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Effect of a decrease in pH on responses mediated by P2 receptors in the rat mesenteric arterial bed

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Abstract

The present study investigated the effect of acidosis (reduction in pH of the Krebs' solution from 7.4 to 6.9) on responses to vasoconstrictors and vasodilators, with a focus on purines, in the rat isolated perfused mesenteric arterial bed. α,β-Methylene ATP (α,β-meATP) (10 μM), a selective P2X receptor agonist, elicited a desensitizing vasocontraction, which was not significantly affected by a reduction in pH to 6.9. Contractions to ATP were also not significantly different at pH 6.9 compared to pH 7.4. In contrast, contractile responses to noradrenaline, methoxamine, and vasopressin were greatly attenuated at pH 6.9 (by 48–83%; $P < 0.01$). At raised tone, vasorelaxations to ADP at P2Y receptors, and to calcitonin gene-related peptide (CGRP), were not different at pH 7.4 and pH 6.9. These data indicate that a reduction in pH (to 6.9) differentially affects responses to vasoconstrictors in the rat mesenteric arterial bed. There is no effect on contractions mediated via P2X receptors, but contractions to noradrenaline, methoxamine and vasopressin are greatly attenuated.

Keywords: Acidosis; ATP; P2X receptor; pH; Purine; Mesenteric arterial bed; Vasoconstriction

1. Introduction

Cell surface receptors for purines and pyrimidines, P2 receptors, are widely distributed in the cardiovascular system, where they can mediate potent effects on the heart and on blood vessel contractility (Olsson and Pearson, 1990; Burnstock, 1989; Ralevic, 1998). P2 receptors comprise ionotropic P2X receptors and G protein-coupled P2Y receptors, and are activated by the endogenous ligands ATP, ADP, UTP and UDP (Ralevic and Burnstock, 1998). Activation of P2X receptors (principally $P2X_1$ receptors) and P2Y receptors ($P2Y_2$, $P2Y_4$ and $P2Y_6$ receptors) expressed on vascular smooth muscle elicits vasoconstriction, whilst activation of P2Y receptors ($P2Y_1$ and $P2Y_2$ receptors) present on the vascular endothelium elicits vasorelaxation (see Ralevic and Burnstock, 1998). ATP and UTP in particular can each potently activate both vasocontractile and vasorelaxant P2 receptors to elicit opposing actions on blood vessel tone. Whether they evoke preferentially vasocontraction or vasorelaxation depends on a number of factors including the relative potencies of each at contractile P2X and P2Y versus vasorelaxant P2Y receptors. However, there is evidence that P2 receptor sensitivity can change with deviations from physiological pH.

Studies of endogenous P2X receptors and recombinant P2X receptors expressed in *Xenopus* oocytes have shown that the sensitivity of P2X receptors can be potently influenced by changes in extracellular pH (Li et al., 1996; King et al., 1996, 1997; Stoop et al., 1997). Both increases and decreases in sensitivity have been reported. For example, the sensitivity of ATP at the recombinant $P2X_2$ receptor is enhanced 5–10 fold by acidification of the bathing solution to pH 6.5 (King et al., 1996), whilst acidification decreases the effect of ATP at recombinant $P2X_1$, $P2X_3$ and $P2X_4$ receptors (Stoop et al., 1997). Low pH has also been shown to favour desensitization of ATP currents at $P2X_2$ receptors expressed in HEK293 cells (Stoop and Quayle, 1998). At endogenous P2X receptors in the guinea pig vas deferens, acidification of the bathing solution (to 6.9) augmented the contractile response to ATP, but depressed that induced by noradrenaline (Nakanishi et al., 1997). Little is known about the effect of change in extracellular pH on responses mediated by native P2 receptors in cardiovascular tissues.

Hypoxia and ischaemia, conditions associated with a decrease in pH, can evoke the release of purines and

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pyrimidines from a variety of different cells in blood vessels and in the circulation, indicating a role for purines in maintaining vascular tone during such challenges of homeostasis. Indeed, there is a considerable body of evidence for cardioprotective actions of adenosine released during hypoxia and ischaemia (see Olsson and Pearson, 1990; Green and Grover, 1998; Matherne et al., 1998). Moreover, lowering the pH to below 7.4 enhances the cardiovascular actions of adenosine (Merrill et al., 1978; Hiley et al., 1995), providing a direct link between cardioprotection, acidosis and purinergic signalling. ATP is also released from cells during hypoxia and ischaemia (Burnstock, 1989) and is an important source of adenosine following metabolic breakdown, but additionally has potent direct effects on vascular tone via P2 receptors.

The aim of the present study, therefore, was to investigate pharmacologically the effect of a decrease in ambient pH on responses mediated by P2 receptors in blood vessels. The study was carried out at P2 receptors in the rat isolated mesenteric bed (Ralevic and Burnstock, 1988, 1996). In order to determine whether possible effects of pH were specific to ATP and P2 receptors, the effect of acidosis was additionally investigated on contractile responses to noradrenaline and vasopressin, receptor-independent contractions to KCl and relaxations to calcitonin gene-related peptide (CGRP). The effect of low pH on sympathetic neurogenic contraction was additionally investigated. The pH of the Krebs' solution was lowered by a reduction in the concentration of $NaHCO_3$ and by addition of HCl.

2. Materials and methods

2.1. Isolated mesenteric arterial bed preparation

Adult male rats were killed by decapitation after asphyxiation with CO_2. Mesenteric beds were isolated and set up for perfusion as described previously (Ralevic et al., 1995). In brief, the abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was cut, blood flushed from the preparation with approximately 0.5 ml of Krebs' solution, the intestine dissected away from the mesenteric vasculature and the preparation mounted on a stainless steel grid (7×5 cm) in a humid chamber. The preparation was perfused at a constant flow rate of 5 ml min^{-1} using a peristaltic pump (model 7554-30, Cole–Parmer Instrument Co., Chicago, IL). The perfusate was Krebs' solution (pH 7.4) of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2–5% CO_2 and maintained at 37°C. Responses of the mesenteric arterial beds were measured as changes in perfusion pressure (mm Hg) with a pressure transducer (model P23XL, Viggo-Spectramed, Oxnard, CA) on a side arm of the perfusion cannula, and recorded on a polygraph (model 7D, Grass Instrument Co., Quincy, MA). Preparations were allowed to equilibrate for approximately 30 min prior to experimentation. Agonists were applied as doses by bolus injection (50 μl) into an injection port proximal to the preparation, or by perfusion (α,β-methylene ATP, α,β-meATP). The pH of the Krebs' solution was reduced to pH 6.9 by partial omission of $NaHCO_3$ (the concentration was reduced to 5 mM), with osmolarity maintained by equimolar addition of NaCl. As $NaHCO_3$ and Ca^{2+} form ion pairs in solution, $[Ca^{2+}]$ may vary when the $[NaHCO_3]$ is altered (Fry et al., 1994). Thus, we additionally investigated the effect of reducing pH to 6.9 by addition of HCl.

2.2. Experimental protocol

The effect of reducing pH by both partial omission of $[NaHCO_3]$ and by addition of HCl on contractile responses of the rat mesenteric arterial bed was investigated using the following protocol. Contractile responses evoked by electrical field stimulation, applied at 64 Hz, 90 V, 1 ms, for 5 s every 2 min, which produces robust and reproducible responses, were initially investigated in Krebs' solution at pH 7.4 and then in Krebs' solution at pH 6.9, after 10 min equilibration. After return to pH 7.4, a dose–response curve was generated for noradrenaline (0.5–15 nmol) and then at pH 6.9, after which the solution was again returned to pH 7.4. The above procedure was repeated with methoxamine (5–50 nmol), ATP (5 and 50 nmol), α,β-meATP (0.5 nmol), and KCl (15–75 μmol), with 10 min between response curves. Maximal responses to the agonists are not achieved in the mesenteric arterial bed at doses greater than those used in the present study and at limits reached by cost and solubility (Ralevic and Burnstock, 1988; Ralevic et al., 1995). The dose-interval was 5 min, except for doses of ATP and α,β-meATP, which were applied at 15-min intervals in order to avoid desensitization. The limited dose-range of ATP and α,β-meATP used was also in order to avoid desensitization, and the doses for both purines were submaximal (Ralevic and Burnstock, 1988). In preliminary studies, full recovery of responses to the vasoconstrictors was shown with a return to pH 7.4 from pH 6.9. In another group of preparations, two consecutive response curves were generated to vasopressin (5–500 pmol) either in Krebs' solution at pH 7.4 (time controls), or where the second response curve was generated at pH 6.9 brought about by a reduction in $[NaHCO_3]$ or by addition of HCl. In another group, a submaximal concentration of α,β-meATP (10 μM) was perfused for 3 min on three occasions at 1-h intervals, either in control conditions (pH 7.4), or where the third perfusion was carried out at pH 6.9 caused by reducing $[NaHCO_3]$. The effect of pH 6.9 brought about by reducing $[NaHCO_3]$ only was investigated on vasorelaxant responses to ADP and CGRP in a separate group of preparations pre-constricted with methoxamine (10–50 μM).

2.3. Data analysis

Contractile responses were measured as increases in perfusion pressure (mm Hg) above baseline. Vasorelaxant responses were measured as decreases in perfusion pressure and expressed as the percentage reduction of methoxamine raised tone. Responses to electrical field stimulation at 64 Hz in Krebs solution at pH 7.4 and pH 6.9 were calculated as the mean of three consecutive contractile responses. In the experiments with perfusion of α,β-meATP, the maximal response and the time taken for the response to decrease to half of the maximal value was measured. Analysis of variance was used to determine whether there was a significant difference between the dose–response curves and, if appropriate, post hoc Student's *t*-test was used to see where the differences lay. The percentage change in amplitude of the response at pH 6.9 compared to pH 7.4 of a dose of agonist eliciting contraction of approximately 40 mm Hg under control conditions (pH 7.4) was additionally calculated. This value was chosen as it is in the same range as the amplitude of the contractile responses evoked by ATP and α,β-meATP at the greatest doses tested. All data are expressed as the means ± S.E. Data were considered significant when $P < 0.05$.

2.4. Drugs used

α,β-meATP (lithium salt), ADP, ATP, CGRP, methoxamine (hydrochloride), norepinephrine (arterenol bitartrate), prazosin (hydrochloride) and vasopressin were from Sigma, Guanethidine (Ismelin) was from Ciba Laboratories, Surrey. All drugs were dissolved in distilled water.

3. Results

3.1. Effect of reducing pH on contractile responses to ATP and α,β-meATP

A reduction in pH from 7.4 to 6.9, produced by a reduction in the concentration of $NaHCO_3$, had no significant effect on vasocontraction to doses of ATP (5 and 50 nmol; $n = 9$) (Fig. 1a). The contractile response to 0.5 nmol α,β-meATP was also not significantly different at pH 6.9 compared to the control response at pH 7.4 ($n = 9$) (Fig. 1b). Similarly, when pH was reduced to 6.9 with HCl, there was no significant difference between responses to doses of ATP (Fig. 1c) and α,β-meATP (Fig. 1d) compared to those at pH 7.4 ($n = 9$).

Perfusion of α,β-meATP (10 μM) for 3 min evoked a contractile response that desensitized rapidly during the period of perfusion; at pH 7.4, the time taken until this had reversed to half of the maximal response was 1.14 ± 0.14 min ($n = 10$). There was no significant difference between the contractile response evoked by α,β-meATP on each of three consecutive occasions under control conditions (70.3

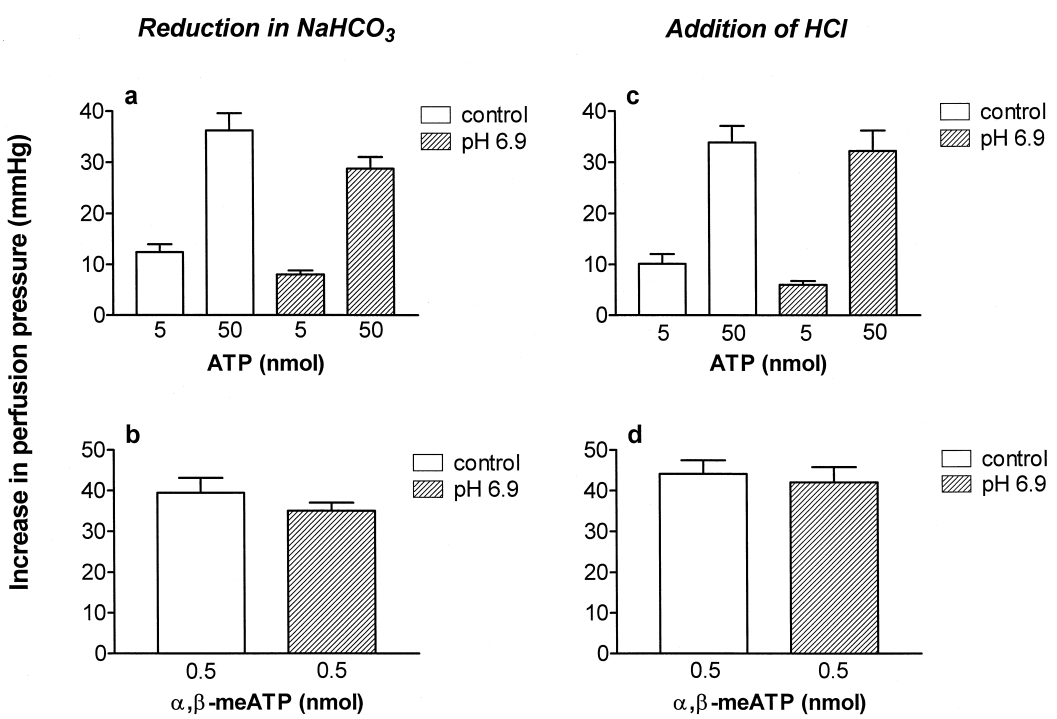

Fig. 1. Effect on contractile responses to doses of ATP (a, c) and α,β-meATP (c, d) of a decrease in pH to 6.9, produced by either a reduction in the perfusate concentration of $NaHCO_3$ ($n = 9$) or addition of HCl ($n = 9$), in the rat isolated mesenteric arterial bed. There was no significant difference between responses to either ATP or α,β-meATP generated at a reduced pH of 6.9 compared to control responses at pH 7.4.

± 9.1, 66.3 ± 13 and 77.2 ± 10.1 mm Hg; $n = 4$) or when the third response in the series was carried out at pH 6.9 (77.2 ± 10.1, 73.2 ± 11.1 and 64.7 ± 8.9 mm Hg; $n = 6$) (Fig. 2). There was also no significant difference in the time taken for the response to α,β-meATP to reverse to half of the maximal value under control conditions (1.1 ± 0.1, 1.3 ± 0.3 and 1.4 ± 0.3 min; $n = 4$) or when the third response in the series was carried out at pH 6.9 (1.1 ± 0.1, 1.0 ± 0.1 and 1.2 ± 0.2 min; $n = 6$) (Fig. 2).

3.2. Effect of reducing pH on contractile responses to noradrenaline and methoxamine

Contractile responses to noradrenaline (0.5–150 nmol) at pH 6.9, produced by a reduction in the concentration of $NaHCO_3$, were significantly attenuated compared to those generated at pH 7.4 (Fig. 3a). The contraction to a dose of noradrenaline producing a response in the same range as that evoked by the purines (1.5 nmol noradrenaline; 37 ± 5.9 mm Hg at pH 7.4) was reduced by 67 ± 6% ($P < 0.01$, $n = 9$). Reducing pH to 6.9 by the addition of HCl also significantly attenuated contractions to noradrenaline (Fig. 3c). The contractile response to a dose of noradrenaline producing a response in the same range as that evoked by the purines (1.5 nmol noradrenaline; 37 ± 4.8 mm Hg at pH 7.4) was reduced by 48 ± 3% ($P < 0.001$; $n = 9$).

Contractile responses to methoxamine (5–500 nmol) at pH 6.9 produced by a reduction in the concentration of $NaHCO_3$ were attenuated compared to those generated at pH 7.4 (Fig. 3b). The contractile response to doses of methoxamine producing contraction in a similar range as that evoked by the purines (5–15 nmol methoxamine; 35 ± 3.7 mm Hg at pH 7.4) was reduced by 83 ± 2% ($P < 0.001$; $n = 9$). Reducing pH to 6.9 by the addition of HCl also significantly attenuated contractions to methoxamine (Fig. 3d). The contractile response to a dose of methoxamine producing a response in a similar range as that evoked by the purines (5 nmol methoxamine; 41 ± 5.5 mm Hg at pH 7.4) was reduced by 53 ± 6% ($P < 0.001$; $n = 9$).

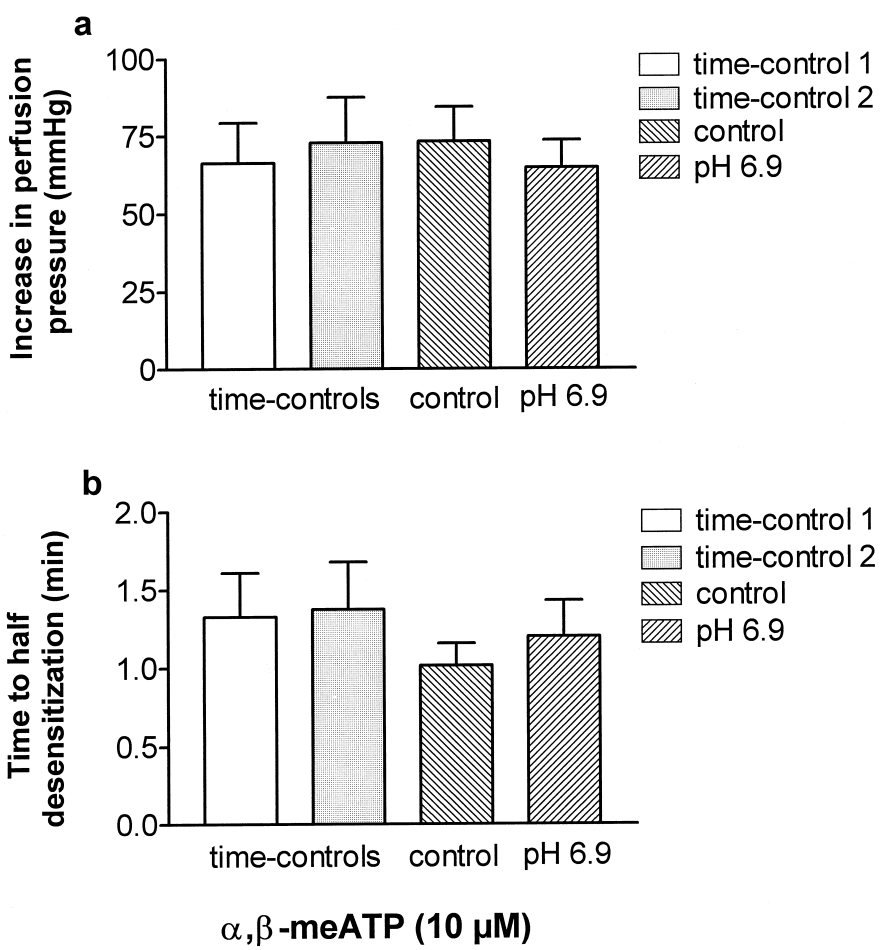

Fig. 2. Effect on the contractile response to perfusion of α,β-meATP (10 μM) of a decrease in pH to 6.9 produced by a reduction in the perfusate concentration of $NaHCO_3$ in the rat isolated mesenteric arterial bed. (a) There was no significant difference between contractile responses to α,β-meATP (10 μM) generated in preparations under control conditions (time controls; pH 7.4, $n = 4$) or in preparations where there was a switch from pH 7.4 (control) to pH 6.9 ($n = 6$). (b) There was also no significant effect of reducing pH on the time taken for the response to α,β-meATP to return to half of its maximal value.

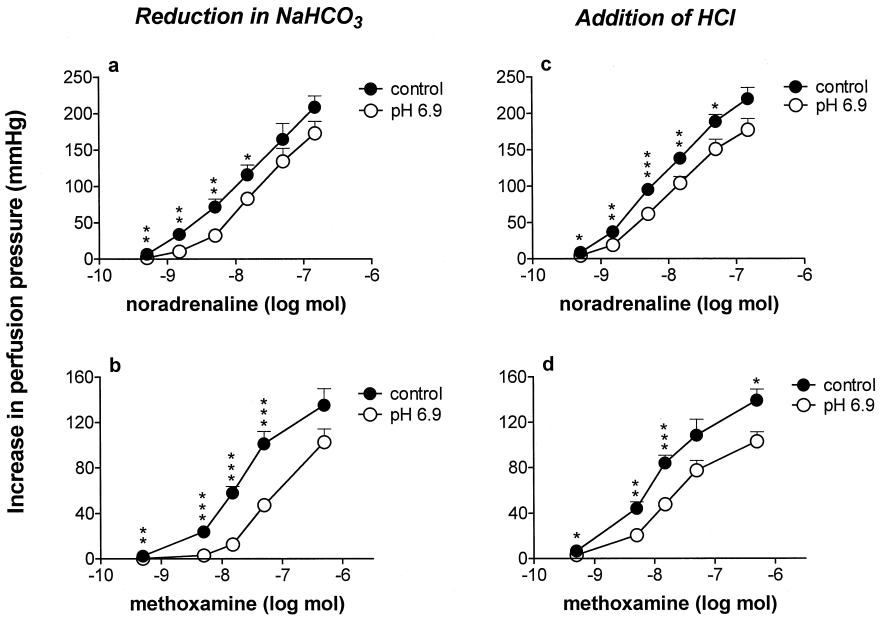

Fig. 3. Effect on contractile responses to noradrenaline (a, c) and methoxamine (b, d) of a decrease in pH to 6.9 produced by either a reduction in the perfusate concentration of $NaHCO_3$ ($n = 9$) or by addition of HCl ($n = 9$), in the rat isolated mesenteric arterial bed. There was a significant reduction in the negative logarithm of the dose of noradrenaline and methoxamine required to elicit a response of 50 mm Hg under both conditions of change in pH to 6.9. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

3.3. Effect of reducing pH on contractile responses to vasopressin and KCl

Contractile responses to vasopressin were attenuated at pH 6.9, produced by a reduction in [$NaHCO_3$], compared to those at pH 7.4 (Fig. 4a). The contractile response to doses of vasopressin producing a response in a similar range as evoked by the purines (0.015–0.15 nmol vasopressin; 51 ± 7.7 mm Hg at pH 7.4) was reduced by $68 \pm 11\%$ ($P < 0.01$; $n = 7$). Reducing pH to 6.9 by the

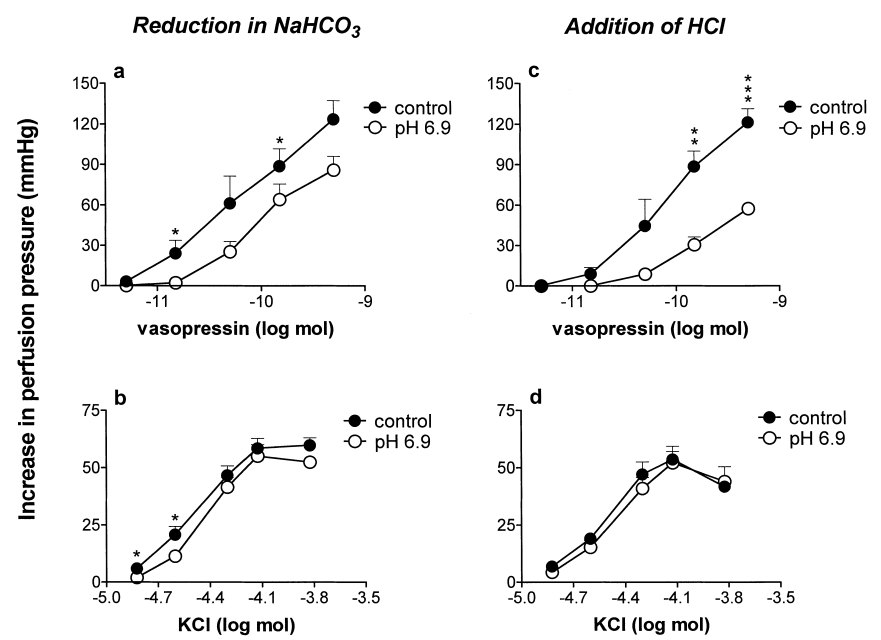

Fig. 4. Effect on contractile responses to vasopressin (a, c) and potassium chloride (KCl, b, d) of a decrease in pH to 6.9 produced by either a reduction in the perfusate concentration of $NaHCO_3$ ($n = 7$–9) or by addition of HCl ($n = 5$–9), in the rat isolated mesenteric arterial bed. There was a significant reduction in the negative logarithm of the dose of vasopressin required to elicit a response of 50 mm Hg under both conditions of change in pH to 6.9. There was a significant reduction in the negative logarithm of the dose of KCl required to elicit a response of 50 mm Hg at pH 6.9 generated by a reduction in $NaHCO_3$, but not by addition of HCl. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

addition of HCl also significantly attenuated contractions to vasopressin (Fig. 4c). The contractile response to doses of vasopressin producing a response in the same range as that evoked by the purines (0.015–0.05 nmol vasopressin; 29 ± 2.3 mm Hg at pH 7.4) was reduced by $83 \pm 8\%$ ($P < 0.01$; $n = 5$). There was no significant difference between two consecutive response curves to vasopressin generated under control conditions (pH 7.4). There was also no significant difference under control conditions in the response to doses of vasopressin producing contraction in the same range as that evoked by the purines (0.015–0.15 nmol vasopressin; 50 ± 12.6 mm Hg at pH 7.4) ($n = 6$).

There was a small but significant difference in the contractile dose–response curve to KCl at pH 6.9 produced by reducing the concentration of $NaHCO_3$ compared to that at pH 7.4 (Fig. 4b). When pH was reduced to 6.9 by the addition of HCl, the responses were not significantly different at pH 7.4 and pH 6.9 (Fig. 4d).

3.4. Effect of reducing pH on contractile response to electrical field stimulation

Electrical field stimulation (64 Hz, 90 V, 1 ms, for 5 s every 2 min) elicited transient vasoconstrictor responses that were blocked by guanethidine (5 μM) or prazosin (1 μM), indicating that they are mediated by noradrenaline released from sympathetic nerves. The response to electrical field stimulation was significantly attenuated by perfusion with Krebs' solution at pH 6.9, produced by a reduction in the concentration of $NaHCO_3$; inhibition compared to the preceding control response on each of two occasions was: $46.1 \pm 6.1\%$ and $49.6 \pm 4.8\%$ ($n = 9$).

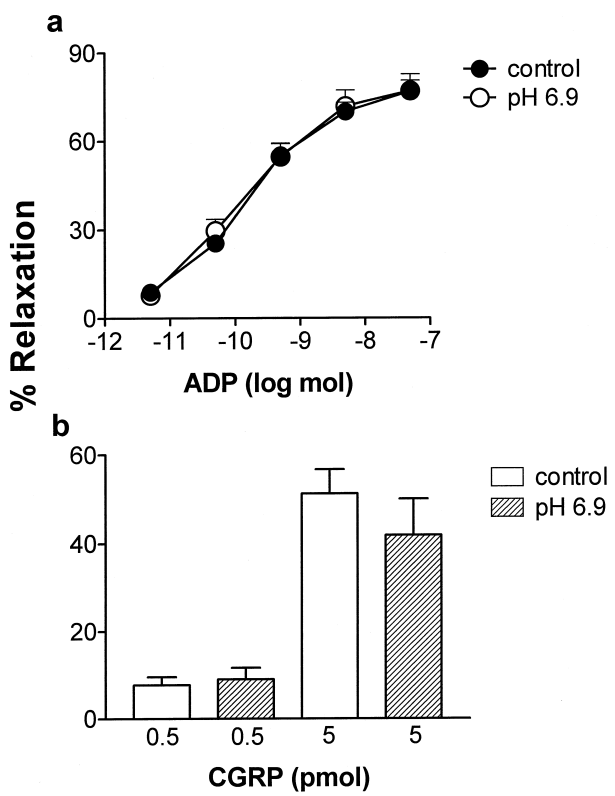

Fig. 5. Effect on vasorelaxant responses to (a) ADP and (b) CGRP of a decrease in pH to 6.9 produced by a reduction in the perfusate concentration of $NaHCO_3$ in the rat isolated mesenteric arterial bed ($n = 9$–10). There was no significant effect on responses generated at pH 6.9 compared to control responses generated at pH 7.4.

3.5. Effect of reducing pH on relaxant response to ADP and CGRP

Vasorelaxant responses to ADP and CGRP were not significantly different at pH 6.9, produced by a reduction in the concentration of $NaHCO_3$, compared to the responses obtained at pH 7.4 ($n = 9$–10) (Fig. 5).

4. Discussion

The results of this study demonstrate that lowering extracellular pH has differential effects on responses to vasoconstrictors of the rat mesenteric arterial bed. Whilst there is a marked attenuation of responses mediated by activation of G protein-coupled receptors for noradrenaline and vasopressin, there is no significant effect of acidosis on contractile responses mediated via ionotropic P2X receptors. Vasorelaxant responses mediated by P2Y ($P2Y_1$) receptors are also unaffected by a decrease in pH.

The main original finding of the present study is that contraction of the rat mesenteric arterial bed mediated by activation of P2X receptors is insensitive to a decrease in extracellular pH from 7.4 to 6.9. Neither the amplitude nor the rate of desensitization of the response was affected by low pH. This is in marked contrast to the pronounced inhibition of contractile responses that was observed for noradrenaline, methoxamine and vasopressin. Whilst α,β-meATP is selective for P2X receptors, ATP may additionally act at contractile P2Y-like receptors in the rat mesenteric arterial bed (Windscheif et al., 1994), but the present data indicate that these either do not contribute greatly to the response or that they are unaffected by a decrease in pH to 6.9. Acidosis induces vasorelaxation by underlying mechanisms that are complex and incompletely understood. Reduced intracellular Ca^{2+} concentration, opening of ATP-sensitive K^+ channels and hyperpolarization, and a release of factors from the vascular endothelium may be involved (Austin and Wray, 1995; Ishizaka and Kuo, 1996; Pen et al., 1998). Moreover, a decrease in extracellular pH can cause a decrease in intracellular pH (Austin and Wray, 1995). All of these factors could potentially contribute to the inhibitory effects of acidosis on agonist-induced contractile responses in blood vessels that have been reported in the present study and by others (Stokke et al., 1984; Loutzenhiser et al., 1990; Austin and Wray, 1995). However, as responses to the vasoconstrictors used in the present study were not uniformly affected by acidosis, it is

unlikely that acidotic vasodilatation per se is responsible for the attenuation of constrictor responses to noradrenaline, methoxamine and vasopressin.

Different signalling mechanisms underly vasoconstriction mediated by adrenoceptors and P2X purine receptors. In the rat mesenteric arterial bed noradrenaline and methoxamine mediate vasoconstriction primarily via α_1-adrenoceptors coupled to G proteins and mobilization of intracellular Ca^{2+} through the generation of inositol 1,4,5-trisphosphate (IP_3) via phospholipase C. Noradrenaline can additionally act at vasorelaxant β-adrenoceptors present in rat mesenteric arteries (Graves and Poston, 1993; Randall and McCulloch, 1995), but the results with methoxamine, a selective α_1-adrenoceptor agonist, indicate effects of low pH on adrenoceptor-mediated contractions. In contrast, P2X receptors are ligand-gated ion channels, which mediate contraction by influx of Ca^{2+} from the extracellular space, causing membrane depolarization and further Ca^{2+} influx (Evans and Surprenant, 1996; Kennedy, 1998). Thus, it seems likely that this difference in signalling pathways underlies the "sparing" of contractile responses mediated by P2X receptors at low pH, and implies that acidosis affects G protein-coupling or events downstream from this in the contractile process. The fact that acidosis had little or no effect on receptor-independent contractile responses to KCl would tend to support this hypothesis. The mechanism by which acid pH attenuates some contractile responses is unclear. Loutzenhiser also reported attenuation by acid pH of contractile responses to noradrenaline and not to KCl in rat aorta, and suggested that acid stimulates an increase in intracellular Ca^{2+} sequestration by the sarcoplasmic reticulum that opposes the action of noradrenaline (Loutzenhiser et al., 1990).

Differences were observed in the degree of attenuation of responses to NA, methoxamine and vasopressin caused by low pH induced by a reduction in [$NaHCO_3$] compared to the addition of HCl. $NaHCO_3$ and Ca^{2+} can form ion pairs in solution, thus, [Ca^{2+}] may vary when the [$NaHCO_3$] is altered (Fry et al., 1994). However, this is unlikely to have contributed to the observed differences as the small increase in [Ca^{2+}] would, if anything, attenuate the inhibitory effect of low pH. Alternatively, whilst reducing [$NaHCO_3$] produces a stable change in pH, a tendency was observed for the pH of the Krebs' solution with added HCl to drift, which may account for the quantitative differences between the data. Nonetheless, under each of these conditions of low pH the lack of effect on responses mediated by P2X receptors compared to those mediated by the other vasoconstrictors was clear.

Both increases and decreases in the sensitivity of recombinant P2X receptors have been reported. Acidification decreases the effects of ATP at recombinant $P2X_1$, $P2X_3$ and $P2X_4$ receptors (Stoop et al., 1997). In contrast, the sensitivity of the recombinant $P2X_2$ receptor increases with acidification (King et al., 1996, 1997). Enhancement of ATP-activated currents by protons has also been shown for endogenous P2X receptors (thought to be $P2X_2P2X_3$ heteromers) in rat sensory ganglia (Li et al., 1996, 1997), and in transfected HEK293 cells expressing heteromeric $P2X_2P2X_3$ receptors (Stoop et al., 1997). The predominant P2X receptor subtype expressed in vascular smooth muscle is the $P2X_1$ receptor, which is sensitive to α,β-meATP and undergoes rapid desensitization both in recombinant and endogenous systems (Collo et al., 1996). However, expression of mRNAs for $P2X_2$ and $P2X_4$ receptors in vascular smooth muscle has also been reported (Nori et al., 1998). Thus, the formation of heteromeric P2X receptors may account for the relative insensitivity to pH of responses to ATP and α,β-meATP observed in the present study. Interestingly, an apparent mismatch has also been reported between the effect of acidification on responses mediated by the endogenous P2X receptor in the vas deferens and by its main counterpart, the $P2X_1$ receptor (Valera et al., 1994), as acidification augments (Nakanishi et al., 1997) and attenuates (Stoop et al., 1997) responses to ATP mediated at the endogenous and recombinant receptors, respectively.

Sympathetic neurogenic contraction to electrical field stimulation in the rat mesenteric arterial bed is blocked by prazosin, indicating an important role of α_1-adrenoceptors in this response. Acid pH greatly attenuated the contractile response to electrical field stimulation, and the results showing significant effects of pH on responses to noradrenaline and methoxamine indicate that at least part of this action is mediated post-junctionally. It is also possible, however, that acid pH inhibits pre-junctionally sympathetic neurotransmission. Given that responses mediated by endogenous vascular P2X receptors are unaffected by low pH, it would be interesting to investigate the effects of acidosis on responses to electrical field stimulation in blood vessels in which there is a large purinergic component of sympathetic neurotransmission. Indeed, in the guinea pig vas deferens, the initial phasic contraction to electrical field stimulation and the phasic contraction to exogenous ATP is augmented by acidification of the bathing solution (to pH 6.9 with HCl), whilst the tonic contraction induced by noradrenaline is attenuated (Nakanishi et al., 1997).

In mesenteric arterial beds with tone raised with methoxamine, there was no effect of low pH on endothelium-dependent relaxation to ADP and endothelium-independent relaxation to CGRP. The ADP receptor is likely to be a $P2Y_1$ receptor (Ralevic and Burnstock, 1996). Others have shown that endothelium-dependent vasorelaxation to acetylcholine is unaffected by a decrease in pH to 6.9 in the rat isolated mesenteric arterial bed (Hiley et al., 1995). There is, however, an augmentation of responses to adenosine (Hiley et al., 1995) and to the A_2 selective agonist *N*-ethylcarboxamido adenosine (unpublished observations) in this vascular preparation, indicating selectivity in the effect of pH on vasorelaxant purine receptors. These results additionally suggest that a reduction in pH does not

inhibit coupling of receptors to G proteins, indicating that the inhibitory effect of pH on contractile responses mediated by noradrenaline, methoxamine and vasopressin may lie distal to this in the signalling pathway.

Chronic acidosis is known to occur in certain pathophysiological conditions including endotoxaemia in experimental animals (Salzman et al., 1994; Kellum et al., 1997) and humans (Riddington et al., 1996), and a fall in pH to as low as 6.98 has been reported for gastric intramucosal pH in patients with systemic endotoxaemia (Riddington et al., 1996). Blunting of noradrenaline-induced contractile responses and IP_3 production in tail arteries of rats with chronic acidosis has been reported (Fox et al., 1992). It is tempting to speculate that the relative sparing of the P2X receptor from the inhibitory effect on contraction of acute acidosis demonstrated in the present study may also occur in chronic acidosis, and thus, that purinergic signalling may assume especial significance in the control of blood flow under pathophysiological conditions.

In conclusion, this study has demonstrated that lowering extracellular pH from 7.4 to 6.9 has no effect on vasoconstriction mediated via ionotropic P2X receptors in the rat mesenteric arterial bed. This is in contrast to the marked attenuation by acid pH of contractile responses mediated by G protein-coupled adrenoceptors and vasopressin receptors. Thus, purines may have an important role in maintaining vascular tone in acidotic challenges of homeostasis.

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