● Original Article ●

# Involvement of ATP-Sensitive Potassium Channel in Acidosis-Induced Inhibition of α2-Adrenoceptor-Mediated Arteriole Constriction

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PURPOSE: The α2-adrenoceptor-mediated constriction is controlled by ATP-sensitive potassium channels, and is easily inhibited by acidosis. The purpose of this study was to examine the role of ATP-sensitive potassium channels in the selective interaction between α2-adrenoceptors and acidosis.
METHODS: Arterioles from Wistar-Kyoto rat cremaster muscle were microcannulated, pressurized, and isolated in a tissue bath for measurement of changes in lumen diameter. Concentration-dependent constriction by the ATP-sensitive potassium channels antagonist glibenclamide (10 nM to 10 μM) was evaluated under four different conditions: 1) untreated (pH=7.4), 2) acidosis (pH=7.0), 3) in the presence of the α2-adrenoceptor agonist UK-14,304 at pH 7.4, and then pH 7.0, and 4) in the presence of UK-14,304, with or without nitroprusside at pH=7.4.
RESULTS: Glibenclamide administration produced concentration-dependent arteriole constriction (Protocols 1 and 2). However, in the presence of nitroprusside, even 10 μM glibenclamide produced no constriction (Protocol 4). Acidosis inhibited α2-adrenoceptor arteriolar constriction, resulting in returning arteriolar diameter to baseline. Glibenclamide administration produced concentration-dependent arteriole constriction in Protocol 3.
CONCLUSIONS: Acidosis inhibits α2-adrenoceptor constriction by stimulating the ATP-sensitive potassium channels. (J. Jpn. Coll. Angiol., 2004, **44**: 21-27)

**Key words:** α2-Adrenoceptor, Acidosis, Signal transduction, Arteriole constriction, ATP-sensitive potassium channels

## INTRODUCTION

Alpha-adrenoceptors consist of two subtypes: α1 and α2-adrenoceptors. Both receptor subtypes are found in vascular smooth muscle cells, and are involved in vasoconstriction. However, the distribution of these receptor subtypes is not uniform. The α1-adrenoceptors are predominantly present in relatively larger arteries, such as conduit arteries and muscular arteries, whereas α2-adrenoceptors are more predominant in smaller vessels, such as precapillary sphincter muscles[1~4]. The physiologic significance of this heterogeneous distribution of α-adrenoceptors has yet to be established. However, marked differences in the responses of α1 and α2-adrenoceptors to changes in tissue metabolism have previously been reported[5]. Arteriole constriction mediated by α2-adrenoceptors is highly sensitive to factors such as changes in perivascular tissue metabolism. Tissue acidosis, caused by decreased blood flow or increased metabolism, more readily inhibits arteriolar constriction mediated by α2-adrenoceptors than that mediated by α1-adrenoceptors. As a result, it increased tissue blood flow[4, 6~8]. The mechanism by which acidosis inhibits α2-adrenoceptor-mediated arteriolar constriction, however, remains unclear. In addition, ATP-sensitive potassium channels are known to play a role in α2-adrenoceptor mediated arteriole constriction[5, 9]. These ATP-sensitive potassium channels were first identified in myocardial tissue in 1983, and channel opening is known to

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be regulated by intracellular concentrations of ATP. Thus, interest has focused on these channels as important couplers of cell metabolism to cell-membrane potential. Decreased intracellular ATP produced by tissue ischemia activates ATP-sensitive potassium channels[9,10]. Acidosis causes relaxation of porcine coronary arteriole by opening ATP-sensitive potassium channels[11, 12].

The objective of the present study was to determine whether ATP-sensitive potassium channels play a role in the inhibition by acidosis of α2-adrenoceptor-induced arteriolar constriction.

## MATERIALS AND METHODS

Ten-week-old male Wistar-Kyoto rats (n=62; mean weight, 239±14 g) were anesthetized using pentobarbital (100 mg/kg by intramuscular administration). The right cremaster muscle was resected and placed in a dissection bath filled with 3-[N-morpholino]-propanesulfonic acid (MOPS) solution (0-4°C; NaCl 145 mmol/L, KCl 4.7 mmol/L, $CaCl_2 \cdot 2H_2O$ 2.0 mmol/L, $MgSO_4 \cdot 7H_2O$ 1.2 mmol/L, glucose 5.0 mmol/L, pyruvate 2.0 mmol/L, MOPS 2.0 mmol/L, EDTA 0.02 mmol/L, $NaH_2PO_4$ 1.2 mmol/L, and 1% w/v bovine serum albumin; Sigma Chemical Co., St. Louis, MO). Arterioles with an internal diameter of about 100 μm in the right cremaster muscle were selected, then sections measuring about 2 mm in length and without side branches were carefully and rapidly dissected under stereoscopic microscopy[8, 9]. Resected arterioles were transfered to a perfusion chamber filled with the above solution at 0-4°C. Both ends of the arteriole were cannulated with a glass pipette (outside diameter: 60 μm) filled with Krebs solution (NaCl 118.5 mmol/L, KCl 4.7 mmol/L, $CaCl_2 \cdot 2H_2O$ 2.55 mmol/L, $MgSO_4 \cdot 7H_2O$ 1.2 mmol/L, $KH_2PO_4$ 1.19 mmol/L, and dextrose 11.6 mmol/L) to adjust internal pressure. Ends were secured with 11-0 nylon sutures, and specimens were suspended in the chamber[13].

The following were added to the Krebs solution: propranolol (1 μM) to antagonize β-adrenoceptors, angiotensin II (10 pM) to maintain sensitivity of α2-adrenoceptors; indomethacin (3 μM) to inhibit the vasodilatory responses to prostacyclin, $N^G$-monomethyl-L-arginine (L-NMMA) (300 μM) to inhibit the endothelium-dependent vasodilatory responses to NO[7], and prazosin (10 nM) to antagonize α1-adrenoceptors[7, 14]. Prazosin (Sigma) and UK-14,304 (Research Biochemicals) were dissolved in Krebs solution; propranolol and nitroprusside (Sigma) were dissolved in saline; angiotensin II (Sigma) was dissolved in water, and glibenclamide was dissolved in dimethyl sulfoxide (DMSO). Only arterioles with the following characteristics were examined: no leaking from the arteriole; ≧30% intrinsic tone when warmed to 34°C; and normal myogenic responses to the application of 15 mmHg, and 30 mmHg of pressure. Arterioles from 32 rats (52%) met these criteria and were examined. The internal pressure was maintained at 40 mmHg (physiologic internal pressure of arterioles). Arteriole diameter was measured every 2 minutes using an inverted microscope (Diaphot-TMD, Nikon) coupled to a CCD camera, with a video monitor and an Argus 10 image processor. Changes in arteriolar diameter were monitored when each drug was added to the Krebs perfusion solution using a Harvard microinfusion pump[15].

Single-pass perfusion in the chamber with Krebs solution was maintained at a rate of 4 ml/min. Unless indicated, the perfusion chamber was maintained at pH 7.4, with a $pO_2$ of 70 mmHg, a $pCO_2$ of 40 mmHg, and at 34°C. The pH in the perfusion chamber was continuously monitored. When acidosis was established by adding $N_2$ and $CO_2$ gas to the perfusion chamber, the $pO_2$ was maintained at 70 mmHg. The pH was adjusted by increasing the concentration of $CO_2$ and decreasing the one of $N_2$. On completion of each experiment, the absence of leaking and normal myogenic responses to the application of 15 mmHg and 30 mmHg of pressure were confirmed. Arterioles were also maximally dilated with nitroprusside (100 μM), then the absence of any difference with the baseline arteriolar diameter and intrinsic tone was confirmed. If these criteria were not met, the experiment was excluded[13, 16]. Arterioles from 28 rats (45%) met these criteria and were included in the analysis.

### Experiments

Experiments compared the concentration-response curves for 10 nM to 10 μM of glibenclamide, an ATP-sensitive potassium channel antagonist, under the following 4 protocol conditions:

Protocol 1: pH maintained at 7.4.

**Table 1** Arteriole Diameter, Intrinsic Tone, and Myogenic Tests in Each Protocol

| | Start of experiment ($\mu$m) | Intrinsic tone (%) | Myogenic test (+15 mmHg) (%) | Myogenic test (+30 mmHg) (%) |
|---|---|---|---|---|
| Protocol 1 (n=7) | 97±9 | 69±2 | 92±1 | 86±1 |
| Protocol 2 (n=7) | 93±6 | 69±2 | 91±2 | 87±2 |
| Protocol 3 (n=6) | 97±6 | 69±1 | 92±2 | 88±1 |
| Protocol 4 (n=8) | 91±10 | 69±2 | 93±2 | 88±1 |

(n.s)

Protocol 2: pH maintained at 7.0 to produce acidosis.
Protocol 3: UK-14,304 (1 $\mu$M), a selective $\alpha$2-adrenoceptor agonist, was added to produce $\alpha$2-adrenoceptor-mediated arteriole constriction. According to our preliminary experiment (data not shown), this concentration produce 70% to 80% of the maximal constriction that can be achieved with UK-14,304.

The pH of the Krebs solution was then decreased from 7.4 to 7.0 by increasing the reservoir $CO_2$ tension, but with no change in the $O_2$ concentration. Acidosis inhibited $\alpha$2-adrenoceptor-mediated arteriole constriction. Then, despite the addition of UK-14,304, the arteriolar diameter returned to the baseline value (i.e., the diameter before the addition of UK-14,304).

Protocol 4: At pH 7.4, UK-14,304 (1 $\mu$M), a selective $\alpha$2-adrenoceptor agonist, was added to produce $\alpha$2-adrenoceptor-mediated arteriolar constriction. Nitroprusside (0.1 to 1 $\mu$M) was then added, so that even during $\alpha$2-adrenoceptor stimulation, the vessel diameter returned to the baseline value.

### Statistical Analysis of the Data

Glibenclamide was added to the observation chamber at progressively increasing concentrations of 10 nM, 100 nM, 1 $\mu$M, and 10 $\mu$M. The internal arteriolar diameter was measured at 1-minute intervals. When no further changes were observed after a period > 2 minutes, the arteriolar diameter was recorded for the specific concentration of glibenclamide. All results are expressed as the mean ± standard deviation (mean ± SD). Data comparing vessel diameter, intrinsic tone, and myogenic responses were examined by analysis of variance followed by Bonferroni's test. The percentage of arteriolar constriction is expressed as a ratio, with 100% representing the stable arteriolar diameter after the development of intrinsic tone at an arteriolar internal pressure of 40 mmHg. An unpaired two-tail t-test was used to compare percentage ratios of vessel constriction at maximum concentrations of agents for each of the protocols. Statistical significance was defined as a value for p of less than 0.05. The multiple regression model was used for statistical analysis of the concentration-dependent constriction curves for glibenclamide in each protocol[1~4].

## RESULTS

**Table 1** compares baseline vessel diameter, intrinsic tone, myogenic test responses to the application of 15 mmHg and 30 mmHg of pressure, and diameter after maximum vasodilation with nitroprusside between the 4 protocols, prior to warming the vessels at the start of each experiment. There were no significant differences for any of these parameters among the protocols. This finding confirmed the absence of any significant differences in the characteristics of the vessels used in each experiment. All experiments were performed under similar conditions.

No significant differences were observed in the control arteriolar diameter or arteriolar diameter following dilation with nitroprusside or by acidosis (prior to glibenclamide administration) before the administration of drugs in the 4 experimental protocols (**Table 2**). **Fig. 1** summarizes the mean changes in each experiment. The administration of glibenclamide to untreated arterioles that developed intrinsic tone at an internal pressure of 40 mmHg produced arteriole constriction in a concentration-dependent manner in the range from 10 nm to 10 $\mu$M (Protocol 1) (**Fig. 2**). However, for arterioles incubated at pH 7.4 and constricted by UK-

**Table 2** Arteriole Diameters of Controls and Before Administration of Glibenclamide in Each Protocol

| | Control (μm) | Before Administration of glibenclamide (μm) |
|---|---|---|
| Protocol 1 (n=7) | 67±6 | 66±6 |
| Protocol 2 (n=7) | 66±5 | 66±5 |
| Protocol 3 (n=6) | 67±6 | 67±6 |
| Protocol 4 (n=8) | 63±8 | 64±7 |

(n.s)

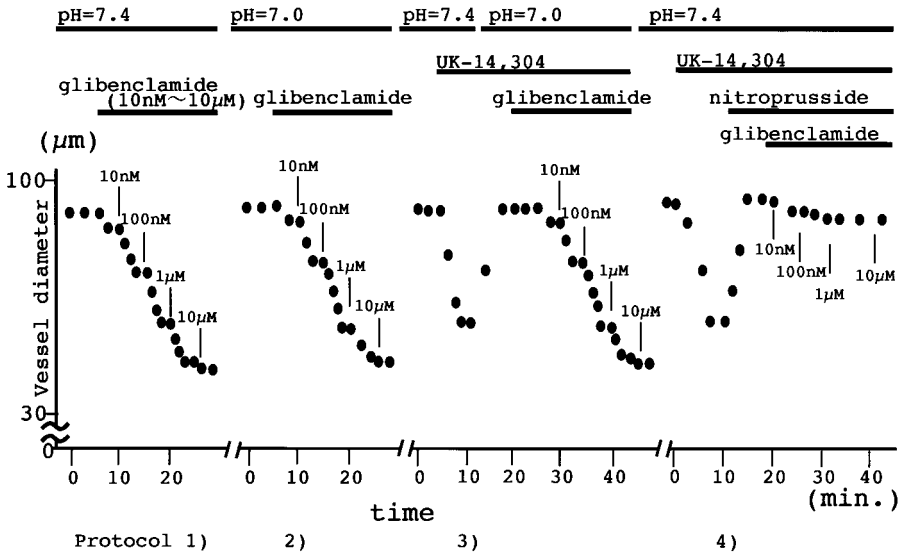


**Figure 1** Changes in arteriolar diameter over time for each protocol.
Protocol 1：At pH 7.4, glibenclamide (10 nM to 10 μM) produced concentration-dependent arteriole constriction.
Protocol 2：At pH 7.0, glibenclamide (10 nM to 10 μM) produced concentration-dependent arteriole constriction.
Protocol 3：After UK-14,304 was administered to constrict arterioles at pH 7.4, pH was decreased to 7.0 (acidosis) to dilate arterioles. Glibenclamide (10 nM to 10 μM) produced concentration-dependent arteriole constriction.
Protocol 4：After UK-14,304 was administered to constrict arterioles at pH 7.4, nitroprusside was administered to dilate arterioles. Glibenclamide (10 nM to 10 μM) produced no arteriole constriction.

14,304 administration (55.7 ± 7.4%), followed by dilation to the baseline diameter by nitroprusside (0.5 ± 0.5 μM), glibenclamide at a concentration of 10 μM produced no constriction (Protocol 4). The arteriole constriction with glibenclamide (Protocol 4) was inhibited significantly compared with the results from Protocols 1 and 2 ($p<0.01$). After arterioles were constricted with 1 μM UK-14,304, the Krebs solution pH was changed from 7.4 to 7.0. Acidosis inhibited α2-adrenoceptor-mediated arteriole constriction, resulting in returning arteriolar diameter to baseline while the administration of glibenclamide produced concentration-dependent arteriolar constriction (Protocol 3). The 10 μM concentration of glibenclamide constricted the vessel to 75.5 ± 10.1% of the baseline diameter. No significant difference was observed with respect to the degree of constriction produced by glibenclamide 10 μM from untreated arterioles (**Fig. 3**).

## DISCUSSION

The mechanism of arteriolar constriction mediated by α2-adrenoceptors involves inhibition of ATP-sensitive potassium channels, which is mediated by an inhibitory GTP-binding protein. Depolarization of vascular smooth muscle cell mem-

branes then results in calcium influx through L-type voltage-gated calcium channels[9, 17]. In this signal transduction pathway, the mechanism of arteriolar constriction downstream from the L-type voltage-gated calcium channels reportedly does not change, even under acidotic conditions that completely inhibit $\alpha$2-adrenoceptor-mediated arteriolar constriction[4, 7]. Therefore, acidotic inhibition of $\alpha$2-adrenoceptor-mediated arteriolar constriction probably involves a signal transduction mechanism located between the receptor and the L-type voltage-gated calcium channel. As mentioned previously, ATP-sensitive potassium channels play a role in $\alpha$2-adrenoceptor-mediated arteriolar constriction, most likely as a signal transduction step between the receptor and the L-type voltage-gated calcium channel. ATP-sensitive potassium channels are stimulated by the change of tissue metabolism[9, 10]. A similar stimulation by acidosis appears to be the mechanism of which acidosis inhibits $\alpha$2-adrenoceptor-mediated arteriolar constriction[9]. Moreover, ATP-sensitive potassium channels are not involved in the $\alpha$1-adrenoceptor-mediated arteriole constriction. This is consistent with the finding that acidosis exerts no effect on the inhibition of arteriolar constriction mediated by $\alpha$1-adrenoceptors. Therefore, the focus of this study was on the role of ATP-sensitive potassium channels in peripheral resistance vessel constriction. We hypothesized that acidosis inhibits $\alpha$2-adrenoceptors

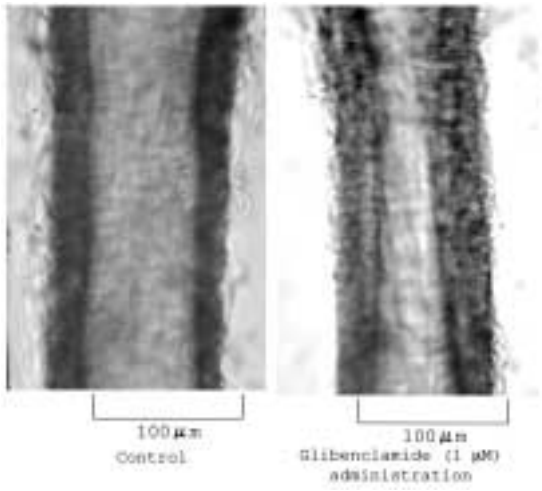


**Figure 2** Changes in Arteriole at protocol 1.
Administration of glibenclamide produced concentration-dependent arteriole constriction.

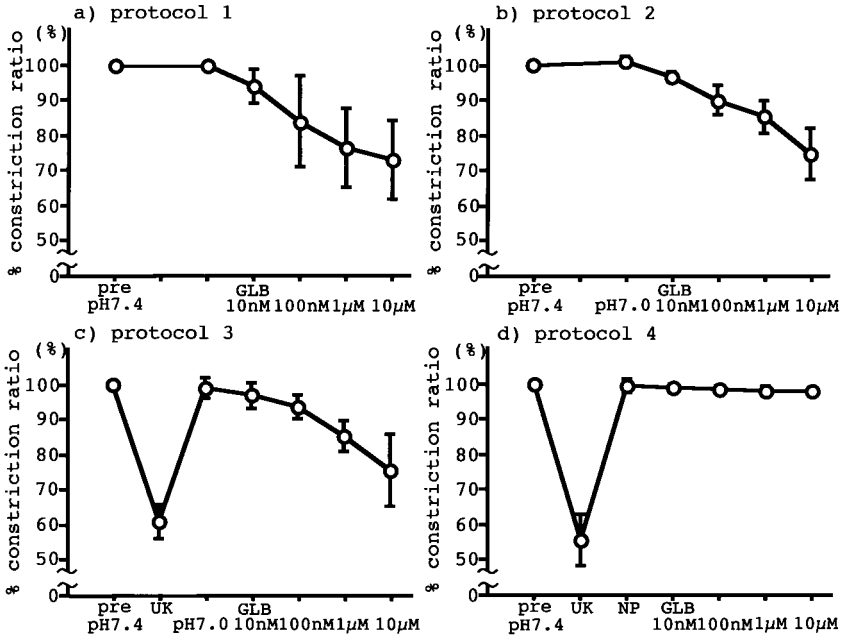


**Figure 3** Concentration-response curves for treatment with glibenclamide (GLB).
For arterioles with UK-14,304-induced constriction that was then reversed by nitroprusside (NP), arteriolar constriction was inhibited significantly compared to the other three protocols ($p<0.01$).

arteriole constriction by stimulating ATP-sensitive potassium channels, and attempted to confirm this hypothesis.

Glibenclamide, the ATP-sensitive potassium channel inhibitor used in this study, produced concentration-dependent arteriolar constriction. This was mediated by glibenclamide inhibition of ATP-sensitive potassium channels, resulting in depolarization of vascular smooth muscle cell membranes and calcium influx through L-type voltage-gated calcium channels. Glibenclamide produced the same degree of arteriole constriction at pH 7.4 and pH 7.0. For all experimental protocols, intrinsic tone was developed over 30%. In this condition, intrinsic tone was not affected under acidotic conditions at pH 7.0[8].

Thus, signal transduction downstream of ATP-sensitive potassium channels and arteriole constriction were unaffected by acidosis. However, for arterioles constricted by UK-14,304, followed by nitroprusside administration, which produced a return to the baseline arteriole diameter, glibenclamide produced no constriction. This was because UK-14,304 administration produced arteriole constriction by inhibiting ATP-sensitive potassium channels. Nitroprusside was then added while the ATP-sensitive potassium channels were inhibited by UK-14,304. This stimulated cyclic GMP production and returned the arteriole diameter to the baseline value. Under this condition, glibenclamide was unable to further inhibit the ATP-sensitive potassium channels, which were already inhibited. However, for arterioles constricted by the administration of UK-14,304, followed by a decrease in the pH from 7.4 to 7.0, glibenclamide did cause arteriole constriction. The degree of constriction in this experiment was the same as the arteriole constriction produced by glibenclamide alone at either pH 7.4 or pH 7.0.

If ATP-sensitive potassium channels are inhibited by the administration of UK-14,304 at pH 7.4 and also inhibited by UK-14,304 under acidotic conditions at pH 7.0, then as in the protocol in which the administration of nitroprusside returned the arteriolar diameter to baseline, we expect that glibenclamide should not produce arteriole constriction. However, when α2-adrenoceptors were inhibited at pH 7.0, even though UK-14,304 was continuously present, glibenclamide still produced arteriole constriction. This can be explained by the fact that the ATP-sensitive potassium channels that had been inhibited by UK-14,304 were no longer inhibited under acidotic conditions. This result provides strong support for our hypothesis that acidosis inhibits α2-adrenoceptor-mediated arteriole constriction by stimulation of ATP-sensitive potassium channels. However, in this study, the effect of endothelium-dependent vasodilatory responses could not be excluded except for the inhibition of NO by L-NMMA. This mechanism remains to be examined in the future. Furthermore, we cannot exclude a possible site of action at a higher point upstream, such as the interaction between GTP-binding proteins and the α2-adrenoceptors. Further studies are needed to clarify these points.

Tissue acidosis develops as a result of decreased blood flow and ischemia. Correction of tissue acidosis requires restoration blood flow. As stated previously, α2-adrenoceptors are predominantly present in small vessels that play an important role in tissue perfusion. Small artery constriction occurs in response to stimulation of these α2-adrenoceptors, preventing improved tissue perfusion. For some reason, tissue ischemia and the resultant acidosis stimulates ATP-sensitive potassium channels, in turn inhibiting α2-adrenoceptor mediated arteriole constriction. This leads to arteriole dilation and increased tissue blood flow. Increased blood flow may improve the acidosis. Acidic inhibition of α2-adrenoceptor arteriole constriction acts as a physiologic mechanism to maintain tissue homeostasis[18, 19]. In a study utilizing porcine coronary arteries, Ishizaka et al.[11, 12] reported that acidotic stimulation of ATP-sensitive potassium channels results in hyperpolarization, which plays a role in maintaining blood flow. Acidotic stimulation of ATP-sensitive potassium channels and its role in maintaining blood flow may be observed in vessels other than the rat cremaster muscle arterioles used in our study, extending to other organs and other species. However, Ishizaka et al.[11] reported that the dilator response did not occur in cremaster muscle arterioles in response to changes in pH in an experimental system similar to ours. The discrepancy between our data and Ishizaka's findings may be due to differences in the species of animal used differences in the vascular beds (the cremaster artery vs. the coronary artery).

In conclusion, acidosis inhibits α2-adrenoceptor-mediated constriction by stimulating ATP-sensitive potassium channel.

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