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Dopamine Mimics the Cardioprotective Effect of Ischemic Preconditioning via Activation of α_1-Adrenoceptors in the Isolated Rat Heart

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Summary

The aim of the present study was to clarify whether pharmacological preconditioning with dopamine protects the heart against ischemia and whether this effect is mediated through dopaminergic receptors (D_1 and D_2) or α_1-adrenoceptors. Isolated perfused rat hearts were either non-preconditioned, preconditioned with 5 min ischemia, or treated for 5 min with dopamine (1, 5 or 10 μM) before being subjected to 45 min of sustained ischemia followed by 60 min reperfusion. Postischemic functional recovery and infarct size were used as indices of the effects of ischemia. Treatment with the lower concentration of dopamine (1 μM), did not provide any protection to the ischemic myocardium. On the other hand, treatment with 5 μM dopamine resulted in significantly improved functional recovery, whereas administration of dopamine (10 μM) resulted in significantly improved functional recovery as well as reduction of infarct size. Pretreatment with the mixed D_1/D_2 dopaminergic receptor antagonist haloperidol or the β-adrenoceptor selective antagonist propranolol did not attenuate the protective effect of pharmacological preconditioning with 10 μM dopamine with respect to both functional recovery and infarct size reduction. On the other hand, the cardioprotective effect of dopamine was blocked when the α_1-adrenoceptor selective antagonist, prazosin, was administered. In conclusion, pharmacological preconditioning with dopamine protects the myocardium against ischemia and this effect seems to be mediated through activation of α_1-adrenoceptors.

Key words
Heart • Preconditioning • Dopamine • Ischemia

Introduction

Brief periods of acute myocardial ischemia protect the heart against subsequent episodes of prolonged ischemia/reperfusion. This phenomenon is termed ischemic preconditioning (IP) and was first demonstrated in a canine model by Murry *et al.* (1986). In experimental animals and humans, ischemic

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preconditioning has been shown to protect the heart by reducing infarct size and improving functional recovery (Wu *et al.* 2001, Eisen *et al.* 2004). Although several stages in the signal transduction of preconditioning are understood, the comprehensive mechanism remains unclear. Numerous reports have suggested that IP leads to the release of agonists that bind to cell surface receptors and activate signaling pathways such as protein kinase C and PI-3-kinase (Nakano *et al.* 2000, Krieg *et al.* 2002, Argaud and Ovize 2004, Murphy 2004). Released factors include adenosine (Liu *et al.* 1991), opioids (Fryer *et al.* 2001), catecholamines (Mitchell *et al.* 1995) and bradykinin (Goto *et al.* 1995). Furthermore, exogenous administration of many G protein-coupled receptor agonists, such as adenosine, opioids, bradykinin, phenylephrine or noradrenaline and acetylcholine, was found to mimic ischemic preconditioning (Liu *et al.* 1991, Goto *et al.* 1995, Fryer *et al.* 2001, Oldenburg *et al.* 2002, Vasara *et al.* 2002). Thus, for therapeutic reasons, it is important to identify clinically accessible agonists of preconditioning.

Dopamine, an endogenous catecholamine, is known to exert important cardiovascular effects. These effects result from its direct action on dopaminergic D_1 and D_2 receptors as well as from its action on α- and β-adrenoceptors. In general, dopamine at low doses acts through the dopaminergic receptors whereas with increasing doses β_1- and α_1-adrenoceptors are activated (Smit 1989, Girbes and Hoogenberg 1998, Murphy 2000). Intravenous dopamine is used as a positive inotrope in the treatment of acute heart failure and cardiogenic shock (Doggrell 2002). The existence of dopamine receptor subtypes in the heart has been suggested by pharmacological, biochemical and molecular techniques (Ozono *et al.* 1996, 1997, Zhang *et al.* 1996, Ouedraogo *et al.* 1998, Amenta *et al.* 2001, Cavallotti *et al.* 2002, Gomez *et al.* 2002). However, the physiological role of cardiac dopaminergic receptors has not been elucidated. Thus, it would be of interest to determine whether dopaminergic receptors contribute to cardioprotection and whether dopamine protects against ischemia. In the present study, we investigated whether pharmacological preconditioning with dopamine protects the heart against ischemia and whether this effect is mediated through dopaminergic or adrenergic receptors. Functional recovery of hearts and infarct size were used as indices of protection from ischemia/reperfusion injuries in a model of the isolated rat heart.

Methods

Animals

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revized 1996). A total of 61 male Wistar rats (250-300 g) were used. Animals were anesthetized with sodium pentothione (100 mg/kg body weight) intraperitoneally.

Perfusion protocol

The hearts were rapidly excised and placed immediately in cold perfusion buffer (0 °C) before being mounted on a non-recirculating Langendorff apparatus for retrograde perfusion. The ischemic time between excision and mounting was less than 1 min. Hearts were perfused with oxygenated (95 % O_2 - 5 % CO_2) normothermic (37 °C) Krebs-Henseleit bicarbonate (KHB) buffer at a constant pressure of 100 cm H_2O. KHB buffer had the following ion concentration (in mM): 25 $NaHCO_3$, 4.7 KCl, 118.5 NaCl, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 2.5 $CaCl_2$ and 10 glucose, pH 7.4. The perfusion apparatus was water-jacketed to maintain a constant perfusion temperature of 37 °C while the hearts were bathed in KHB buffer at 37 °C during prolonged ischemic periods. Hearts were allowed to beat spontaneously throughout the experiment. Ischemia was achieved by clamping the aortic perfusion catheter so that coronary flow was reduced to zero.

To determine left ventricular pressure, a latex balloon was inserted into the left ventricle through an incision in the left atrial appendage. The balloon was tied securely into place and filled with water to give an end-diastolic pressure between 6 and 10 mm Hg. The adjusted volume remained constant throughout the experiment. The balloon was connected to a pressure transducer *via* water filled polyethylene tubing. Left ventricular pressure and heart rate were monitored continuously and recorded on a computer. Coronary flow rate was determined by collecting the coronary effluent in a graduated cylinder. Lethal arrhythmias, such as ventricular fibrillation at the onset of reperfusion following the sustained ischemic insult, were converted to normal rhythm by tapping the ventricle.

Left ventricular function was assessed by left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), the product heart rate×left ventricular developed pressure (HR x LVDP)

and the coronary flow. Developed pressure is defined as peak systolic minus end-diastolic pressure.

Experimental protocols

All hearts were allowed to stabilize for 20 min before undergoing any treatment. Baseline measurements were recorded at the end of this period. Preliminary experiments were carried out in order to evaluate the effect of ascorbic acid on cardiac contractile function.

The hearts were randomly assigned to one of 8 groups: (1) the non-preconditioned control (NP) was subjected to 45 min of ischemia followed by 60 min of reperfusion; (2) the ischemic preconditioning (IP) group underwent one cycle of 5 min global ischemia and 10 min reperfusion before the 45 min sustained ischemic insult; (3) the DOPA 1 μM group was treated with dopamine 1 μM for 5 min followed by a 10 min drug-free period before the 45 min of sustained ischemia; (4) the DOPA 5 μM group and (5) the DOPA 10 μM group were treated with dopamine 5 μM and 10 μM, respectively, as for group 3; (6) the DOPA 10 μM-HALO group received dopamine (10 μM) as for group 3 and the dopamine receptor antagonist haloperidol (10 μM) which was administered 5 min before dopamine, during the 5 min perfusion with dopamine and also during the first 5 min of dopamine-free period before ischemia; (7) the DOPA 10 μM-PRO group was treated with both dopamine (10 μM) as for group 3 and the β-selective adrenoceptor antagonist propranolol. Propranolol (10 μM) was administered 5 min before dopamine, during the 5 min perfusion with dopamine and also during the first 5 min of dopamine-free period before ischemia; (8) the DOPA 10 μM-PRA group was treated with both dopamine (10 μM) as for group 3 and the α_1-selective adrenoceptor antagonist prazosin (1 μM), which was administered 5 min before dopamine, during the 5 min perfusion with dopamine and also during the first 5 min of dopamine-free period before ischemia.

Infarct size measurement

At the end of the reperfusion period, hearts were frozen in liquid nitrogen to facilitate slicing into 2 mm thick transverse sections across the long axis. All hearts had approximately the same size (1.2 cm, the atria and great vessels were removed), therefore six slices were taken. Slices were incubated in 1 % triphenyl tetrazolium chloride (TTC) in phosphate buffer (pH 7.4) for 30 min at 37 °C. After staining the slices were immersed in 10 % formalin to enhance the contrast between stained and unstained tissue. Tissue stained brick red was taken as viable and pale or white tissue was taken as necrotic. The stained slices were photographed, photos were magnified (×4) and used for the estimation of infarcted tissue. The slices were also viewed under a stereoscope and traced on acetate paper. The area of left ventricle and the area of infarcted tissue were measured by planimetry from both the photographs and tracings independently and compared. Volumes were obtained by multiplying the area by the thickness of the slice. Infarct size was expressed as a percentage of left ventricular volume for each heart.

Chemicals

Biochemical reagents, dopamine, the β-adrenoceptor selective antagonist propranolol, the α_1- adrenoceptor selective antagonist, prazosin, and the dopaminergic receptor antagonist haloperidol were obtained from Sigma-Aldrich (Deisenhofen, Germany). General laboratory chemicals were obtained from Merck (Darmstadt, Germany). Dopamine was diluted in solution containing 1 mM ascorbic acid.

Statistical analysis

Statistical analyses were performed using the Instat program (GraphPad Software, San Diego, CA, USA). Results are given as means ± S.E.M. Groups were compared by one-way ANOVA. If a significant value of F was obtained, the Dunnet test was used to identify individual group differences. A difference was considered statistically significant when $P<0.05$.

Results

Baseline and function during interventions

Table 1 summarizes the baseline hemodynamics of all groups included in the study. No significant differences were observed between groups after 20-min stabilization period for any of the hemodynamic parameters examined (LVDP, coronary flow, HR and the rate-pressure product). Administration of dopamine at 5 or 10 μM, haloperidol, propranolol or prazosin caused temporary changes in hemodynamic parameters that, however, returned to baseline levels by the end of the drug-free period. In preliminary experiments, it was established that ascorbic acid used in dopamine solutions had no effects on cardiac contractile function.

Table 1. Baseline hemodynamic characteristics

Group	n	LVDP (mm Hg)	CF (ml/min)	HR (beats/min)	HR x LVDP (beats x mm Hg x min^{-1})
NP	8	101 ± 6	13.2 ± 1.7	213 ± 10	21600 ± 2398
IP	10	114 ± 10	12.9 ± 1.7	244 ± 9	27835 ± 2950
DOPA 1μM	6	114 ± 7	11.9 ± 0.6	240 ± 15	26619 ± 2892
DOPA 5μM	13	124 ± 8	13.0 ± 0.7	241 ± 10	29795 ± 2400
DOPA 10 μM	6	108 ± 6	13.0 ± 1.8	204 ± 10	21794 ± 1530
DOPA 10 μM-HALO	6	120 ± 7	14.0 ± 2.7	219 ± 23	26925 ± 2429
DOPA 10 μM-PRO	6	95 ± 6	13.0 ± 1.0	252 ± 13	23992 ± 2800
DOPA 10 μM-PRA	6	106 ± 5	12.8 ± 1.2	240 ± 12	25560 ± 2535

Values are expressed as mean ± S.E.M., n, number of hearts in each group; LVDP, left ventricular developed pressure; CF, coronary flow; HR, heart rate; NP, non preconditioned; IP, ischemic preconditioning; DOPA 1 μM, 5 μM or 10 μM, treatment with 1, 5 or 10 μM dopamine; HALO, haloperidol; PRO, propranolol; PRA, prazosin.

Table 2. Functional parameters at the end of 60 min reperfusion period

Group	n	% LVDP	LVEDP (mm Hg)	HR (beats/min)	% HR x LVDP	CF (ml/min)
NP	8	32.11 ± 2.37	57.11 ± 3.71	167 ± 20	26.00 ± 4.89	6.3 ± 0.8
IP	10	60.62 ± 5.99 *	32.00 ± 4.48 *	194 ± 11	52.26 ± 6.62 *	7.7 ± 1.4
DOPA 1 μM	6	22.77 ± 6.26	47.00 ± 9.92	171 ± 10	13.01 ± 2.57	6.6 ± 0.4
DOPA 5 μM	13	54.79 ± 4.75 *	30.69 ± 4.98 *	199 ± 16	42.12 ± 6.14	7.1 ± 0.5
DOPA 10 μM	6	61.90 ± 5.12 *	32.71 ± 7.39 *	192 ± 15	57.23 ± 6.71 *	9.2 ± 1.4
DOPA 10 μM-HALO	6	57.44 ± 4.56 *	15.86 ± 5.64 *	174 ± 12	43.41 ± 5.45	7.6 ± 0.8
DOPA 10 μM-PRO	6	63.00 ± 8.89 *	36.67 ± 10.74	212 ± 16	54.49 ± 9.76 *	8.7 ± 0.7
DOPA 10 μM-PRA	6	45.48 ± 1.44$^{\#}$	42.33 ± 5.36	172 ± 14	32.62 ± 2.68 $^{\#}$	7.3 ± 0.4

Values are expressed as mean ± S.E.M.; n, number of hearts in each group; LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; CF, coronary flow; HR, heart rate; NP, non preconditioned; IP, ischemic preconditioning; DOPA 1 μM, 5 μM or 10 μM, treatment with 1, 5 or 10 μM dopamine; HALO, haloperidol; PRO, propranolol; PRA, prazosin, * $P<0.05$ compared with NP group, $^{\#}$ $P<0.05$ compared with DOPA 10 μM group.

Pharmacological preconditioning with dopamine

Different groups of hearts were perfused with different concentrations of dopamine (1, 5 or 10 μM). Postischemic left ventricular function (Table 2) was assessed by LVDP (expressed as a percentage of the individual baseline value), LVEDP, HR, HR x LVDP (expressed as a percentage of the individual baseline value) and coronary flow. At the end of reperfusion period, LVDP recovered significantly better in IP group compared with the NP control, as well as in groups which were treated with dopamine at 5 μM (DOPA 5 μM) and 10 μM (DOPA 10 μM). In these groups, LVEDP was also significantly lower. However, the recovery of rate-pressure product was significantly enhanced only in the IP group and DOPA 10 μM group as compared with the NP control group. Coronary flow was not affected by either IP or preconditioning with 5 or 10 μM dopamine. Treatment with dopamine at 1 μM did not protect the hearts from the effects of ischemia; the recovery of hearts in this group was not different from that of the NP control hearts (Table 2).

In hearts treated with 1 μM dopamine (DOPA 1 μM), the infarct size (53.44±2.46 %) was not different from that observed in the NP controls (47.62±2.75 %). In addition, no significant difference was observed in infarct size between NP control and DOPA 5 μM hearts (32.79±7.77 %), which is in contrast with the observed high functional recovery in this group of hearts. On the

other hand, treatment with dopamine at 10 μM protected the ischemic myocardium, reducing infarct size to the same extent as observed in the IP group (19.77±2.95 and 11.96±5.86 % in the IP and DOPA 10 μM groups, respectively) (Fig. 1).

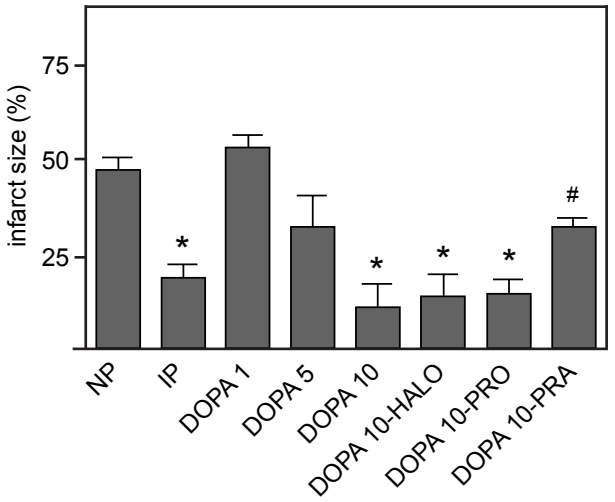

Fig. 1. Infarct size, expressed as percentage of left ventricular volume, for non-preconditioned hearts (NP), ischemic preconditioned hearts (IP), hearts treated with 1, 5 or 10 μM dopamine (DOPA 1, DOPA 5 and DOPA 10, respectively) and hearts pretreated with haloperidol, propranolol or prazosin in the 10 μM dopamine protocol (DOPA 10-HALO, DOPA 10-PRO and DOPA 10-PRA, respectively). Data are mean ± S.E.M. (n = 6-13). * P<0.05 compared with NP group, # P<0.05 compared with DOPA 10 μM groip

Effects of dopaminergic and adrenergic antagonists on dopamine-induced protection

It is known that dopamine at low concentrations (< 10^{-5} M) acts through the dopaminergic D_1 and D_2 receptors, whereas at higher concentrations, β- or α_1-adrenoceptors could also be stimulated (Smit 1989, Girbes and Hoogenberg 1998, Murphy 2000). Thus, we used specific antagonists of the above receptors in order to test whether they reverse the cardioprotective effect of the treatment with dopamine. In these experiments, the higher concentration of dopamine (10 μM) was used because this concentration induced cardioprotection against ischemia by means of both functional recovery and limited infarct size area (Table 2, Fig. 1).

Haloperidol is used as mixed D_1-like and D_2-like receptor antagonist (Schwartz *et al.* 1998). Blockade of dopaminergic receptors with haloperidol did not affect the protective effect of preconditioning with dopamine at 10 μM with respect to both LVDP recovery and infarct size reduction. Postischemic hemodynamics as well as infarct size were not different among the IP, DOPA 10 μM and DOPA 10 μM-HALO groups (Table 2, Fig. 1), suggesting that pharmacological preconditioning with dopamine was not mediated through dopaminergic receptors. In addition, significantly better postischemic recovery and reduced infarct size (15.20±3.61 %) was observed when the β-adrenoceptor antagonist propranolol was used (DOPA 10 μM-PRO group) compared with the NP controls (Table 2, Fig. 1). However, blockade of α_1-adrenoceptors with the selective antagonist prazosin attenuated the protective effect of preconditioning with dopamine at 10 μM. Functional recovery of DOPA 10 μM-PRA group was significantly decreased compared with the DOPA 10 μM group (Table 2), whereas the infarct size was increased (32.85±1.78 %). These results indicate that α_1-adrenoceptors but not β-adrenoceptors are involved in the dopamine-induced cardioprotection.

Discussion

Dopamine regulates cardiovascular functions by actions on the central and peripheral nervous systems, vascular smooth muscle, the heart and the kidney *via* different dopamine receptor subtypes (Velasco and Luchsinger 1998, José *et al.* 1999, Amenta *et al.* 2001, Doggrell 2002). The heart, as well as the kidney, is a potentially important target of dopamine administered exogenously as a therapeutic agent in patients with compromised hemodynamic function, while dopamine infusions are used widely for the management of cardiovascular disorders and renal dysfunction in intensive care units (Emilien *et al.* 1999, Doggrell 2002). Thus, a range of dopamine agonists is in various stages of preclinical and clinical development. In addition, there are several attempts to effectively use the endogenous protective mechanism of ischemic preconditioning in clinical settings. In this regard, the development of preconditioning mimetic agents is of great interest. It has been shown that α_1-adrenergic agonists, used as substitution for ischemic preconditioning, protect against postischemic myocardial dysfunction (Banerjee *et al.* 1993, Mitchell *et al.* 1995), reduce infarct size (Tsuchida *et al.* 1994, Thornton *et al.* 1993, Bankwala *et al.* 1994) and reduce the incidence of reperfusion-induced ventricular fibrillation and ventricular tachycardia (Tosaki *et al.* 1995). Recently, we have confirmed that in an experimental model of isolated rat heart, pharmacological preconditioning with α_1-adrenoceptor agonist phenylephrine results in cardioprotection

comparable to that seen in IP hearts, although the activation of α_1-adrenergic receptors is not essential for the mediation of ischemic preconditioning (Vasara *et al.* 2002). Hence, it seemed of interest to examine whether pharmacological preconditioning with dopamine mimics ischemic preconditioning and whether this effect is mediated by activation of dopamine receptors.

Dopamine at low concentrations is reported to act through the dopaminergic receptors, whereas at higher concentrations ($>10^{-5}$ M) it also stimulates β- and/or α-adrenoceptors (Emilien *et al.* 1999, Murphy 2000). Dopamine receptors are cell surface receptors coupled with G-proteins and classified into two main families based on biochemical, pharmacological and molecular characteristics. The dopamine D_1-like receptor family includes D_1 and D_5 receptor subtypes, which are linked to stimulation of adenylate cyclase. The dopamine D_2-like receptor family includes D_2-, D_3-, and D_4- receptor subtypes, which are linked to inhibition of adenylate cyclase or not related with this enzyme activity (José *et al.* 1999, Amenta *et al.* 2001). Expression of the dopamine receptor varies from one tissue to another in the same animal. The D_1 receptors are localized in the rat heart, although in low abundance, as demonstrated by microscopic immunohistochemistry, electron microscopic immunocytochemistry and *in situ* amplification of mRNA (Ozono *et al.* 1996). Regarding D_2 receptors, they have also been identified in rat heart using combined binding techniques and light microscopy autoradiography (Ricci *et al.* 1998, Cavallotti *et al.* 2002). In addition, Gomez *et al.* (2002) using autoradiographic techniques identified functionally active D_3 and D_4 receptors in the guinea pig heart.

In this study, we used dopamine at different concentrations (1, 5 or 10 μM) in order to examine whether dopamine mimics the protection of IP, and whether the effects of dopamine are mediated through dopaminergic receptors or through β- or α_1- adrenoceptors. We chose the same experimental model used in our previous study (Vasara *et al.* 2002) where it was found that the stimulation of α_1-adrenoceptors mimics the protection conferred by ischemic preconditioning, and we assessed protection with respect to both postischemic functional recovery and infarct size. From the results of the present study, it is evident that pharmacological preconditioning of isolated rat hearts with dopamine at 10 μM results in cardioprotection comparable to that seen in ischemic preconditioned hearts (Table 2, Fig. 1). Treatment with dopamine at 10 μM not only improved postischemic functional recovery but also significantly reduced infarct area. Hearts perfused with dopamine at 5 μM also demonstrated improved postischemic functional recovery; however, infarct size in these hearts, although reduced, was not found significantly different when compared with the non-preconditioned ones. On the other hand, hearts perfused with dopamine at the lower concentration (1 μM), presented relatively low postischemic recovery and infarct size comparable to that observed in non-preconditioned hearts. It is known that dopamine binds to the D_4 receptor with a high affinity (K_d = 30 nM; Seeman and van Tol 1994) and at a dopamine concentration of 1 μM, it is most likely that only dopaminergic receptors are stimulated. Thus, it seems that dopaminergic receptors are not responsible for the dopamine-induced protection of the ischemic myocardium, which was observed clearly in the DOPA 10 μM group and in a limited extent in the DOPA 5 μM group. This concept was confirmed when haloperidol, which is a mixed D_1-like and D_2-like dopamine receptor antagonist (Schwartz *et al.* 1998), was used. Treatment with haloperidol did not block the protective effects exerted by 10 μM dopamine (Table 2, Fig.1). In a canine model of coronary artery occlusion, Vegh *et al.* (1998) showed reduction of ischemia and reperfusion-induced arrhythmias induced by a dopamine receptor agonist. However, they concluded that this protection is due to the inhibition of noradrenaline release and not due to the direct stimulation of dopaminergic receptors.

The protective effect of 10 μM dopamine was not attenuated in hearts treated with the β-adrenoceptor antagonist propranolol, whereas it was partially blocked when the α_1-adrenoceptor antagonist prazosin was used. These results indicate that in pharmacological preconditioning with dopamine, protection assessed by both postischemic recovery and reduced infarct size is associated with activation of α_1-adrenoceptors. In conclusion, dopamine mimics ischemic preconditioning and protects the heart against ischemia/reperfusion injuries. However, it seems that its action is not mediated through dopaminergic receptors but through α_1-adrenoceptors.

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