4; NS), suggesting that the potentiation is related to the raised [HCO₃⁻] rather than raised PCO₂. The response to PGF₂α was therefore also examined in the absence of CO₂ in the aerating gas, using HEPES PSS with and without 24 or 48 mM [HCO₃⁻] (adjusted to pH 7.4). The addition of HCO₃⁻...
did potentiate PGF$_{2\alpha}$-induced tension, but only by a factor of 2 (HEPES: 5.1±0.6% KPSS; HEPES, 24 mM HCO$_3^-$: 10.2±1.2% KPSS; n=5; p<0.01). Increasing [HCO$_3^-$] to 48 mM had no further effect (n=4; data not shown).

Changes in intracellular pH (pHi) during hypercapnic acidosis (10% CO$_2$, 24 mM [HCO$_3^-$], pH 7.12), and euhydric hypercapnia were examined using BCECF. Fig. 4 shows a typical trace representative of three others, demonstrating that although hypercapnic acidosis caused the expected intracellular acidicification of about 0.23 pH units, euhydric hypercapnia also caused acidicification (~0.15 pH units), although extracellular pH was normal. There was some recovery in pHi towards normal over 40 min, but nevertheless, pH was still acid at the end of this period. Following return to normal PSS, there was a small alkaline overshoot of similar size to the extent of recovery during the preceding 40 min, presumably reflecting increased intracellular [HCO$_3^-$].

It has been suggested that depolarisation-induced vasoconstriction of rat IPA is potentiated under euhydric hypercapnia via a reduction in nitric oxide production from the endothelium [10], whereas agonist-induced vasoconstriction is not [9]. We examined whether the potentiation of PGF$_{2\alpha}$-induced vasoconstriction observed here was also due to inhibition of nitric oxide production. IPA were incubated in 100 μM L-NAME for 20 min. L-NAME itself potentiated the response to 3 μM PGF$_{2\alpha}$ (22.5±4.2% KPSS). Nevertheless, in the presence of L-NAME, euhydric hypercapnia still caused a significant further potentiation of the response to PGF$_{2\alpha}$ (69.8±9.3% KPSS; n=9; p<0.001; Fig. 2). We also examined the effects of removal of the endothelium by rubbing the IPA lumen with a human hair; adequate removal was ascertained by lack of relaxation to 1 μM acetylcholine following constriction with PGF$_{2\alpha}$. As with L-NAME, removal of the endothelium potentiated the response to PGF$_{2\alpha}$ in normal PSS (31.6±4.6% KPSS, n=6). Following transition to euhydric hypercapnia, endothelium-denuded IPA exhibited a stable baseline for 40 min. The subsequent response to PGF$_{2\alpha}$ was further potentiated (57.8±4.1% KPSS, n=6, p<0.005), and did not differ significantly from that of intact IPA in the presence euhydric hypercapnia and L-NAME.

The data suggest that both the potentiation of PGF$_{2\alpha}$-induced vasoconstriction and the slow spontaneous vasoconstriction are due to depolarisation. It would therefore be predicted that mild depolarisation with 20 mM [K$^+$] should mimic the effect of euhydric hypercapnia and enhance the response to PGF$_{2\alpha}$. Depolarisation with 20 mM [K$^+$] caused an increase in tension of 10.4±1.9% KPSS (n=6). Under these conditions, the response to 3 μM PGF$_{2\alpha}$ was significantly potentiated (65.1±5.8% KPSS, n=6, p<0.001). Conversely, euhydric hypercapnia substantially enhanced vasoconstriction to 20 mM [K$^+$] (Fig. 5, n=3). Both of these findings are consistent with the hypothesis that euhydric hypercapnia causes smooth muscle depolarisation in IPA.

### 3.3. Mechanism of high [HCO$_3^-$] induced depolarisation in IPA

We examined the effect of DIDS, a nonspecific inhibitor of anion transport and Cl$^-$ channels. DIDS (100 μM) itself caused a small but significant reduction in 3 μM PGF$_{2\alpha}$-induced tension (Control: 8.0±0.6% KPSS; DIDS: 5.3±0.7% KPSS; n=7; p=0.04); however, it completely abolished potentiation in euhydric hypercapnia (4.9±1.2% KPSS; n=7; Fig. 2). We investigated involvement of Na$^+$-dependent HCO$_3^-$ transport by removal of extracellular Na$^+$, using as substitutes the imperanent cation NMDG and choline HCO$_3^-$ for NaCl and NaHCO$_3$, respectively (remaining [Na$^+$]<0.5 mM). Na$^+$ substitution itself caused a small
increase in the response to PGF$_{2\alpha}$, but again completely abolished any further potentiation in euhydrmic hypercapnia (Low Na$^+$, control: 19.8±7.0% KPSS; Low Na$^+$, euhydrmic hypercapnia: 17.6±4.1% KPSS; $n=4$; $p<0.01$; Fig. 2).

The carbonic anhydrase inhibitor acetazolamide is reported to have an IC$_{50}$ of ~1 $\mu$M, although doses between 100 $\mu$M and 1 mM are commonly used[14]. At 10 $\mu$M, acetazolamide had no effect on IPA reactivity ($n=4$; data not shown). At 100 $\mu$M, it caused a significant reduction in PGF$_{2\alpha}$-induced tension under control conditions (4.8±0.8% KPSS, $n=4$), and also reduced the potentiation in euhydrmic hypercapnia such that this was no longer significant from control (15.0±4.9% KPSS; NS; Fig. 2).

Increasing the outward electrochemical gradient for Cl$^-$/C0$_{Cl}$ by substitution of extracellular Cl$^-$ with either methyl sulphonate or iodide elicited a slowly developing but powerful vasoconstriction in IPA ($n=9$; data not shown), similar to the spontaneous vasoconstriction elicited by euhydrmic hypercapnia. This implies that a significant Cl$^-$ conductance exists in IPA under basal conditions. The potential role of Cl$^-$ channels was examined by use of anthracene-9-carboxylic acid (A9C), which has been described as a blocker of Ca$^{2+}$-activated Cl$^-$ channels [15]. A9C (200 $\mu$M) itself caused a small suppression of PGF$_{2\alpha}$-induced tension, but completely abolished its potentiation by euhydrmic hypercapnia (A9C, control: 6.0±1.5% KPSS; A9C, euhydrmic hypercapnia: 6.4±2.7% KPSS; $n=4$; NS; Fig. 2).

3.4. Effect of euhydrmic hypercapnia on systemic arteries

To establish whether the potentiation of PGF$_{2\alpha}$-induced tension by euhydrmic hypercapnia was specific to IPA, we performed experiments in small mesenteric and renal arteries. As shown in Fig. 6, no potentiation of PGF$_{2\alpha}$-induced tension was observed in mesenteric arteries (Control: 5.1±1.1% KPSS; Euhydrmic hypercapnia: 4.4±1.1% KPSS; $n=10$; NS); similar results were obtained for renal arterioles.
arteries (Control: 9.7±1.6% KPSS; Euhydric hypercapnia: 7.3±1.3% KPSS; n=5; NS). The lack of potentiation was not due to suppression of contraction by high CO₂-induced intracellular acidification, because depolarisation of mesenteric arteries with 20 mM [K⁺] caused significant and similar degrees of potentiation of the response to PGF₂α in both control conditions and euhydric hypercapnia (Fig. 7). This also indicates that it is the mechanism causing the depolarisation in response to euhydric hypercapnia that is specific to IPA, and not the effects of the depolarisation itself.

4. Discussion

The key novel observation from this study is that euhydric hypercapnia (10% CO₂, 48 mM [HCO₃⁻], pH 7.4) substantially increased the vasoreactivity of rat small IPA (Fig. 2), but did not affect vasoreactivity of small mesenteric or renal arteries (Fig. 7). The data strongly suggest that the mechanism involves smooth muscle depolarisation. In contrast, neither euhydric hypocapnia nor hypercapnic acidosis had any significant effect on the response of IPA to low concentrations of PGF₂α. The latter is consistent with previous reports from McLoughlin et al. [7–9] showing that agonist-induced vasoconstriction of pulmonary arteries is resistant to acidosis, which they have suggested is due to the greater importance of Rho-kinase-mediated Ca²⁺-sensitisation mechanisms in this tissue [16]. PGF₂α has been reported to induce vasoconstriction in pulmonary artery primarily via Ca²⁺-sensitisation rather than voltage-gated Ca²⁺ entry [8,17], and this is consistent with our data which show that the L-type channel blocker diltiazem had no effect on constriction induced by 3 µM PGF₂α in normal PSS.

IPA exhibited two patterns of response after exposure to euhydric hypercapnia, with the majority showing a stable basal tension for 40 min, followed by potentiation of both PGF₂α- and 20 mM [K⁺]-induced vasoconstriction. In ~20% of IPA, however, a slow spontaneous constriction developed after a consistent delay of ~20 min. Both potentiation and spontaneous constriction were reversed by diltiazem, suggesting that both responses were due to the same mechanism, namely, activation of voltage-dependent L-type Ca²⁺ channels by depolarisation. Moreover, depolarisation with 20 mM [K⁺] mimicked the effect of euhydric hypercapnia.

The reason why some IPA exhibit a spontaneous constriction during euhydric hypercapnia whilst the majority did not is not clear, as there were no obvious differences between groups. The inability to predict which IPA would spontaneously constrict, coupled with the variable nature of the response, made further experimentation difficult. However, the relationship between depolarisation and constriction is very steep in IPA and effectively has a threshold, so small differences in the extent of depolarisation have profound effects on constriction, or whether or not it occurs at all (e.g., Ref. [18]). Euhydric hypercapnia may depolarise IPA close to this threshold, but in some cases exceed it and so cause constriction. This does not easily explain the apparently consistent nature of the delay before the spontaneous constriction, which needs further study.

Our findings contrast with those reported by Sweeney et al. in rat conduit pulmonary artery [9] and IPA [8], where euhydric hypercapnia did not alter phenylephrine- or PGF₂α-induced vasoconstriction, respectively. There are, however, differences in protocol that might explain these conflicting results. In both studies, Sweeney et al. examined the effects of euhydric hypercapnia after agonist-induced vasoconstriction was established, as opposed to the current study where preparations were preincubated with euhydric hypercapnia for 40 min before challenging with PGF₂α. Moreover, the dose of agonist and size of vasoconstriction were greater, at the EC₅₀ or EC₇₀ [8,9] compared to ~EC₁₀ in the present study. We have previously shown that vasoconstriction of IPA to higher concentrations of PGF₂α (50 µM) has a significant component (~50%) that is verapamil-sensitive, indicating that PGF₂α does induce depolarisation at higher concentrations [19]. This could potentially mask any depolarisation induced by euhydric hypercapnia. Finally, Sweeney et al. [8,9] measured the response 15–20 min after initiation of euhydric hypercapnia, rather than the 40 min used here. If euhydric hypercapnia causes a slowly developing response (see below), 20 min might not have been sufficient to show any significant effect under the above conditions.

The delay in onset and slow nature of the spontaneous constriction plus the reduced potentiation of PGF₂α-induced tension after 20 min implies that the underlying mechanism is slowly developing. This is reminiscent of the phenomenon reported by Gordon et al. [6], whereby an acute (5–10 min) hypercapnic acidosis had no effect on PVR in intact
piglets, but when sustained for up to an hour caused a significant rise in basal PVR and potentiated HPV. There could also be parallels with the slow increase in PVR that has been reported during hypercapnia in humans [2]. However, we did not show any alterations in either basal tension or the response to PGF$_{2\alpha}$ of IPA for up to 40 min of hypercapnic acidosis. On the other hand, both of these studies were performed in intact organisms, and although renal compensation is unlikely to be effective within hours, some degree of extra-renal compensation may occur much faster, with a rise in plasma [HCO$_3^-$] due to titration of body bicarbonate buffers [20]. It is not possible to say whether plasma [HCO$_3^-$] did increase in these studies [2,6], as these data were not provided.

It could be argued that both the potentiating effect of euhydric hypercapnia that we report here and the hypercapnia-associated increases in PVR reported in the previous two studies are due to vasoconstriction in response to hypercapnia per se, and that the reason we do not see an effect of hypercapnic acidosis is that, in our preparation, this vasoconstriction is suppressed by extracellular or intracellular acidosis. This would seem unlikely on two grounds. The first is that PGF$_{2\alpha}$-induced vasoconstriction in rat IPA has been shown elsewhere to be unaltered by either hypercapnic acidosis, as also reported here, or an equivalent normocapnic acidosis [8]. The second is that the difference in degree of intracellular acidosis that we observed in IPA during hypercapnic acidosis and euydryc hypercapnia was relatively small (~0.08 pH units), and the amount of recovery of pHi after 40 min was also small (Fig. 4). It would seem implausible that these small differences in pHi could have such profound effects on PGF$_{2\alpha}$-induced tension, especially in the light of the study of Hyvelin et al. [16], where it was shown that agonist-induced Rhokinase-mediated Ca$^{2+}$ sensitisation in pulmonary artery was essentially unaffected by changes in cytosolic pH.

Nevertheless, we attempted to determine whether the observed effects of euydric hypercapnia were related to the hypercapnia itself or the increase in [HCO$_3^-$], by using HEPES buffered PSS with the addition of either CO$_2$ or HCO$_3^-$ alone. HEPES PSS gassed with 10% CO$_2$ (pH 7.4) did not affect PGF$_{2\alpha}$-induced tension, again suggesting that the potentiation of PGF$_{2\alpha}$-induced tension is not related to hypercapnia per se. Conversely, HCO$_3^-$ containing HEPES gassed with air did show potentiation, albeit only by a factor of 2. Even so, these data might be construed as suggesting that it is primarily the increase in extracellular [HCO$_3^-$] that is responsible for observed effects of euydric hypercapnia. However, these results using HEPES should perhaps be interpreted with some caution, as it is not clear how removal of extracellular CO$_2$ or HCO$_3^-$ would affect the relative intracellular concentrations of this buffer pair. HEPES is also known to suppress contraction in vascular smooth muscle [21].

Although Sweeney et al. [8,9] have reported that euydric hypercapnia does not enhance agonist-induced vasoconstriction, the same group have also reported that it does enhance depolarisation-induced vasoconstriction, which they suggested was mediated via inhibition of endothelium-derived nitric oxide production by hypercapnia [10]. However, we found that although both inhibition of nitric oxide synthase with L-NAME and removal of the endothelium potentiated the response to PGF$_{2\alpha}$, this was considerably less than that induced by euvyrdic hypercapnia, and neither L-NAME nor removal of the endothelium prevented further potentiation during subsequent euvyrdic hypercapnia (see above and Fig. 2). This strongly suggests that neither nitric oxide nor the endothelium plays a role in the potentiation of PGF$_{2\alpha}$-induced tension observed here.

As the response to euydric hypercapnia is slowly developing, the depolarisation is unlikely to be related to any direct effect on ion channels. A more plausible explanation is that it is directly or indirectly related to a change in the composition of intracellular fluid as a result of a transport process, and the implication that the raised [HCO$_3^-$] is critical suggests the Na$^+$-dependent HCO$_3^-$-Cl$^-$ transporter, which has been shown to be important both for recovery from intracellular acidosis and HPV in cat small IPA [11]. Consistent with this, both DIDS, a nonspecific inhibitor of anion transport, and removal of extracellular Na$^+$ abolished the potentiation of PGF$_{2\alpha}$-induced tension during euhydric hypercapnia. Our HEPES experiments could be interpreted as showing that both HCO$_3^-$ and CO$_2$ are required. It has been suggested that carbonic anhydrases associated with certain HCO$_3^-$ transporters may regulate their action, and perhaps act as sensors for intracellular CO$_2$/HCO$_3^-$ balance [22]. We found that inhibition of carbonic anhydrase with acetazolamide suppressed the potentiation of PGF$_{2\alpha}$-induced tension (Fig. 2), perhaps consistent with a regulatory function on the transporter. Interestingly, like the Na$^+$-dependent HCO$_3^-$-Cl$^-$ transporter, carbonic anhydrase has also been implicated in HPV [23].

It has been suggested that Cl$^-$ channels may play a significant role in the regulation of pulmonary artery tone [24,25]. DIDS also blocks Cl$^-$ channels, and it is possible that the depolarisation associated with euydric hypercapnia could be ultimately due to activation of an outward Cl$^-$ current. Consistent with this hypothesis, blockade of Ca$^{2+}$-activated Cl$^-$ channels with A9C completely abolished the potentiation of PGF$_{2\alpha}$-induced tension (Fig. 2). The mechanism by which increased HCO$_3^-$ transport is linked to activation of these Cl$^-$ channels remains to be ascertained. We believe that it is unlikely to be due a rise in intracellular Ca$^{2+}$ secondary to an initial depolarisation caused by increased transporter activity, as one would predict that in that case there would still be some potentiation of PGF$_{2\alpha}$-induced tension in the presence of A9C. Moreover, modulation of Na$^+$-dependent HCO$_3^-$-Cl$^-$ transport in vascular smooth muscle has been shown to have little effect on membrane potential [26].
In summary, our results suggest that euhydric hypercapnia enhances the vasoreactivity of rat small IMA, but not mesenteric or renal arteries, by mechanisms involving enhanced HCO_3^- transport, activation of Ca^{2+}-activated Cl^- channels and subsequent depolarisation. The most logical initial stimulus is the effect of an increased transmembrane HCO_3^- gradient on the normal intracellular alkalinisation mechanisms activated by the fall in pHi. The fact that this phenomenon appears to be specific to pulmonary arteries is of particular interest, especially considering that it has been suggested that both the Na^+-dependent HCO_3^-Cl^- transporter and Ca^{2+}-activated Cl^- channels play an important role in the regulation of pulmonary vascular tone and HPV [11,24,25]. It is also of note that both A9C-inhibitable Cl^- channels and HCO_3^-Cl^- transport have been implicated in the response of the carotid body chemosensor to hypercapnia and hypoxia [27]. From a functional and clinical standpoint, these findings may also at least partly explain why chronic hypercapnia with a compensated acidosis is associated with promotion of pulmonary hypertension and increased mortality in COPD patients [1].

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References


