Acidosis reduces the function and expression of $\alpha_{1D}$-adrenoceptor in superior mesenteric artery of *Capra hircus*

Ipsita Mohanty, Sujit Suklabaidya¹, Subas Chandra Parija²

Abstract:

Objective: The objective of this study was to characterize the $\alpha_1$-adrenoceptor ($\alpha_1$-AR) subtypes and evaluate the effect of acidosis on $\alpha_1$-AR function and expression in goat superior mesenteric artery (GSMA).

Materials and Methods: GSMA rings were mounted in a thermostatically controlled (37.0°C ± 0.5°C) organ bath containing 20 ml of modified Krebs-Henseleit solution, maintained at pH$_7.4$ of 7.4, 6.8, 6.0, 5.5, 5.0, and 4.5. Noradrenaline (NA) and phenylephrine (PE)-induced contractile responses were elicited in the absence or presence of endothelium and prazosin at pH$_7.4$ of 7.4, 6.0, and 5.0. The responses were recorded isometrically by an automatic organ bath connected to PowerLab and analyzed using Labchart 7.1.3 software. Expression of $\alpha_{1D}$-AR was compared at physiological and acidic pH$_7.4$ using reverse transcription-polymerase chain reaction (RT-PCR).

Results: NA- and PE-induced contractile responses were attenuated proportionately with a decrease in extracellular pH (pH$_e$). i.e. 7.4 → 6.8 → 6.0 → 5.5 → 5.0 → 4.5. Endothelium denudation increased the contractile response at both normal and acidic pH$_7.4$. Prazosin (1 nM, 10 nM, and 0.1 µM) inhibited the NA- and PE-induced contractile response at pH$_7.4$ and the blocking effect of prazosin was potentiated at pH$_7.4$ of 6.0 and 5.0. RT-PCR analysis for $\alpha_{1D}$-AR in GSMA showed that the mRNA expression of $\alpha_{1D}$-AR was decreased under acidic pH$_7.4$ as compared to physiological pH$_7.4$.

Conclusion: (i) Adrenergic receptor mediates vasoconstriction in GSMA under normal physiological pH$_7.4$, and $\alpha_{1D}$-AR is the possible subtype involved in this event (ii) acidosis attenuates the vasocontractile response due to reduced function and expression of $\alpha_{1D}$-AR and also increased the release of endothelial-relaxing factors.

Key words: Acidosis, *Capra hircus*, prazosin, superior mesenteric artery, $\alpha_{1D}$-adrenoceptor

In vascular bed, both the $\alpha_1$- and the $\alpha_2$-adrenoceptor (AR) subtypes are present postsynaptically, where they mediate vasoconstriction and maintain the peripheral vascular resistance, although the $\alpha_1$-AR is the predominant receptor in vascular smooth muscle.[1,2] The $\alpha_1$ subtypes involved in mesenteric vessel contraction may be $\alpha_{1A}$ or $\alpha_{1B}$ or $\alpha_{1D}$ or due to the activation of more than one subtype,[3] while $\alpha_2$-AR agonist-mediated contraction is mostly absent or even restricted to small arteries/arterioles.[4] Hence, a great variability does exist for the function and expression of $\alpha_1$-AR subtypes in mesenteric artery of different species. $\alpha_1$-AR subtypes generated vasoconstriction in superior mesenteric artery of goat which is far from its functional and molecular identification. Hence, our research was designed to characterize the $\alpha_1$-AR subtype on the basis of functional and reverse transcription-polymerase chain reaction (RT-PCR) analysis in the goat superior mesenteric artery (GSMA).

Extracellular pH (pH$_e$) maintains the blood flow through controlling the contractile state of vascular smooth muscle cells (VSMCs).[4] Acidosis exerts a profound effect on the vascular tone by modulating both the vasocontractile and vasodilatory mechanisms.[5] Vascular myocytes are highly sensitive to pH$_e$. Vasodilation by pH$_e$ < 7.4 (acidosis) and vasoconstriction for pH$_e$ > 7.4 (alkalosis) have been reported in mesenteric artery.[6,7] The vasodilatory effects of acidosis have been well described in animals both in vivo and in vitro.[8,9] A series of in vitro studies reveal that acidosis could affect the agonist-induced vasoconstriction by an increase or decrease or no change[10,11] in the maximal...
response or sensitivity to an α-agonist. Thus, the effect of pH on vasoconstrictive mechanism is often disparate and may vary depending on the species, strain, vascular location, and experimental model. Because acidosis alters vasoconstriction mediated by α-AR, our objective was to assess the effect of acidosis on α-AR agonist-mediated vasoconstriction and α-AR gene expression in GSMA rings.

Materials and Methods

Ethical Guidelines
This work has been approved by the Institutional Animal Ethical Committee (Registration No: 433/CPCSEA/20/06/2001 vide ID No. 130/CVS/dt. 31.03.2015 for conducting randomized animal tissue experiments.

Materials
Noradrenaline (NA) (Merck, India), phenylephrine (PE) hydrochloride (Sigma, USA), and prazosin (MP Biochemicals, India) were the drugs employed for isometric contraction study. The drug solutions were prepared fresh in triple distilled water except NA and prazosin, which were soluble in 0.1N HCl solution. The following components such as 100 bp DNA ladder (SRL, India), 1x gel-loading dye (SRL, India), acrylamide (SRL, India), ammonium persulfate (SRL, India), chloroform (SRL, India), diethyl pyrocarbonate (Genetix, India), dNTPs (Applied Biosystem, USA), ethidium bromide (Sigma, USA), high capacity cDNA synthesis kit (Applied Biosystems, USA), isopropanol (E-Merck, India), multi scribe reverse transcriptase (Applied Biosystem, USA), nuclease-free water (Promega, UK), RNAase Zap (Life Technologies, USA), RNAlater (Life Technologies, USA), SYBR Green (Applied Biosystem, USA), Taq DNA polymerase (Applied Biosystem, USA), and trizol reagent (Ambion, USA) were used for RT-PCR study.

Preparation of Superior Mesenteric Artery and Tension Recording
After the careful exposure of goat intestinal mesentery, a branch of the superior mesenteric artery adjacent to the duodenum and jejunum just before its branching into the inferior branch was dissected out and placed in cold aerated modified Krebs-Henseleit solution (MKHS) with the following composition: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 11.9 mM NaHCO₃, 1.2 mM KH₂PO₄, and 11.1 mM d-glucose (pH 7.4). Further, 1N HCl solution was added to MKHS so as to adjust the pH at 6.8 or 6.0 or 5.5 or 5.0 or 4.5. Arteries were cleared of fat and connective tissues. Endothelium was removed by cotton swab method. The arterial ring of 1.5–2 mm was then mounted between two fine force transducer (Model: MLT0201, AD instrument, Australia) and analyzed using Chart 7.1.3 software.

Isometric Contraction Study
Phenylephrine- or noradrenaline (1 nM–100 μM)-induced concentration-related contractile response at pH of 7.4 or 6.8 or 6.0 or 5.5 or 5.0 for 45 min, NA or PE (1 nM–100 μM) was added to the bath in a cumulative manner at an increment of 1 log unit at 4 min interval to obtain concentration-related contractile (CRC) response. Net tension (gm) due to each concentration was recorded and plotted against Log (M) concentration of NA/PE to elicit a sigmoid CRC response curve for comparison. Mean maximal response (%Eₘₐₓ/Eₘₐₓ) and EC₅₀ were calculated for GSMA rings under different pH ranges and compared. About 6–8 GSMA rings were used for each pH.

Noradrenaline- or phenylephrine (1 nM–100 μM)-induced concentration-related contractile response in goat superior mesenteric artery in the absence or presence of prazosin (1 nM, 10 nM, and 0.1 μM) at pH of 7.4 or 6.0 or 5.0
Arterial rings were preincubated with each concentration of prazosin (1 nM, 10 nM, 0.1 μM) for 30 min prior to exposure to NA/PE in each pH. NA/PE (1 nM–100 μM) was added with an increment of 1 log unit in a cumulative manner into the bath at 4 min interval. The CRC response curves of NA/PE were elicited in the presence of prazosin, and shift of the CRCs was compared with nontreated control. Eₘₐₓ/Eₘₐₓ, mean threshold concentration, and EC₅₀ of agonists in the presence of prazosin were calculated for GSMA rings to evaluate the blocking effect of antagonists under different pH ranges.

Noradrenaline- or phenylephrine (10 μM)-induced contractile response in goat superior mesenteric artery rings in the absence or presence of endothelium at a pH of 7.4 or 6.0 or 5.0
To assess the influence of acidic pH on endothelium, a submaximal dose of NA/PE (10 μM) was added to the 20 ml bath to obtain the first phasic followed by a sustained contractile response in both endothelium intact (ED⁺) and denuded (ED⁻) GSMA rings. The mean peak tension (gm) and plateau tension (gm) were recorded for both ED⁺ and ED⁻ rings and compared at pH of 7.4, 6.0, and 5.0. Under each pH, about 6 tissues were used.

Isolation of Total RNA from Tissue Samples
Goat arterial rings were incubated in MKHS adjusted to pH of 7.4, 6.0, and 5.0 at 37°C for 3 h and thereafter were collected in RNAlater (Life Technologies, USA). Total RNA was isolated from the mesenteric tissue using Trizol reagent (Ambion, USA) according to the manufacturer’s instructions. The purity and concentration of total RNA were measured by a spectrophotometer at 260 and 280 nm. Ratios of absorption (260:280 nm) of all the samples were between 1.8 and 2.0.

cDNA Synthesis and Reverse Transcription Polymerase Chain Reaction
First-strand cDNA synthesis was performed from 2 μg of total RNA using a high-capacity cDNA synthesis kit according to the manufacturer’s instructions (Applied Biosystems, USA). Synthesized cDNA was stored at -20°C until further use. Gene-specific primers were designed for
α<sub>1<sub>,-adrenoceptor (ADRA1D) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) from the corresponding NCBI Reference Sequence (XM_005688233.1; XM_005680968.1) using online Primer 3 software. Primers for ADRA1D (Forward: TGAAGTACCCCTCCATCAT, Reverse: TAGGGACTAGAAAGAGCAC) and GAPDH (Forward: GAGATCAGAAGGTGGTGA; Reverse: CATAACGGAAAATGACCTTG) were commercially procured from Eurogentec, USA. Two microliter of each CDNA samples was used as a template for performing RT-PCR. For all the PCR reactions, the program was as follows: 94°C for 5 min, 94°C for 30 s, 55°C for 20 s, and 72°C for 30 s and a final extension at 72°C for 5 min. PCR products were resolved on 2% agarose gel at 60 V, and the presence of amplicons (GAPDH-175 bp; ADRA1D-195 bp) was documented using gel documentation system (BioRad, USA). The data shown were obtained with 35 PCR cycles.

Statistical Analysis

E<sub>max</sub> and E<sub>max</sub> are the mean maximal responses in the absence and in the presence of the antagonist, respectively. The data were expressed as percentage of the maximum response to agonist obtained in the absence of antagonist (control) and analyzed by the interactive nonlinear regression through the computer program GraphPad Prism (GraphPad Prism Software, San Diego, CA, USA). E<sub>max</sub> and LogEC<sub>50</sub>/EC<sub>50</sub> were calculated through GraphPad Prism and compared for significance level using a t-test calculator - GraphPad Quick Calcs. P < 0.05 was considered statistically significant.

Results

Noradrenaline- or Phenylephrine (10 µM)-induced Contractile Response at pH of 7.4, 6.8, 6.0, 5.5, 5.0, and 4.5

NA or PE (10 µM) induced a first phasic contraction followed by a sustained (plateau) contractile response in GSMA rings, which was progressively attenuated with a decrease in pH. NA (10 µM)-induced sustained contractile response at pH 7.4 (1.72 ± 0.11 g) was significantly (P < 0.05) decreased at pH 6.8 (1.28 ± 0.23 g), pH 6.0 (0.75 ± 0.05 g), pH 5.5 (0.70 ± 0.19 g), pH 5.0 (0.22 ± 0.02 g), and pH 4.5 (0.06 ± 0.02 g). Similarly, PE (10 µM)-induced sustained contractile response at pH 7.4 (1.12 ± 0.09 g) was significantly decreased at pH 6.8 (0.71 ± 0.02 g), pH 6.0 (0.38 ± 0.07 g), pH 5.5 (0.33 ± 0.03 g), pH 5.0 (0.17 ± 0.04 g), and pH 4.5 (0.07 ± 0.01 g) [Figure 1a].

Noradrenaline- (10 µM)-induced Concentration-related Contractile Response at pH of 7.4, 6.0, and 5.0 in Endothelium Intact (ED<sup>+</sup>) and Denuded (ED<sup>−</sup>) Goat Superior Mesenteric Artery Rings

NA (10 µM)-induced contractile response in ED – GSMA rings was significantly (P < 0.05) increased at pH 7.4 (1.91 ± 0.05 g), pH 6.0 (1.68 ± 0.14 g), and pH 5.0 (0.48 ± 0.07 g) as compared to ED + GSMA rings (at pH 7.4: 1.29 ± 0.04 g; pH 6.0: 0.98 ± 0.04 g; and pH 5.0: 0.22 ± 0.03 g). Similarly, PE (10 µM)-induced contractile response in ED – GSMA rings was significantly (P < 0.05) increased at pH 7.4 (1.96 ± 0.46 g), pH 6.0 (0.50 ± 0.01 g), and nonsignificantly increased at pH 5.0 (0.29 ± 0.03 g), as compared to ED + GSMA rings (at pH 7.4: 1.11 ± 0.09 g; pH 6.0: 0.25 ± 0.04 g; and pH 5.0: 0.02 ± 0.01 g) [Figure 1b and c].

Noradrenaline- and Phenylephrine (1 nM–100 µM)-induced Concentration-related Contractile Response at pH<sub>o</sub> of 7.4, 6.8, 6.0, 5.5, 5.0, and 4.5

Table 1 compares the E<sub>max</sub> and pD<sub>2</sub> of NA and PE at pH<sub>o</sub> of 7.4, 6.8, 6.0, 5.5, 5.0, and 4.5. The NA-induced CRC response curve elicited at pH<sub>o</sub> 7.4 (E<sub>max</sub>, 2.28 ± 0.20 g) was shifted to the right with a significant (P < 0.05) decrease in E<sub>max</sub> at pH<sub>o</sub> 6.8 (1.81 ± 0.14 g), pH<sub>o</sub> 6.0 (1.62 ± 0.14 g), pH<sub>o</sub> 5.5 (1.37 ± 0.14 g), pH<sub>o</sub> 5.0 (0.49 ± 0.02 g), and pH<sub>o</sub> 4.5 (0.12 ± 0.01 g) [Figure 2a].

The PE-induced CRC response curve elicited at pH<sub>o</sub> 7.4 (E<sub>max</sub>, 2.18 ± 0.24 g) was shifted to the right with a significant (P < 0.05) decrease in E<sub>max</sub> at pH<sub>o</sub> 6.8 (0.79 ± 0.07 g), pH<sub>o</sub> 6.0 (0.68 ± 0.06 g), pH<sub>o</sub> 5.5 (0.67 ± 0.1 g), pH<sub>o</sub> 5.0 (0.38 ± 0.03 g), and pH<sub>o</sub> 4.5 (0.31 ± 0.01 g) [Figure 2b].

Noradrenaline- (1 nM–100 µM)-induced Concentration-related Contractile Response Either in the Absence or Presence of Prazosin (1 nM, 10 nM, and 0.1 µM) at pH<sub>o</sub> of 7.4, 6.0, and 5.0

Table 2 compares E<sub>max</sub> and EC<sub>50</sub> of NA calculated from its CRC response curve in the absence or presence of prazosin at pH<sub>o</sub> of 7.4, 6.0, and 5.0. NA-induced CRC response curve elicited at pH<sub>o</sub> 7.4 (E<sub>max</sub>, 2.28 ± 0.20 g) was shifted to the right with a significant (P < 0.05) decrease in E<sub>max</sub> in the presence of 1 nM prazosin (1.56 ± 0.02 g), 10 nM prazosin (1.17 ± 0.16 g), and 0.1 µM prazosin (1.07 ± 0.02 g) [Figure 3a].

At a pH<sub>o</sub> of 6.0, NA-induced CRC response curve (E<sub>max</sub>, 1.62 ± 0.14 g) was shifted to the right with a significant (P < 0.05) decrease in E<sub>max</sub> in the presence of 1 nM prazosin.
Table 1: Noradrenaline- and phenylephrine (1 nM–100 µM)-induced maximal contractile response at pH 7.4 (control), 6.8, 6.0, 5.5, 5.0, and 4.5 in goat superior mesenteric artery rings

|      | NA | PE |
|------|----|----|
| pH 7.4 | 2.28±0.20 | 5.29±0.14 |
| pH 6.8 | 1.81±0.14 | 5.27±0.11 |
| pH 6.0 | 1.62±0.14 | 4.72±0.11 |
| pH 5.5 | 1.37±0.14 | 4.74±0.11 |
| pH 5.0 | 0.49±0.02 | 4.82±0.12 |
| pH 4.5 | 0.12±0.01 | 4.46±0.15 |

Note: *P < 0.05 versus pH 7.4 (control). Data were calculated within column between rows with respect to pH 7.4 (control), data were expressed as mean gram tension ± standard error, NA = Noradrenaline, PE = Phenylephrine.
Table 2: Noradrenaline- and phenylephrine (1 nM-100 µM)-induced maximal contractile response in the absence and presence of prazosin (1 nM, 10 nM, and 0.1 µM) at pH₇,4, 6.0, and 5.0 in goat superior mesenteric artery rings

| Treatment | pH₇,4 | pH₆,0 | pH₅,0 | pH₇,4 | pH₆,0 | pH₅,0 |
|-----------|------|------|------|------|------|------|
| NA Control | 2.28±0.20 (n=22) | 1.62±0.14₆ (n=16) | 0.49±0.02₆ (n=16) | 5.13 (2.72-9.67)×10⁻⁵ | 1.92 (1.16-3.19)×10⁻⁵ | 1.58 (0.89-2.61)×10⁻⁵ |
| 1 nM Prazosin | 1.56±0.02₆ (n=6) | 0.72±0.11₆ (n=6) | 0.46±0.01₆ (n=6) | 6.93 (4.87-9.86)×10⁻⁶ | 3.31 (3.09-3.47)×10⁻⁶ | 2.22 (1.34-3.69)×10⁻⁵ |
| 10 nM Prazosin | 1.17±0.16 (n=6) | 0.38±0.04₆ (n=5) | 0.41±0.02₆ (n=5) | 3.19 (1.14-8.92)×10⁻² | 4.47 (2.46-4.57)×10⁻² | 2.83 (1.23-6.53)×10⁻⁵ |
| 0.1 µM prazosin | 1.07±0.02 (n=7) | 0.23±0.05 (n=5) | 0.06±0.02 (n=6) | 4.89 (4.68-5.19)×10⁻⁷ | 7.43 (2.44-2.67)×10⁻⁵ | 4.69 (2.66-8.30)×10⁻⁵ |
| PE Control | 2.18±0.24 (n=15) | 0.68±0.06₆ (n=12) | 0.38±0.03₆ (n=9) | 2.66 (1.06-6.68)×10⁻⁵ | 2.72 (1.42-5.24)×10⁻⁶ | 2.97 (1.37-6.43)×10⁻⁵ |
| 1 nM Prazosin | 1.18±0.04 (n=6) | 0.45±0.06 (n=5) | 0.39±0.003 (n=6) | 1.10 (0.76-1.60)×10⁻⁵ | 5.19 (2.11-2.73)×10⁻⁶ | 2.49 (1.94-3.21)×10⁻⁵ |
| 10 nM Prazosin | 1.08±0.02 (n=6) | 0.30±0.02 (n=4) | 0.39±0.004 (n=5) | 2.10 (1.51-2.92)×10⁻² | 1.02 (0.91-1.15)×10⁻⁵ | 3.61 (2.31-5.65)×10⁻⁵ |
| 0.1 µM Prazosin | 1.02±0.12 (n=10) | 0.19±0.06 (n=4) | 0.21±0.01 (n=7) | 6.03 (2.04-7.81)×10⁻⁶ | 2.24 (0.32-5.73)×10⁻⁵ | 3.96 (2.43-6.45)×10⁻⁵ |

1P<0.05 versus pH 7.4 (control). Data were compared within row between columns with respect to pH 7.4. 2P<0.05 versus control (nontreated). Data were compared within columns between rows with respect to nontreated control, data were expressed as mean gram tension ± SE. n=Number of experiments, PE=Phenylephrine, NA=Noradrenaline, SE=Standard error, CL=Confidence limit.

Reduction of pH₇,4 (7.4 → 6.8 → 6.0 → 5.5 → 5.0 → 4.5) attenuated the NA- and PE-induced CRC response successively and significantly in GSMA rings. At a pH₇,4 of 4.5, NA- and PE-induced mean maximal response (E₉₅) was decreased to 5% and 14%, respectively, of the contractile response measured at pH₇,4 as 100%. A single dose of NA/PE (10 µM)-induced contractile response was decreased (NA: 1: 0.74: 0.43: 0.40: 0.12: 0.03; PE: 1: 0.63: 0.33: 0.29: 0.15: 0.06) with a decrease in pH 7.4 → 6.8 → 6.0 → 5.5 → 5.0 → 4.5. It is interesting to note that there was a progressive development of vascular shock with a decrease in the pH₇,4 to 5.0-4.5, which is partially reversible within 90 min after exposing GSMA rings to MKHS maintained at a normal physiological pH. These clearly demonstrate that hyperacidity could induce a state of vascular shock that almost completely abolishes α₁D-AR-mediated vasotonic function in GSMA. Hence, α₁-AR function is suppressed in a proportional fashion by reduction in pH₇,4. Based on our observation that a moderate inhibition of α₁-AR mediated contractile response. Decrease in the mean tension of NA and PE with an increase in the concentration of prazosin confirms the involvement of α₁D-AR in mediating the contraction to these agonists. At a pH₇,4 of 7.4, a higher dose ratio (EC₉₅ of agonist with antagonist/EC₉₅ of agonist) of NA (1.35) in the presence of lower concentration of prazosin (1 nM) was increased to 6.2 at its higher concentration (0.1 µM). Similarly, a lower dose ratio of PE (4.13) in the presence of lower concentration of prazosin (1 nM) was increased to 22.7 at its higher concentration (0.1 µM). Hence, the potential blockade properties of prazosin at nanomolar concentration for NA and at micromolar concentration for PE indicate that NA and PE could be inducing contractile response in GSMA by interacting with (i) high- and low-affinity binding sites of a particular α₁-AR subtype, (ii) or two different α₁-AR subtypes possibly α₁a and α₁dD-AR as reported in the mesenteric artery of rats and rabbits (iii) or only α₁dD-AR as reported in goat ruminal artery.

In GSMA at a pH₇,4 of 7.4, antagonism of NA- and PE-mediated responses by three different concentrations (1 nM, 10 nM, and 0.1 µM) of prazosin was surmountable and fully consistent with simple competition, as indicated by clear-cut rightward shift with consequent reduction of the E₉₅ and EC₉₅ of NA- and PE-CRC response curve. Decrease in the affinity of NA and PE with an increase in the concentration of prazosin confirms the involvement of α₁D-AR in mediating the contraction to these agonists. At a pH₇,4 of 7.4, a higher dose ratio (EC₉₅ of agonist with antagonist/EC₉₅ of agonist) of NA (1.35) in the presence of lower concentration of prazosin (1 nM) was increased to 6.2 at its higher concentration (0.1 µM). Similarly, a lower dose ratio of PE (4.13) in the presence of lower concentration of prazosin (1 nM) was increased to 22.7 at its higher concentration (0.1 µM). Hence, the potential blockade properties of prazosin at nanomolar concentration for NA and at micromolar concentration for PE indicate that NA and PE could be inducing contractile response in GSMA by interacting with (i) high- and low-affinity binding sites of a particular α₁-AR subtype, (ii) or two different α₁-AR subtypes possibly α₁a and α₁dD-AR as reported in the mesenteric artery of rats and rabbits (iii) or only α₁dD-AR as reported in goat ruminal artery.
Mohanty, et al.: Acidosis reduces α1D-adrenoceptor expression in GSMA

sites to bring about cellular acidosis. Hence, it is the acidic environment but not HCl as a whole does impart any direct change in the receptor. The β1 receptor’s number and function have been observed earlier in cardiac muscles and coronary arteries when the pH is reduced to 6.0 by the addition of HCl or ascorbic acid into the perusing medium. Affinity of receptor to its agonist (s) varies with (i) a number of receptors or number of binding sites that bind to agonists and (ii) a number of G-protein coupled receptors that activate second messenger. In addition, the cycle of dephosphorylation and phosphorylation

Figure 3: Effect of acidosis on the blocking effect of prazosin on noradrenaline- and phenylephrine-induced concentration-related contractile response in goat superior mesenteric artery. (a-c) Noradrenaline (1 nM–100 µM)-induced concentration-related contractile response curve in the absence or presence of prazosin (1 nM, 10 nM, and 0.1 µM) elicited at pH 7.4 (a), 6.0 (b), and 5.0 (c). (d-f) Phenylephrine (1 nM–100 µM)-induced concentration-related contractile response curve in the absence or presence of prazosin (1 nM, 10 nM, and 0.1 µM) elicited at pH 7.4 (d), 6.0 (e), and 5.0 (f). Data were expressed as mean gram tension ± standard error. *P < 0.05 versus nontreated group (control).
Endothelium denudation significantly increased the contractile response to NA at pH₁ of 7.4, 6.0, and 5.0 by 67%, 84%, and 118%, respectively, indicating that reduced vasotonic response to NA in acidosis is due to increased function of endothelium that, in turn, augments the release of endothelium-dependent relaxing factors, which exert a subtractive influence on vasconstrictor response. However, such increase in vasotonic response to PE in endothelium-denuded GMSA rings was observed only at a pH₀ of 5.0. Furthermore, the estimation of mRNA level for α₁-AR in the goat mesenteric artery using RT-PCR showed a significant decrease in α₁A/D transcript expression as a consequence of acidosis. Thus, our observation reveals that the attenuated contractility, registered in acidosis at the isolated organ bath experiments, could originate from a reduced expression level of α₁-ARs. Most studies now agree that acidosis exerts a direct effect on the vascular smooth muscle via ion channels (ATP-sensitive K⁺ channels, in particular), which may act in conjunction with reduced receptor sensitivity, resulting in attenuated maximal receptor-mediated responsiveness. Hence, the possibility of alteration of different cell signaling molecules as influenced by acidosis is yet far from a scientific approach that needs further study.

In the presence of prazosin, NA- and PE-mediated contractile responses were inhibited differentially at pH₀ 6.0 and 5.0 in a dose-dependent manner. Prazosin (1 nM, 10 nM, and 0.1 μM) reduced 100% maximal contractile response induced by NA at pH₁ 7.4-68%, 51%, and 46%; at pH₀ 6.0-44%, 23%, and 14%; and at pH₀ 5.0-93%, 84%, and 12%, respectively. Similarly, prazosin (1 nM, 10 nM, and 0.1 μM) attenuated maximal contractile response induced by PE at pH₁ 7.4-85%, 50%, and 47%; at pH₀ 6.0-66%, 44%, and 27%; and at pH₀ 5.0-99%, 98%, and 55%. The α₁-AR blocking effect of prazosin is proportionately increased with respect to an increase in its concentration within a particular pH. While comparing the influence of acidosis on the blocking effect of prazosin, it was interesting to note that the blocking effect of prazosin at different concentrations was further increased at pH₀ 6.0, but not at pH₀ 5.0, as compared to pH₁ 7.4. At pH₀ 5.0, blocking effect of prazosin was only increased at 0.1 μM. From the above observation, it is quite obvious to mention that in GMSA, the increased blocking effect of prazosin is due to decreased function and expression of α₁D-AR under acidosis.

Integrating both the results from the functional study and change in gene expression, it could be concluded that a decrease in pH₁ (7.4 → 4.5) in GMSA attenuated vasotonic response to NA and PE due to an additive effect of (i) enhanced release of endothelial-relaxing factors and (ii) reduced α₁D-AR receptor expression in VSMCs of GMSA.

An important clinical cardiovascular complication due to extreme acidemia is low blood pressure (hypotension), and this could be attributed to decreased vascular contraction arising from attenuated sympathetic response and reduced expression level of α₁-ARs in this vascular bed as evidenced from the present vascular model. The possible clinical implication is that the therapeutic use of vasoconstrictor-like α₁-receptor agonist would not be a primary choice to reverse hypotensive crisis arising from the extreme acidemia. The possible therapeutic use of alternative vasoconstrictor agents could be approached with experimental validations to counter cardiovascular complication due to extreme acidemia.

Acknowledgments
I Mohanty is an INSPIRE Fellow (IF130735), GOI. The authors are thankful to Dr. Shantibhusan Senapati, Scientist of ILS, for providing necessary laboratory facilities to conduct quantitative polymerase chain reaction.

Financial Support and Sponsorship
This study was funded by DST, GOI.

Conflicts of Interest
There are no conflicts of interest.

References
1. Docherty JR. Subtypes of functional alpha1-adrenoceptor. Cell Mol Life Sci 2010;67:405-17.
2. Langer SZ, Hicks PE. Alpha-adrenoceptor subtypes in blood vessels: Physiology and pharmacology. J Cardiovasc Pharmacol 1984;6:S547-58.
3. Zhong H, Minneman KP. Alpha1-adrenoceptor subtypes. Eur J Pharmacol 1999;375:261-76.
4. Klöckner U, Isenberg G. Calcium channel current of vascular smooth muscle: Physiology and pharmacology. J Cardiovasc Pharmacol 1984;6:S547-58.
5. Ma Z, Qi J, Fu Z, Ling M, Li L, Zhang Y. Protective role of acidic pH-activated chloride channel in severe acidosis-induced contraction from the aorta of spontaneously hypertensive rats. PLoS One 2013;8:e61018.
6. Austin C, Wray S. Interactions between Ca(2+) and H(+) and functional consequences in vascular smooth muscle. Circ Res 2000;86:355-63.
7. Iarysev VN, Karachentseva OV. The contractile activity of the isolated mesenteric artery at different pH values of the perfusion solution. Ross Fiziol Zh Im I M Sechenova 1996;82:28-36.
8. Ives SJ, Robert HI, Andtbacka R, Noyes D, Morgan RG, Gifford JR, et al. α₁-adrenergic responsiveness in human skeletal muscle feed arteries: The impact of reducing extracellular pH. Exp Physiol 2013;98:256-67.
9. Celotto AC, Restini CB, Capellini VK, Bendhack LM, Evora PR.

Figure 4: Quantitative polymerase chain reaction analysis showing α₁D-adrenoceptor (ADRA1D) expression levels for goat superior mesenteric artery rings incubated at normal and acidic pH (pH 7.4, 6.0, and 5.0). Glycerinaldehyde-3-phosphate dehydrogenase was used as an internal control.
Acidosis induces relaxation mediated by nitric oxide and potassium channels in rat thoracic aorta. Eur J Pharmacol 2011;656:88-93.

10. Rohra DK, Saito SY, Ohizumi Y. Extracellular acidosis results in higher intraacellular acidosis and greater contraction in spontaneously hypertensive rat aorta. Eur J Pharmacol 2003;465:141-4.

11. Rohra DK, Saito SY, Ohizumi Y. Strain-specific effects of acidic pH on contractile state of aortas from Wistar and Wistar Kyoto rats. Eur J Pharmacol 2003;476:123-30.

12. Medgett IC, Hicks PE, Langer SZ. Effect of acidosis on alpha 1- and alpha 2-adrenoceptor-mediated vasoconstrictor responses in isolated arteries. Eur J Pharmacol 1987;135:443-7.

13. Heintz A, Koch T, Deussen A. Intact nitric oxide production is obligatory for the sustained flow response during hypercapnic acidosis in guinea pig heart. Cardiovasc Res 2005;66:55-63.

14. Rosolowsky M, Pfister SL, Buja LM, Clubb FJ Jr., Campbell WB. Method of removal of aortic endothelium affects arachidonic acid metabolism and vascular reactivity. Eur J Pharmacol 1991;193:293-300.

15. Costa P, Bressolle F, Sarrazin B, Mosser J, Navratil H, Galtier M. Moxisylyte plasma kinetics in humans after intracavernous administration. Biopharm Drug Dispos 1992;13:671-9.

16. Pereira FJ, Will JA. Functional characterization of post-junctional adrenergic receptor subtypes in bovine intra-mammary arteries. J Vet Pharmacol Ther 1997;20:434-41.

17. Belloli C, Badino P, Arioli F, Odore R, Re G. Adrenergic regulation of vascular smooth muscle tone in calf digital artery. J Vet Pharmacol Ther 2004;27:247-54.

18. Piascik MT, Perez DM. α1,3-adrenergic receptors: New insights and directions. J Vet Pharmacol Ther 2001;28:403-10.

19. Gow IF, Mitchell E, Wait M. Adrenergic receptors in the bovine mammary artery. Biochem Pharmacol 2003;65:1747-53.

20. Kathirvel K, Behera PC, Fallai S, Mohanty J, Parija SC. Pharmacological and molecular identification of α1A/D-adrenoceptor in goat ruminal artery. Int J Drug Dev Res 2010;2:64-53.

21. Hrometz SL, Edelmann SE, McCune DF, Olges JR, Hadley RW, Perez DM, et al. Expression of multiple alpha1-adrenoceptors on vascular smooth muscle: Correlation with the regulation of contraction. J Pharmacol Exp Ther 1999;290:452-63.

22. Methven L, Simpson PC, McGrath JC. Alpha1A/B-knockout mice explain the native alpha1D-adrenoceptor’s role in vasoconstriction and show that its location is independent of the other alpha1-subtypes. Br J Pharmacol 2009;158:1663-75.

23. Van der Graaf PH, Deplanne V, Duquenne C, Angel I. Analysis of alpha1-adrenoceptors in rabbit lower urinary tract and mesenteric artery. Eur J Pharmacol 1997;327:25-32.

24. McGillivray-Anderson KM, Faber JE. Effect of acidosis on contraction of microvascular smooth muscle by alpha 1- and alpha 2-adrenoceptors. Implications for neural and metabolic regulation. Circ Res 1990;66:1643-57.

25. Tateishi J, Faber JE. Inhibition of arteriole alpha 2- but not alpha 1-adrenoceptor constriction by acidosis and hypoxia in vitro. Am J Physiol Heart Circ Physiol 1995;268:H2068-76.

26. Capellini VK, Restini CB, Bendhack LM, Evora PR, Celotto AC. The effect of extracellular pH changes on intracellular pH and nitric oxide concentration in endothelial and smooth muscle cells from rat aorta. PLoS One 2013;8:e62887.

27. Hyvelin JM, O’Connor C, McLoughlin P. Effect of changes in pH on wall tension in isolated rat pulmonary artery: Role of the RhoA/Rho-kinase pathway. Am J Physiol Lung Cell Mol Physiol 2004;287:L673-84.