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Acidosis-induced relaxation of human internal mammary artery is due to activation of ATP-sensitive potassium channels

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Abstract

Metabolic acidosis is associated with various clinical situations including diabetes mellitus and renal diseases. The aim of this study was to investigate the effects of acidosis on the resting as well as precontracted human left internal mammary artery. The vessels were obtained from the patients undergoing coronary artery bypass grafting surgery at The Aga Khan University Hospital, Karachi. Left internal mammary artery was cut into rings and isometric tension recording experiments were performed. Decrease in pH of the bathing solution from 7.4 to 6.8 had no effect on the resting tension of left internal mammary artery, whereas, acidic pH markedly relaxed the contractions to 24.8 mM KCl and 300 nM phenylephrine. Interestingly, when the KCl- or phenylephrine-contracted rings were treated with 3 μM glibenclamide; an inhibitor of ATP-sensitive potassium (K_{ATP}) channels, the relaxant effect of acidosis was abolished. Similarly, acidosis failed to cause relaxation of 100 nM endothelin-1-induced contraction in Ca^{2+}-free bathing solution or in the presence of a voltage-dependent Ca^{2+} channel inhibitor, verapamil (10 μM), whereas, endothelin-1-induced contraction was attenuated by acidosis in Ca^{2+}-containing normal solution. From all these data, it is concluded that under the acidic pH conditions, opening of K_{ATP} channels occurs; resulting in the hyperpolarization, decrease in Ca^{2+} influx via voltage-dependent Ca^{2+} channels and subsequent relaxation of human left internal mammary artery.

Keywords: Acidosis; ATP-sensitive potassium channel; Human internal mammary artery; pH; Vascular smooth muscle

1. Introduction

The contractile state of vascular smooth muscle can be regulated by many factors including changes in pH (Smith et al., 1989). Although the pH of blood and extracellular fluids is generally maintained at around 7.4, there are various pathophysiological conditions such as diabetes mellitus, renal dysfunction, and pulmonary edema, in which a significant decrease in pH occurs (Austin and Wray, 2000). A fall in pH of blood as low as 6.8 has been reported in certain clinical situations (Gan et al., 1992), producing a corresponding decline in intracellular pH (pH_i) (Ramsey et al., 1994). Experimentally, changes in extracellular pH (pH_o) produce changes in pH_i (Rohra et al., 2003b).

Gaskell (1881) was perhaps the first scientist, who described the effects of acidosis on vascular tissue. Since then, diversity in the effects of acidosis on the contractility of vascular smooth muscle has been described in in vitro studies using vascular preparations from various animals (Berger et al., 1998; Rohra et al., 2003c). For example, in aortas from spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats (Rohra et al., 2002a,b), and rat and dog pulmonary artery (Hyvelin et al., 2004; Höhne et al., 2004), a contractile response to acidosis has been demonstrated. Whereas, in Wistar rat aorta (Rohra et al., 2003c) and

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cerebral artery (Peng et al., 1998) and porcine coronary arterioles (Ishizaka et al., 1999), acidosis induces a relaxation. Acidosis has been suggested to produce vascular relaxation by utilizing various mechanisms such as activation of ATP-sensitive potassium (K_{ATP}) channels (Ishizaka et al., 1999), production of cAMP (Leffler et al., 1999), and a direct inhibition of L-type Ca^{2+} channels in vascular smooth muscle cells (Smirnov et al., 2000). Different subtypes of K^+ channels have been described in vascular smooth muscle cells, but activation of K_{ATP} channels appears to have a major role in relaxation of various types of vessels in response to several stimuli including acidosis (for review; see Brayden, 2002).

Experimentally, acidosis can be induced by producing hypercapnia (Peng et al., 1998; Bayerle-Eder et al., 2000) or simply making the extracellular environment acidic (Rohra et al., 2003a). There are a few studies to document the hypercapnia-induced changes in the diameter of the human vessels, but these were carried out in vivo (Bayerle-Eder et al., 2000). Although the effects of acidosis on the cardiovascular system are surmised to be depressant, no such direct study has been carried out to evaluate the impact of extracellular acidic environment on the vascular reactivity of human vessels. Thus the aim of the present study was to investigate the effects of acidosis on the contractility of human internal mammary artery. To accomplish this aim, isolated vessel techniques were used to eliminate the confounding influences from neurohumoral and local control mechanisms. The findings of the present study for the first time show that extracellular acidosis induces a relaxation of human internal mammary artery which is associated with the activation of K_{ATP} channels.

2. Materials and methods

This study was carried out in accordance with the World Medical Association Declaration of Helsinki regarding the Ethical Principles for Medical Research involving human subjects and was reviewed and approved by the Institutional Ethical Review Committee.

2.1. Tissue preparation

The human left internal mammary artery was obtained from the Cardiothoracic Section of the Department of Surgery, The Aga Khan University Hospital. The arteries of patients, who underwent coronary artery bypass grafting operation, were used in the study. The standard bypass operation involves using the left internal mammary artery as one of the bypass conduits. The artery is harvested in almost all operations and a portion of it is usually discarded after tailoring it to the appropriate length for the recipient (left anterior descending) artery. This normally discarded distal end of the human left internal mammary artery was used in the isometric tension recording experiments. Informed consent prior to surgery was obtained from all the patients whose left internal mammary artery was used for experiments. The artery was quickly immersed in an ice-cold HEPES-buffered physiological salt solution (PSS) and was cleaned of adherent connective tissue. Rings of approximately 3 mm width were made from the artery. The endothelium was removed by gently rubbing the endothelial surface with cotton pellets in order to obviate the involvement of the endothelium and to examine the function of vascular smooth muscle alone. The lack of endothelium was confirmed by failure of carbachol (1 μM) to cause relaxation of phenylephrine (1 μM)-induced contraction.

2.2. Experimental protocol

All experiments were carried out at 37 °C. The rings were fixed between two hooks and suspended in a 5 ml organ bath, containing well aerated (95% O_2 + 5% CO_2) PSS of pH 7.4. The hook anchoring the upper end of the ring was connected to a force displacement transducer (Transbridge 4M, WPI Ltd. UK). The tissues were adjusted to a preloaded resting tension of 1 g and equilibrated for at least 1 h. The arterial rings were contracted 3 times with 64.8 mM KCl to acclimatize the tissue and to verify that the artery is viable. The last contractile response to high KCl was the reproduction of the second one and later it was employed as a standard and other contractions were normalised to it. The pH of the bathing solution was changed from control value of 7.4 to 6.8 by addition of HCl and the pH inside the bath was measured with the help of a micro pH electrode (Model PHR 146, Lazar Research Lab. Inc. CA, USA) attached with a pH meter (Model 611, Orion Research Instruments, Cambridge, USA). In experiments investigating the effect of acidic pH on the contractile state of the left internal mammary artery, the concentrations of 300 nM for phenylephrine and 24.8 mM for KCl were selected in order to obtain a contractile response that should be 70–90% of the 64.8 mM KCl-induced contraction. The tissues showing <70% or >90% of the 64.8 mM KCl-induced contraction were discarded and not included in the study. The effect of acidosis on the vessels in Ca^{2+}-free conditions was investigated in the following protocol. Bathing PSS was changed by Ca^{2+}-free solution containing 0.5 mM EGTA [ethyleneglycol-bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid]. After 10 min, etdothelin-1 was added and when maximum contraction was achieved, the pH of bathing solution was changed from 7.4 to 6.8.

2.3. Solutions

The composition of the PSS was (in mM): NaCl 120, KCl 4.8, $MgSO_4$ 1.3, $CaCl_2$ 1.2, $NaHCO_3$ 25.2, glucose 5.8, KH_2PO_4 1.2 and HEPES 20. 64.8 mM KCl was made by replacing 60 mM NaCl of PSS with equimolar KCl, while Ca^{2+}-free PSS was prepared by omitting Ca^{2+} from normal

PSS and adding 0.5 mM EGTA in order to chelate any residual Ca^{2+}.

2.4. Materials

All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Phenylephrine, endothelin-1 and verapamil were dissolved in water, while glibenclamide was dissolved in dimethyl sulphoxide.

2.5. Statistics

Data from tension recording experiments are expressed as means ± S.E.M. The relaxation of phenylephrine- or KCl-induced contractions caused by acidosis has been expressed as the % of the contraction observed at pH 7.4. *n* represents the number of experiments performed. Data were analysed by Student's *t*-test and the differences were considered statistically significant at $P<0.05$.

3. Results

3.1. Effect of acidosis on the resting tension and the contractile state

Effect of decreasing pH extracellularly on the resting state of the isolated human left internal mammary artery rings was first characterized. Changing pH from 7.4 to 6.8 by adding HCl to the bathing solution failed to produce any effect on the resting tension of the vessel (Fig. 1A).

In order to assess the effects of acidic pH on the precontracted artery, left internal mammary artery rings were contracted with sub-maximal concentrations of either KCl (24.8 mM) or phenylephrine (300 nM). When the contractile response reached a steady level, the pH of the bathing solution was lowered from 7.4 to 6.8. Acidosis caused a relaxation of both KCl (Fig. 1B) and phenylephrine (Fig. 1C) contracted arterial rings. However, the extent of acidosis-induced relaxation was remarkably greater in phenylephrine-induced contraction compared to the one caused by KCl. As quantified in Fig. 2, the magnitude of 24.8 mM KCl-induced contraction at pH 6.8 was 76.0 ± 1.3% ($n=4$) of the contraction of the same stimulus but at pH 7.4. Whereas at similar acidic pH, phenylephrine-induced contraction was 39.2 ± 9.8% ($n=4$) of the one at pH 7.4. The difference between the magnitudes of relaxation of both types of contractions was statistically significant ($P<0.01$).

3.2. Effect of acidosis in the presence of glibenclamide

To evaluate the role of K_{ATP} channels, experiments were performed using a selective K_{ATP} channel inhibitor, glibenclamide (Nelson and Quayle, 1995; Teramoto et al., 2000; Rosenblum, 2003). The KCl- or phenylephrine-precontracted arterial rings were treated with 3 uM glibenclamide, and after 10 min, the pH of the bathing solution was lowered from 7.4 to 6.8. Interestingly, the relaxant effect of acidosis was observed to be nearly abolished in the presence of glibenclamide (Fig. 3). Glibenclamide by itself did not show any effect on the resting tension as well as on the contractions caused by KCl or phenylephrine at pH 7.4 (data not shown).

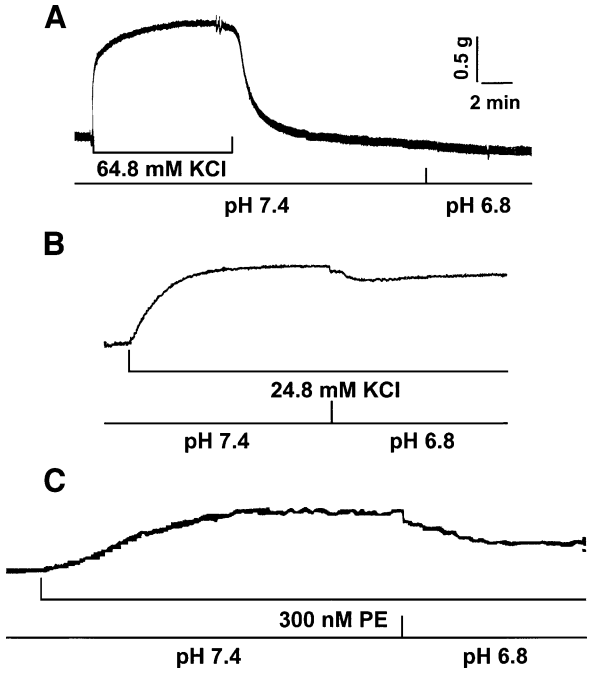

Fig. 1. Representative recordings showing the effects of acidosis on the resting tension as well as on the phenylephrine- and KCl-contracted isolated human internal mammary artery. (A) Arterial rings were precontracted with 64.8 mM KCl 3 times and after washout, the pH of the bathing solution was changed from 7.4 to 6.8 by adding HCl to it. Arterial rings were contracted with either 24.8 mM KCl (B) or 300 nM phenylephrine (C) at pH 7.4. After the peak response was achieved, pH of the bathing solution was decreased to 6.8 by adding HCl. Recordings are the representative of 4–5 independent experiments from different vessels.

3.3. Effect of acidosis in Ca^{2+}-free solution

The role of Ca^{2+} influx in acidosis-induced relaxation was investigated. The artery was contracted in Ca^{2+}-free solution by 100 nM endothelin-1. The level of contraction produced by endothelin-1 in Ca^{2+}-free conditions was markedly low, that was 49.2 ± 4.4% of the 64.8 mM KCl-induced contraction. When the endothelin-1-induced contraction in Ca^{2+}-free solution attained a steady level, the bathing solution was acidified up to pH 6.8. Acidification failed to produce any relaxation of endothelin-1-induced contraction in Ca^{2+}-free medium (Fig. 4A). In separate experiments, the effect of acidosis on endothelin-1-induced contraction in the presence of Ca^{2+}-containing normal PSS was also studied. Under the normal Ca^{2+}-containing conditions, endothelin-1 induced a marked contraction, which was 118.0 ± 6.8% of the 64.8 mM KCl-induced

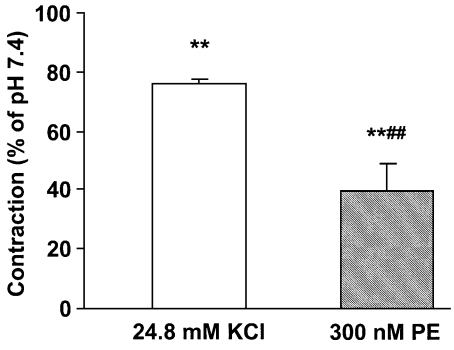

Fig. 2. Quantification of the effects of acidosis on contractile responses to KCl and phenylephrine in isolated human internal mammary artery. The contractions to 24.8 mM KCl or 300 nM phenylephrine at pH 7.4 were considered as 100%. The levels of the contractions at pH 6.8 are plotted as percentage of those at pH 7.4. The data represent the means ± S.E.M. $n=4–5$ experiments from different vessels in each group. $^{**}P<0.01$ versus contractile response at pH 7.4 in respective group; $^{\#\#}P<0.01$ versus KCl-induced contraction at pH 6.8.

contraction. Interestingly and contrary to the observation in Ca^{2+}-free solution, acidification of the bathing solution to 6.8 did relax the contractile response to 100 nM endothelin-1 in Ca^{2+}-containing normal PSS (Fig. 4B). The residual contraction in the continuous presence of endothelin-1 under the acidic conditions was found to be 75.7 ± 4.5%. The acidosis-induced relaxation of endothelin-1-induced contraction in Ca^{2+}-containing normal PSS was again abolished by glibenclamide (data not shown).

3.4. Effect of acidosis in the presence of verapamil

One of the most common mechanism by which vascular smooth muscle contraction is brought about is the rise in intracellular Ca^{2+} following opening of voltage-dependent

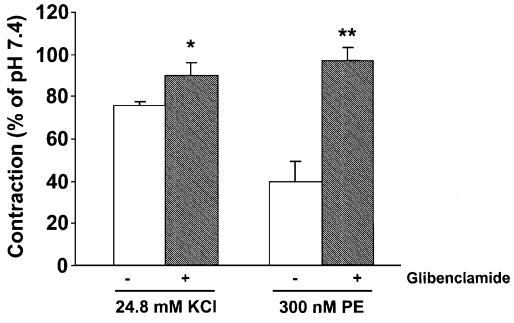

Fig. 3. Effects of acidosis on contractile responses to KCl and phenylephrine in isolated human internal mammary artery in the presence of glibenclamide. Arterial rings were contracted with 24.8 mM KCl or 300 nM phenylephrine at pH 7.4. After the peak response was achieved, ATP-sensitive K^+ channel inhibitor, glibenclamide (3 μM) was added to the bathing solution. 10 min later, pH of the bathing solution was changed to 6.8. The contractions to 24.8 mM KCl or 300 nM phenylephrine at pH 7.4 were considered as 100%. The levels of the contractions at pH 6.8 in the presence or absence of glibenclamide are plotted as percentage of those at pH 7.4. The data represent the means ± S.E.M. $n=4–5$ experiments from different vessels in each group. $^{*}P<0.05$; $^{**}P<0.01$ versus contractile response at pH 6.8 in the absence of glibenclamide, in respective group.

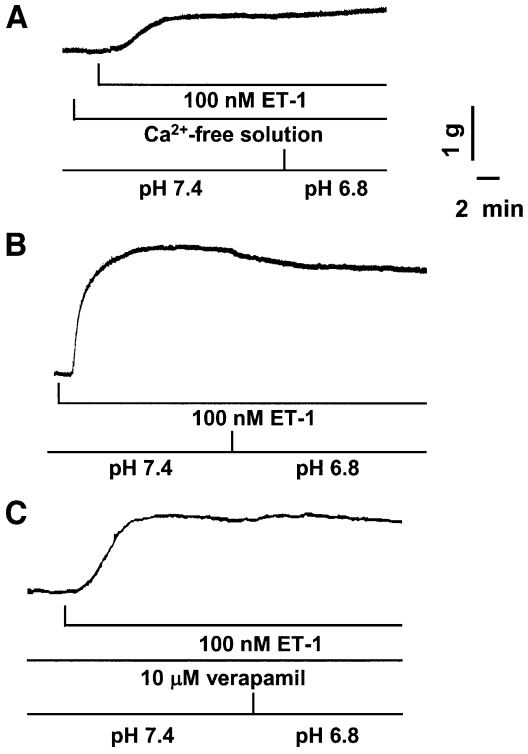

Fig. 4. Representative recordings showing the effects of acidosis on the endothelin-1-induced contraction in Ca^{2+}-free solution, Ca^{2+}-containing normal solution, or in the presence of verapamil in isolated human internal mammary artery. Arterial rings were contracted with 100 nM endothelin-1 in Ca^{2+}-free solution (A), Ca^{2+}-containing normal solution (B), or in the presence of 10 μM verapamil, a voltage-dependent Ca^{2+} channel inhibitor (C). After the peak response was achieved, pH of the bathing solution was decreased to 6.8 by adding HCl. Recordings are the representative of 4 independent experiments from different vessels in each group.

Ca^{2+} channels. To investigate the involvement of Ca^{2+} channels in acidosis-induced relaxation, 10 μM verapamil, a blocker of voltage-dependent Ca^{2+} channels, was added to the bathing solution 10 min prior to the addition of endothelin-1. When the contraction to endothelin-1 reached a steady level (78.6 ± 5.6% of the 64.8 mM KCl-induced contraction), the pH of the bathing solution was changed to 6.8. As shown in Fig. 4C, acidosis in the continued presence of verapamil failed to produce any relaxation of the endothelin-1 contracted vessel.

4. Discussion

This study is the continuation of our previous reports studying the effects of acidosis on the vascular tissue obtained from different animals (for review, Rohra, 2003). We have shown previously the strain-specificity of the effects of acidosis; the vessels of SHR and WKY rats show a contractile response, whereas in Wistar rats, there is a relaxant response towards acidosis (Rohra et al., 2003c,d). Since metabolic acidosis is not an uncommon clinical situation, we investigated the effects of experimental

acidosis on the contractility of human left internal mammary artery.

Decrease in pH of the bathing solution showed no effect on the resting tension of the arterial rings. However, acidosis relaxed the vessels precontracted with KCl or phenylephrine. Since the arteries used in the experiments were devoid of endothelium, the relaxant effect of acidosis seems to be endothelium-independent and direct on the vascular smooth muscle cells. The reason why KCl was used along with phenylephrine for studying the effects of acidosis is that one may argue that acidosis may interfere with the binding of phenylephrine with α-adrenergic receptors or inhibit receptor mediated activation of second messenger pathways. The use of KCl to observe effects of acidosis was a means to bypass receptor activation and any receptor-dependent signal transduction pathways. Since at resting level, the arterial rings were in absolute relaxed state in PSS at pH 7.4, the decrease in pH could not produce a further relaxation, which was revealed when the tissue was precontracted. Under physiological conditions, vascular tone is modulated not only by the local chemical environments (Feigl, 1983) but also by the changes in pressure (Kuo et al., 1988) and hence the arteries are in partially contracted state. Due to this fact, it is reasonable to believe that in vivo, acidosis produces vasodilation. This belief is in consistence with the observations of Bayerle-Eder et al. (2000), who have shown that hypercapnic acidosis induces cerebral and ocular vasodilation in vivo, in humans.

Having characterized the relaxant effect of acidosis, the next step was to investigate the mechanism underlying the acidosis-induced relaxation. K_{ATP} channels are expressed in various cells including vascular smooth muscle cells, where they couple the intermediary metabolism to cellular excitability (Quayle et al., 1997). In this respect, several lines of evidence gathered from the animal tissue studies suggested that vascular smooth muscle K_{ATP} channels may be involved in the mediation of acidosis-induced relaxation in human vessels. First, changes in pH reportedly modulate the open-state probability of several K^+ channel families including K_{ATP} channels (Ishizaka and Kuo, 1996). These same channels have been shown to be involved in the regulation of membrane potential in vascular smooth muscle cells (Nelson and Quayle, 1995). Second, vascular smooth muscle cells hyperpolarize in response to acidosis indicating that factors that regulate membrane potential may be pH-sensitive (Dietrich and Dacey, 1994). Third, K_{ATP} channel activation and subsequent hyperpolarization has been shown to be the cause of relaxation produced by acidosis in isolated arterial preparations taken from animals (Lindauer et al., 2003; Wang et al., 2003). Thus glibenclamide, a selective inhibitor of K_{ATP} channels, was used to examine whether inhibition of K_{ATP} channel activation with this blocker influences the effects of acidosis on human left internal mammary artery. In our study, pretreatment with glibenclamide eliminated the relaxant effect of acidosis on both phenylephrine- and KCl-induced contractions. Glibenclamide has been shown to be a selective inhibitor of K_{ATP} channels (Nelson and Quayle, 1995; Teramoto et al., 2000; Rosenblum, 2003). Furthermore, this compound did not influence the resting tone or the contractions to KCl and phenylephrine at pH 7.4, indicating that the inhibitory effect of glibenclamide on acidosis-induced relaxation is not nonspecific. From these findings, it can be concluded that acidosis induces relaxation of human left internal mammary artery via activation of K_{ATP} channels.

The glibenclamide-sensitive relaxation induced by acidosis was further assessed. We were hypothesizing that if acidosis-induced relaxation is due to opening of K_{ATP} channels, hyperpolarization, and subsequent decrease in Ca^{2+} influx, then acidosis should not produce relaxation of the contraction, which is independent of Ca^{2+} influx. This assumption would also apply if acidosis had the capacity to block voltage-dependent Ca^{2+} channels directly. In order to produce a persistent contraction, KCl is totally and phenylephrine is mainly dependent upon the presence of extracellular Ca^{2+}, therefore KCl did not produce and phenylephrine induced a very small transient contraction under Ca^{2+}-free conditions (unpublished observation). That is the reason that both of these agents could not be used as contractile agents in Ca^{2+}-free solution or in the presence of an inhibitor of voltage-dependent Ca^{2+} channels. To test the hypothesis that acidosis does not produce relaxation of Ca^{2+} influx-independent contraction; endothelin-1 was used as a constrictor agent in Ca^{2+}-free solution or in the presence of verapamil, an inhibitor of voltage-dependent Ca^{2+} channels. Endothelin-1 even in the absence of extracellular Ca^{2+} or in the continued presence of verapamil was able to induce a contraction albeit relatively smaller one. Acidosis failed to cause relaxation of endothelin-1-induced contraction under these conditions. Interestingly, decrease in pH had a relaxant effect on the contractile response to endothelin-1 in the normal Ca^{2+}-containing PSS. From the results of experiments using Ca^{2+}-free solution and verapamil, it can be deduced that acidosis-induced relaxation is consequent upon the inhibition of Ca^{2+} influx via voltage-dependent Ca^{2+} channels. Interpreting all the data from the experiments using glibenclamide, Ca^{2+}-free solution, and verapamil, it can safely be concluded that activation of K_{ATP} channels and subsequent inhibition of Ca^{2+} influx via voltage-dependent Ca^{2+} channels is the mechanism by which a fall in pH produces a relaxation.

In this study, there are certain questions that are not addressed and need some explanation. First, in addition to plasma membrane, K_{ATP} channels are also present in the mitochondrial wall of the vascular smooth muscle cells (Yokoshiki et al., 1998) and glibenclamide is a non-selective inhibitor of both types of K_{ATP} channels (Chen

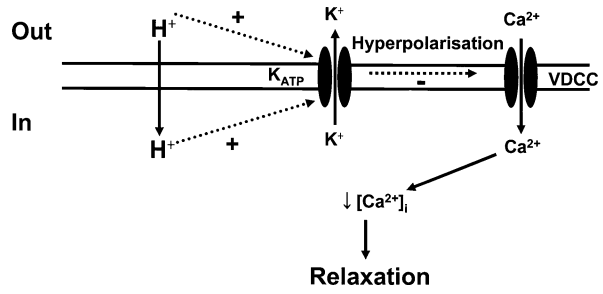

Fig. 5. Simplified scheme illustrating the events occurring under the acidic pH conditions and resulting in relaxation of human internal mammary artery. VDCC; voltage-dependent Ca^{2+} channels.

et al., 2001). Thus, it can be argued that the relaxant effect of acidosis may be due to modulation of mitochondrial activity rather than the function of plasma K_{ATP} channels. Nevertheless, the disappearance of glibenclamide-sensitive relaxation in the presence of verapamil and in Ca^{2+}-free conditions raises the possibilities of the involvement of sarcolemmal K_{ATP} channels in the present study. Second, how extracellular acidosis is sensed and linked to activation of K_{ATP} channels. Recent reports indicating that the pH_i levels in vascular smooth muscle cells may be particularly sensitive to environmental acidosis, with 70–80% of pH_o change transmitted to the cytoplasm (Ramsey et al., 1994), suggest that pH_i represents a potential stimulus for modulating vascular reactivity. We also have conclusively shown that it is the decline in pH_i, which is closely correlated with the effects of acidosis in the aorta from SHR and WKY rats (Rohra et al., 2003b). Because enzymes function optimally within a narrow cytosolic pH range, it is possible that the acidosis may result in the inhibition of certain enzymes of the metabolic pathways. That might lead to depletion of ATP and increase in ADP (Crumrine et al., 1991) and activation of K_{ATP} channels, which are sensitive to increased levels of intracellular ATP (Brayden, 2002). Recently, direct activation of K_{ATP} channels has also been shown by acidosis independent of intracellular ATP levels (Xu et al., 2001). Third, why phenylephrine-induced contraction is markedly sensitive towards relaxant effects of acidosis compared to the one induced by KCl. One explanation of this phenomenon may be that for similar level of contraction, KCl causes greater depolarization and more influx of Ca^{2+} via voltage-dependent Ca^{2+} channels than agonists including α-adrenergic agonists (Neild and Kotecha, 1987; Videbaek et al., 1988). Furthermore, an agent (acidosis in this case) that activates K^+ channels does not efficiently relax a contraction produced by a markedly depolarizing agent like KCl, but does relax contraction produced by a small depolarization (Hamilton et al., 1986; Wu et al., 2001). That may be the reason why KCl-induced contraction is more resistant to the hyperpolarizing effect of acidosis.

In conclusion, our study demonstrates that the acidosis causes the opening of K_{ATP} channels, inhibition of Ca^{2+} influx via voltage-dependent Ca^{2+} channels, and relaxation of human left internal mammary artery. The chain of events occurring under the acidic pH conditions is explained as a schematic diagram in Fig. 5.

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