

Short Report

## The Role of Nitric Oxide and Prostaglandins in the Effect of Adenosine on Contractility, Heart Rate and Coronary Blood Flow in Guinea Pig Isolated Heart

Mohsen Khalili<sup>\*1</sup> and Jamshid Narengkar<sup>2</sup>

<sup>1</sup>Dept. of Physiology and <sup>2</sup>Dept. of pharmacology, School of Medical Science, Shahed University, P.O. Box 14155-7435, Tehran, Iran

Received 2 June 2004; revised 9 March 2005; accepted 4 April 2005

### ABSTRACT

It is a well-established fact that adenosine and its receptor subtypes ( $A_1$  and  $A_2$ ) are involved in changes of contractility, heart rate and coronary blood flow (CBF) under different circumstances. This study was conducted to evaluate the role of nitric oxide and prostaglandins in development of these changes. For this purpose, Nitro-L-Arginine methyl ester (L-NAME), and indomethacin as inhibitors of nitric oxide and prostaglandins synthesis were used respectively. In this respect, guinea pig isolated hearts were randomly divided into control (receiving adenosine) and groups II and III which received L-NAME (100  $\mu$ M) and indomethacin (50 nM) before adenosine application, respectively, using isolated heart setup. The results showed that adenosine increased CBF and decreased heart rate and contractility in control group. In the presence of L-NAME, adenosine was less effective in enhancing the CBF and decreasing cardiac contractility. Furthermore, no significant change was observed in the presence of indomethacin (regarding all of parameters). It can be concluded that nitric oxide (and not prostaglandins) is essential for the effect of adenosine on CBF and cardiac contractility. *Iran. Biomed. J. 9 (4): 177-180, 2005*

*Keywords:* Adenosine, Nitric oxide (NO), Prostaglandins, Isolated heart

### INTRODUCTION

Adenosine plays a key role in the heart under normal and pathophysiological conditions including ischemic heart disease [1]. In an stressful environment like overactivity or hypoxia, this mediator could modify the rate, contractility and coronary blood flow (CBF) of the heart. In this respect, two principle kinds of receptors have been identified:  $A_1$  for exhibition of negative inotropic and chronotropic effects of adenosine and  $A_2$  for enhancement of CBF [2-4].

There are some experimental evidence that the effect of adenosine on the functional tissues cells may be mediated through changing the levels of nitric oxide (NO) (Endothelial derived relaxing factor) and/or prostaglandins (PG) [5, 6]. In this regard, activation of  $A_2$  receptors are followed by

increased production of NO and PG [7]. Since NO and PG themselves could exert inotropic and chronotropic effect in the heart and change CBF [8-10], this study was carried out to evaluate the role of NO and PG in mediation of the effect of adenosine on the cardiac tissue using isolated langendorff heart setup.

### MATERIALS AND METHODS

*Animals.* Adult male guinea pigs (Razi Vaccine and Serum Research Institute, Karaj, Iran), weighing  $400 \pm 50$  g were allocated two to each cage and had free access to food and water ad libitum at a temperature of  $19 \pm 2^\circ\text{C}$  and with a light-dark cycle. The animals were transferred to animal house! at least two weeks before the

\*Corresponding Author; Tel! (+98-21) 8896 4792; Fax: (+98-21) 8896 6310; E-mail: najafabady@yahoo.com

experiment for adaptation purpose. Animal care and handling were performed according to NIH guideline.

**Experimental methods.** The animals were randomly divided into control (receiving adenosine) and group II and III which received Nitro-L-Arginine methyl ester (L-NAME, 100  $\mu$ M) and indomethacin (50 nM) before adenosine application, respectively using langendorff heart setup. The experimental procedure was conducted as described before [8]. Briefly, the animals were anesthetized with sodium nembutal (40 mg/kg), tracheostomized and respired using ventilator (Harvard, UK). Then after cutting the thorax along the anatomic axillary lines, the exposed heart was cannulated in the initial part of aorta. Meanwhile, all of the vessels were cut out. The heart was perfused with freshly prepared Krebs solution (37°C, pH 7.4), saturated before hand with %95 O<sub>2</sub> and %5 CO<sub>2</sub>. The Krebs solution composed of (mM): NaCl, 118.5; NaHCO<sub>2</sub>, 25; KCl, 4.75; MgSO<sub>4</sub>, 1.19; KH<sub>2</sub>PO<sub>4</sub>, 1.18; CaCl<sub>2</sub> (pH 7.4), 2.5 and glucose (11.1). The flowing Krebs solution finally left the heart through the right atrium. In all of the experiments, a 10-min period was allowed before the study.

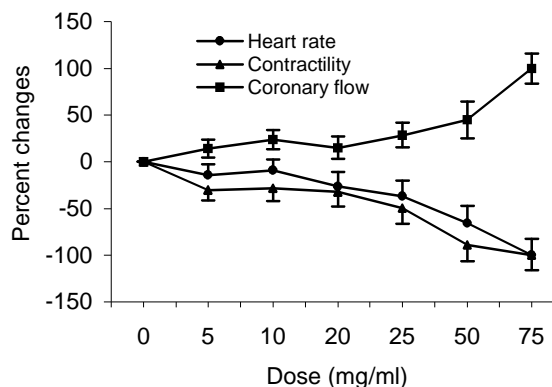
For measurement of inotropic parameter, a small and thin balloon was connected to a catheter entered into the left ventricle via left atrium. The balloon was filled with normal saline (37°C). The catheter conducted the left ventricular parameter  $\Delta V/\Delta P$  to a pressure transducer (Nihon Kohden, Japan). An IBM-compatible computer was used for online data collection and analysis. Simultaneously,  $\Delta V/\Delta P$  signals were sent to a pulse rate tachometer coupler (Narco, USA). The later signals were used for measurement of heart rate (HR). A drop counter transducer (Narco, USA) was also used for evaluation of CBF. In this respect, numbers of exited drops from right atrium were sensed by the transducer and signals were finally integrated.

**Data analysis.** All data were shown as mean  $\pm$  S.E.M. Student's *t*-test (paired and unpaired) was used and  $P < 0.05$  was considered significant.

## RESULTS

The effect of adenosine (5-75 mg/ml) on the HR, contractility, and CBF was determined using isolated heart set up (Fig. 1). In this regard, a clear concentration-dependent response was observed. After adenosine application at doses higher than 20 mg/ml, CBF was significantly increased ( $P < 0.05$ ) and two other parameters (contractility and HR) were significantly decreased ( $P < 0.05$ ).

After L-NAME treatment (100  $\mu$ M), and/or indomethacin (50 nM) no significant changes were observed regarding contractility. With respect to CBF, and HR a significant reduction was found out only after L-NAME treatment (Table 1).



**Fig 1.** Dose-response curve effect of adenosine on the contractility, rate of the heart and coronary blood flow in isolated guinea pig heart. As indicated, the dosage of 25 mg/ml is closed to EC<sub>50</sub> and in dosage of 75 mg/ml the full effect of adenosine on mentioned heart parameters was produced (n = 16).

**Table 1.** Effect of L-NAME (100  $\mu$ M) and indomethacin (50 nM) on the contractility, heart rate, and coronary blood flow of guinea pig isolated heart (n = 14-16).

Groups	Contractility ( $\Delta V/\Delta P$ )	Heart rate (Beats/min)	Coronary blood flow (Drops/min)
Before L-NAME treatment	25.43 $\pm$ 1.10	175.51 $\pm$ 2.22	173.64 $\pm$ 1.09
After L-NAME treatment	26.33 $\pm$ 2.15	179.33 $\pm$ 3.07	152.66 $\pm$ 1.13*
Before indomethacin treatment	21.13 $\pm$ 3.09	168.55 $\pm$ 1.21	164.44 $\pm$ 2.16
After indomethacin treatment	22.53 $\pm$ 1.11	171.28 $\pm$ 2.17	156.41 $\pm$ 1.88

\* $P < 0.05$

**Table 2.** Effect of adenosine on the contractility, heart rate and coronary blood flow of guinea pig isolated heart pretreated with L-NAME (100  $\mu$ M) and indomethacin (50 nM, n = 14-16).

Percent changes	Contractility ( $\Delta V/\Delta P$ )	Heart rate (Beats/min)	Coronary blood flow (Drops/min)
Adenosine (25 mg/ml)	-49.50 $\pm$ 11.56	-36.50 $\pm$ 7.33	+28.49 $\pm$ 5.12
Adenosine + L-NAME	-23.29 $\pm$ 9.22*	-35.20 $\pm$ 6.25	+17.52 $\pm$ 3.99*
Adenosine (25 mg/kg)	-49.50 $\pm$ 8.61	-36.50 $\pm$ 6.85	+28.49 $\pm$ 5.33
Adenosine + indomethacin	-46.32 $\pm$ 9.99	-38.51 $\pm$ 7.21	+27.96 $\pm$ 3.99

(-) and (+) means decrement and enhancement of the heart parameters, respectively. \* $P < 0.05$

Table 2 shows the effect of adenosine on the above mentioned heart parameters, as expressed as % changes after L-NAME or indomethacin pretreatment. In this respect, after L-NAME pretreatment, a significant reduction was found outfor contractility and CBF, but no significant changes were observed regarding HR. Concerning indomethacin pretreatment, no significant changes were observed.

## DISCUSSION

The results of this study demonstrated that adenosine could lead to a significant reduction in HR and contractility and produce a significant increase in CBF. A similar result was also obtained by Ikeda *et al.* [11] using guinea pig isolated heart set up. As an explanation for these results, it has been proved that adenosine  $A_1$  receptors located on sinoatrial node and myocardial tissue may be responsible for observed negative inotropic and chronotropic effects, respectively [1, 12]. Furthermore, adenosine  $A_2$  receptors of coronary endothelial cells could mediate the increased CBF in the presence of adenosine [13, 14]. From a molecular signaling viewpoint, activation of adenosine  $A_1$  and  $A_2$  receptors are followed by an increase and a decrease in the level of intracellular cyclic adenosine monophosphate, respectively [3]. Meanwhile, activation of adenosine  $A_2$  receptors through a cGMP-related pathway could lead to increase synthesis of NO and PG in target tissues [5, 15]. Although there are some controversial reports on the effect of basal NO synthesis inhibition in related to studied heart parameters, our results with respect to enhanced CBF were consistent with Kostic and Schrader [5] and Rubio and Ceballos [7]. Regarding HR and contractility, our data were well matched with those of Mathias, i.e. no significant changes in these parameters [12]. The results of this study showed that coadministration of adenosine and L-NAME has a

significant weaker effect on enhancement of CBF and decrement of contractility than adenosine alone. In this respect, the obtained results for CBF and contractility were consistent with reports of Maddock *et al.* [8] and Kennedy *et al.* [16], respectively. Regarding HR, although NO had no significant effect on the negative chronotropic effect of adenosine, but there are some indications of its efficacy [7, 16]. Finally, although our results indicated that both basal PG and adenosine-induced PG synthesis had no significant effect on the contractility, CBF and HR, but there are some controversial reports on the CBF [7, 8].

In summary, it can be concluded that negative inotropic effect of adenosine and its augmenting effect on CBF is partly mediated through NO.

## ACKNOWLEDGEMENTS

We thank Electronic College of Shaheed Beheshti University (Tehran, Iran) for designing and preparing on-line heart parameters recording computerized board.

## REFERENCES

1. Froldi, G. and Belardinelli, L. (1990) Species-dependent effects of adenosine on heart rate and atrioventricular nodal conduction. Mechanism and physiological implication. *Circ. Res.* 64: 960-978.
2. Belloni, F.L., Belardinelli, L., Halperin, C. and Hintze, T.H. (1989) An unusual receptor mediates adenosine-induced SA nodal bradycardia in dogs. *Am. J. Physiol.* 256: 553-567.
3. Olsson, J. and Pearson, J.D. (1990) Cardiovascular purinoceptors. *Physiol. Rev.* 10: 761-812.
4. Hernandez, J., Loarden, M.L. Ruiz, F. and Riberio, J.A. (1989) Effect of adenosine and its analogues on ventricular automatically induced by a local injury: role of catecholamine and cyclic AMP. *Arch. int. Pharmacodyn. Ther.* 297: 49-59.
5. Kostic, M.M. and Schrader, J. (1992) Role of nitric

- oxide in reactive hyperemia of the guinea pig heart. *Circ. Res.* 70: 208-212.
6. Masatsugu, H. and Hori, L. (1991) Adenosine, the heart and coronary circulation. *Hypertension* 8: 565-574.
  7. Rubio, R. and Ceballos, G. (2003) Sole activation of three luminal adenosine receptor subtypes in different parts of coronary vasculature. *Am. J. Physiol. Heart Circ. Physiol.* 284: 204-214.
  8. Maddock, H.L., Broadley, K.J., Bril, A. and Khandoudi, N. (2002) Effect of adenosine receptor agonist on guinea-pig isolated working heart and the role of endothelium and NO. *J. Pharmacol.* 54: 859-867.
  9. Nanto, S., Kitakaze, M., Takano, Y., Hori, M. and Nagata, S. (1997) Intracoronary administration of adenosine triphosphate increases myocardial adenosine levels and coronary blood flow in man. *Jpn. Circ. J.* 61: 836-842.
  10. Wilke, A., Noll, B. and Maish, B. (1999) Angina pectoris in extracoronary diseases. *Herz* 24: 132-139.
  11. Ikeda, U., Kurosaki, K., Shimpo, M., Okada, K., Saito, T. and Shimada, K. (1997) Adenosine stimulate nitric oxide synthesis in rat cardiac myocytes. *Am. J. Physiol.* 273: 59-65.
  12. Borst, M.M. and Schrader, J. (1991) Adenosine nucleotide release from isolated perfused guinea pig heart and extracellular formation of adenosine. *Circ.Res.* 68: 797-806.
  13. Hein, T.W., Belardinelli, L. and Kuo, L. (1999) Adenosine A (2A) receptors mediate coronary microvascular dilation to adenosine: role of nitric oxide and ATP-sensitive potassium channels. *J. Pharmacol. Exp. Ther.* 291: 655-664.
  14. Olanrewaju, H.A. and Mustafa, S.J. (2000) Adenosine A (2A) and A (2B) receptors mediated nitric oxide production in coronary artery endothelial cells. *Gen. Pharmacol.* 35: 171-177.
  15. Erga, K.S., Seubert, C.N., Liang, H.X., Wu, L., Shryock, J.C. and Belardinelli, L. (2000) Role of A (2A) adenosine receptor activation for ATP mediated coronary vasodilation in guinea-pig isolated heart. *Br. J. Pharmacol.* 130: 1065-1075.
  16. Kennedy, R.H., Hicks, K.K., Brian, J.E. and Seifen, E. (1994) Nitric oxide has no chronotropic effect in right atria isolated from rat heart. *Eur. J. Pharmacol.* 255: 149-156.