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# Relationship between adenosine-induced vascular effects and ATP-sensitive $K^+$ channels

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**KEY WORDS** potassium channels; purinergic $P_1$ receptors; adenosine; pinacidil; glyburide; vascular endothelium; thoracic aorta; potassium chloride

## ABSTRACT

**AIM**: To study the relationship between adenosine (Ade) receptors and adenosine 5′-triphosphate (ATP)-sensitive potassium ($K_{ATP}$) channels in rat aorta. **METHODS**: Isolated rat aorta rings were suspended for isometric force recording. The vascular effects of Ade were assessed in the presence or absence of functional endothelium. The interactions of Ade and pinacidil (Pin) or glibenclamide (Gli) were investigated. **RESULTS**: In isolated aorta preconstricted with KCl 20 mmol·$L^{-1}$, Ade 3 - 300 μmol·$L^{-1}$ induced relaxation in a concentration-dependent manner; and in 48/99 preparations from 32 rats, Ade induced initial transient constriction followed by sustained relaxation. When the functions of $K_{ATP}$ channels were blocked with Gli 1 or 100 μmol·$L^{-1}$, effects of Ade were characterized by vasoconstriction rather than vasorelaxation. The combination of Pin 1 μmol·$L^{-1}$ with Ade 100 μmol·$L^{-1}$ showed no synergic vasodilatory effects and did not affect Ade-induced vasoconstriction. After the removal of endothelium, Ade still induced vasoconstriction and vasorelaxation, and the constrictive effects showed no difference from those in the presence of endothelium, but the potency of vasodilatory effects became weaker with slower decrease in tension. **CONCLUSION**: The activation of $K_{ATP}$ channels is involved in Ade receptor-induced vasodilation.

## INTRODUCTION

Adenosine 5′-triphosphate (ATP) sensitive potassium ($K_{ATP}$) channels, widely distributed in the vascular system, are opened under pathophysiologic conditions such as hypoxia and cellular metabolic impairment, and the channel opening will lead to vasodilation due to membrane hyperpolarization. Adenosine (Ade) receptors can mediate the arterial smooth muscle relaxation. But the mechanism is still controversial. Some experiments *in vivo* indicated that Ade induced vasodilation through activating $K_{ATP}$ channels[1-5]. Other experiments *in vivo* and *in vitro* revealed that Ade receptor-mediated vasorelaxation depended on the functional endothelium, and endothelial release of nitric oxide was involved[6-9]. These contradictions resulted from different experimental conditions, animal models, and measured parameters in different researches.

The present study was to determine the pharmacological characteristics of Ade-induced vasodilatory responses in isolated rat aorta rings, and discuss the interaction between Ade receptors and $K_{ATP}$ channels.

## MATERIALS AND METHODS

**Drug** Ade and pinacidil (Pin) were

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purchased from Sigma Co. Glibenclamide (Gli, lot № 940705), purity >98 %, was synthesized in Tianjin Institute of Pharmaceutical Reseach. Bromacyl acetylcholine was bought from Beijing Chemical Works. Norepinephrine bitartrate injection was from Wuhan Pharmaceutical (Group) Co. Gli was dissolved in $Me_2SO$ and ethanol (1:1).

**Rats** Wistar rats (♂, $n = 66$, weighting 250 g ± $s$ 38 g) were provided by the Laboratory Animal Center of our Academy (Grade Ⅱ, Certificate № 01-3039 conferred by Beijing Municipal Administrative Committee of Animals).

**Preparations of aorta ring** Rats were decapitated. The thoracic aorta was washed in cold Krebs-Henseleit solution: NaCl 122, KCl 4.73, $CaCl_2$ 2.49, $MgCl_2$ 1.19, $NaHCO_3$ 15.5, $KH_2PO_4$ 2.25, glucose 11.5 mmol·$L^{-1}$; pH 7.4 at 25 ℃. The aorta was cut into rings 4-5 mm in length. The endothelium of some rings was removed by rubbing with a moistened cotton bud, and endothelium-denuded aorta rings were tested with norepinephrine and acetylcholine.

**Tension studies** Experiments were conducted with a 10-mL bath system containing Krebs-Henseleit solution at 37 ℃ and bubbled with 95 % $O_2$ + 5 % $CO_2$. Isometric contractions were measured and recorded on equilibrium recorder under an initial tension of 1 g. Adjacent rings from the same aorta were studied in parallel. After a 60-min equilibration period, endothelium-intact or -denuded aorta rings were exposed to KCl 20 mmol·$L^{-1}$, a maintained constriction was allowed to develop. At the plateau of the constriction produced by KCl, diversal drugs were added: 1) Endothelium-intact aorta rings were exposed to Ade 3 – 300 μmol·$L^{-1}$, or solvent using a cumulative protocol. 2) Endothelium-intact or endothelium-denuded aorta rings were exposed to Ade 100 μmol·$L^{-1}$. 3) Two endothelium-intact rings exposed to Gli 1 or 100 μmol·$L^{-1}$ for 10 min were incubated with Ade 100 μmol·$L^{-1}$ or Pin 1 μmol·$L^{-1}$. The other 3 rings to solvent, one of them was used as control and the other 2 were incubated with the same concentration of Ade or Pin. 4) One ring served as control and the other 3 were incubated with Ade 100 μmol·$L^{-1}$, Pin 1 μmol·$L^{-1}$, or Ade 100 μmol·$L^{-1}$ + Pin 1 μmol·$L^{-1}$, respectively.

**Data analysis** Results were expressed as $\bar{x} \pm s$. $EC_{50}$ and its 95 % confidence limits were calculated with weighted probit analysis. Intergroup differences were tested by two-tailed paired $t$ test or by one way ANOVA followed by Dunnett test for multiple comparisons.

## RESULTS

**Vascular effects mediated by Ade receptors** In endothelium-intact aorta rings preconstricted with KCl 20 mmol·$L^{-1}$, excitation of Ade receptors by Ade 3 – 300 μmol·$L^{-1}$ mediated relaxation in a concentration-dependent manner, $EC_{50}$ values (95 % confidence limits) were 63 (44 – 91) μmol·$L^{-1}$ (Fig 1).

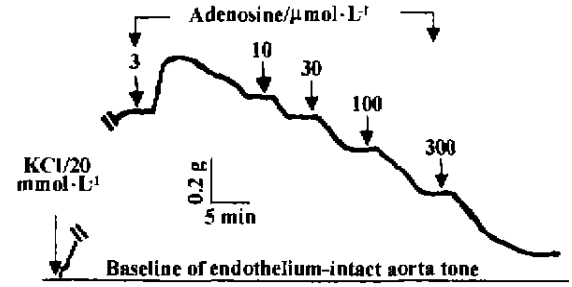


**Fig 1. Adenosine-induced relaxation of the isolated rat aorta preconstricted with KCl 20 mmol·$L^{-1}$.**
***n* = 7 aorta rings isolated from 7 rats. $\bar{x} \pm s$.**

In 48/99 preparations, Ade induced initial transient constriction followed by sustained relaxation (Fig 2). The constriction was only observed in the first exposure of the rings to Ade in cumulative administration protocol (Fig 1).

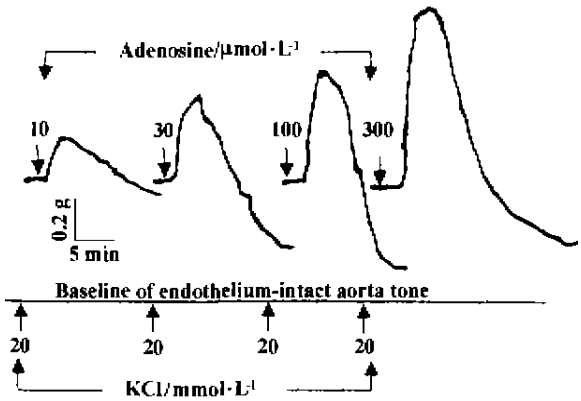


**Fig 2. Dual responses induced by adenosine 3 – 300 μmol · $L^{-1}$ in 48/99 endothelium-intact aorta rings from 32 rats preconstricted with KCl 20 mmol·$L^{-1}$.**

Ade 100 μmol·$L^{-1}$ still produced constriction and relaxation in endothelium-denuded aorta rings. The vasoconstrictive effects showed no difference from those in endothelium-intact aorta rings, but the mild changes in vasodilatory effects were observed after removal of endothelium, the potency became weaker with slower decrease in tension.

### Modulation of Ade receptors by Gli

After the endothelium-intact aorta rings were preconstricted with KCl 20 mmol·$L^{-1}$, when the functions of $K_{ATP}$ channels were blocked by Gli 1 or 100 μmol·$L^{-1}$, the vasodilatory response of Pin 1 μmol·$L^{-1}$ was antagonized. The effects of Ade 100 μmol·$L^{-1}$ were characterized by vasoconstriction rather than vasorelaxation (Fig 3).

### Modulation of Ade receptors by Pin

In isolated endothelium-intact aorta rings preconstricted with KCl 20 mmol·$L^{-1}$, an activator of $K_{ATP}$ channels, Pin 1 μmol · $L^{-1}$ induced dilatory response in a time-dependent manner but did not evoke constrictive response. Adenosine 100 μmol·$L^{-1}$ in combination with Pin 1 μmol·$L^{-1}$ did not alter Ade-induced vasoconstriction. Although the relaxant response of the aorta rings to the combination of the 2 drugs was more potent than that to Ade 100 μmol·$L^{-1}$ alone, it had no difference from that to Pin 1 μmol · $L^{-1}$ alone. When $K_{ATP}$ channels were activated with Pin 1 μmol · $L^{-1}$, further vasodilatory effects of Ade were not observed (Fig 4).

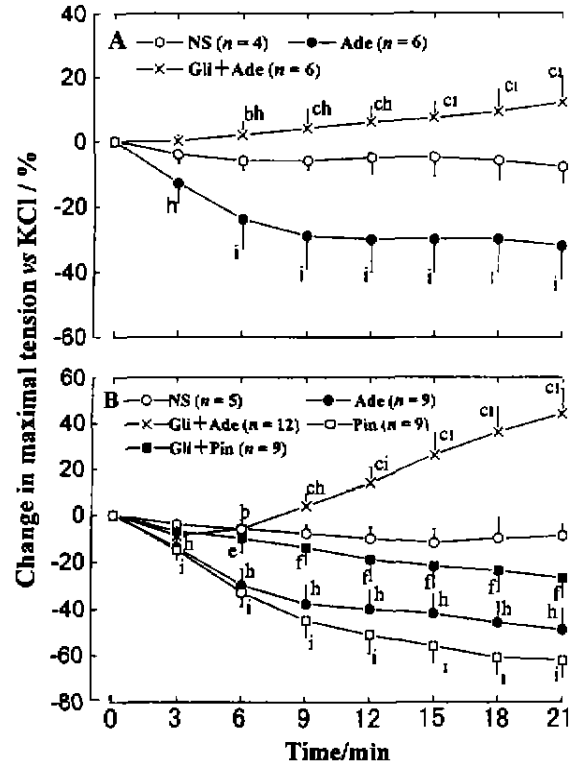


**Fig 3. Effects of Gli 1 (A) and 100 μmol·$L^{-1}$ (B) on Ade (100 μmol·$L^{-1}$)- and Pin (1 μmol·$L^{-1}$)-induced relaxation of isolated rat aorta preconstricted with KCl 20 mmol · $L^{-1}$. $n$ = number of rings (rats). $\bar{x} \pm s$. Maximal tension *vs* KCl: (A) 0.82 g ± 0.07 g, (B) 0.76 g ± 0.06 g. $^bP < 0.05$, $^cP < 0.01$ *vs* Ade. $^eP < 0.05$, $^fP < 0.01$ *vs* Pin. $^hP < 0.05$, $^iP < 0.01$ *vs* NS.**

## DISCUSSION

The vasodilatory effects of Pin on isolated aorta rings, and the antagonism of Gli observed in the present experiments are consistent with those[10,11]. The *in vitro* experimental conditions are reliable. In these experimental conditions, the dual response of isolated aorta rings preconstricted with KCl to Ade was

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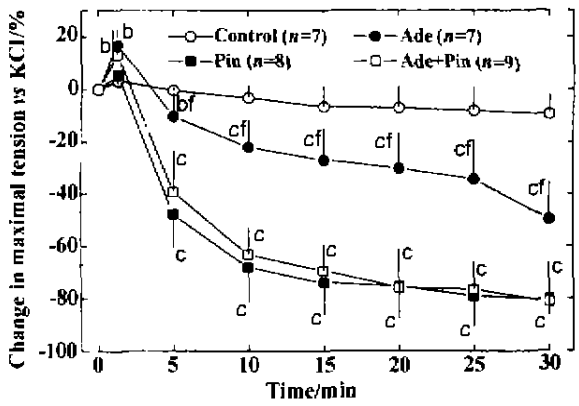


**Fig 4. Effects of Ade 100 μmol·L$^{-1}$, Pin 1 μmol·L$^{-1}$, and Ade + Pin on isolated rat aorta preconstricted with KCl 20 mmol·L$^{-1}$. $n$ = number of rings (rats). $\bar{x} \pm s$. Maximal tension to KCl: 0.84 g ± 0.15 g. $^{b}P<0.05$, $^{c}P<0.01$ vs control. $^{f}P<0.01$ vs Ade + Pin.**

observed. The constrictive phase of the dual response is characterized by (1) subjected to be tolerated, (2) independent on the presence of functional endothelium, (3) not affected by Gli or Pin.

In this study endothelium was involved in Ade-induced vasorelaxation, but it was not indispensable for the vascular effect of Ade.

When $K_{ATP}$ channels were blocked with Gli, Ade receptor-mediated vasodilation was abolished. It indicated that Ade receptors might mediate vasodilation through activating $K_{ATP}$ channels. Under the same experimental conditions, the constrictive response of the aorta preparation to Ade was still observed. It revealed that Ade-induced vasoconstriction was not related to $K_{ATP}$ channels.

The combination of Pin, an activator for $K_{ATP}$ channels, with Ade showed no more potent vasodilatory effects than Pin alone, implying that Ade and Pin induced vasodilation through the same pathway for the modulation of $K_{ATP}$ channels in vascular smooth muscle. It is quite possible that $K_{ATP}$ channels is the effector downstream in mediating vasodilation. Furthermore, Pin did not alter the aortic constrictive response to Ade, it supported that Ade-induced vasoconstriction was not implicated in $K_{ATP}$ channels.

The dual response of isolated aorta rings preconstricted with KCl to Ade observed in our study may be explained by 2 pathways transducing intracellular message: one inducing vasodilation involves $K_{ATP}$ channels while the other inducing vasoconstriction could be related to Ade receptor subtype-mediated phosphatidylinositol turnover. The individual difference of animal and the complexity of Ade-induced vascular effects may be the reasons that the dual responses of aorta rings to Ade were not observed in 51/99 preparations in the present experiments.

In summary, the activation of $K_{ATP}$ channels is involved in Ade receptor-induced vasodilation but not Ade receptor-mediated vasoconstriction.

## REFERENCES

1 Armstead WM. Role of nitric oxide, cyclic nucleotides, and the activation of ATP-sensitive $K^+$ channels in the contribution of adenosine to hypoxia-induced pial artery dilation. J Cereb Blood Flow Metab 1997; 17: 100-8.

2 Gidday JM, Maceren RG, Shah AR, Meier JA, Zhu Y. $K_{ATP}$ channels mediate adenosine-induced hyperemia in retina. Invest Ophthalmol Vis Sci 1996; 37: 2624-33.

3 Kuo L, Chancellor JD. Adenosine potentiates flow-induced dilation of coronary arterioles by activating $K_{ATP}$ channels in endothelium. Am J Physiol 1995; 269: H541-9.

4 Nakhostine N, Lamontagne D. Contribution of prostaglandins in hypoxia-induced vasodilation in isolated rabbit hearts. Relation to adenosine and $K^+$ channels. Pflügers Arch 1994; 428: 526-32.

5 Marshall JM, Thomas T, Turner L. A link between adenosine, ATP-sensitive $K^+$ channels, potassium and muscle vasodilatation in the rat in systemic hypoxia. J Physiol (Lond) 1993; 472: 1-9.

6 Hüsken BCP, Pfaffendorf M, van Zwieten PA.

ATP-sensitive potassium channels in isolated rat aorta during physiologic, hypoxic, and low-glucose conditions.
J Cardiovasc Pharmacol 1997; 29: 130-5.

7 Smits P, Williams SB, Lipson DE, Banitt P, Rongen GA, Creager MA.
Endothelial release of nitric oxide contributes to the vasodilator effect of adenosine in humans.
Circulation 1995; 92: 2135-41.

8 Tabrizchi R, Lupichuk SM. Vasodilatation produced by adenosine in isolated rat perfused mesenteric artery: a role for endothelium. Naunyn Schmiedebergs Arch Pharmacol 1995;352:412-8.

9 Makujina SR, Olanrewaju HA, Mustafa SJ. Evidence against $K_{ATP}$ channel involvement in adenosine receptor-mediated dilation of epicardial vessels. Am J Physiol 1994; 267: H716-24.

10 Wang H, Lu XQ, Zhang YF, Long CL, Wang JH. Effects of pinacidil and nifedipine on isolated rat arteries.
Chin J Pharmacol Toxicol 1997; 11: 229-30.

11 Meisheri KD, Khan SA, Martin JL.
Vascular pharmacology of ATP-sensitive $K^+$ channels: interactions between glyburide and $K^+$ channel openers.
J Vasc Res 1993; 30: 2-12.

## 腺苷的血管效应与腺苷三磷酸敏感性钾通道的关系

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**关键词** 钾通道；嘌呤 $P_1$ 受体；腺苷；吡那地尔；格列本脲；血管内皮；胸主动脉；氯化钾

**目的**：研究大鼠主动脉腺苷受体和腺苷三磷酸(ATP)敏感性钾通道间的关系. **方法**：在离体大鼠主动脉环上观察内皮完整和内皮去除后腺苷受体介导的血管效应及吡那地尔与格列苯脲对腺苷作用的影响. **结果**：腺苷 3 - 300 $\mu mol \cdot L^{-1}$浓度依赖地松弛 KCl 20 $mmol \cdot L^{-1}$预收缩的离体大鼠主动脉环，在 48/99 的标本，腺苷产生先短暂收缩后持续舒张的双向反应. 格列本脲 1 和 100 $\mu mol \cdot L^{-1}$ 阻断 ATP 敏感性钾通道后腺苷的舒张作用被取消而收缩作用仍存在；腺苷 100 $\mu mol \cdot L^{-1}$与吡那地尔 1 $\mu mol \cdot L^{-1}$合用时未产生协同作用；去除内皮不影响腺苷的缩血管效应，但舒血管作用减弱且变慢. **结论**：ATP 敏感性钾通道的激活参与腺苷受体介导血管扩张作用，但与血管收缩无关.

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