Effect of Isoproterenol on Tissue Defense Enzymes, Hemodynamic and Left Ventricular Contractile Function in Rats

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Abstract Present study investigated the effects of isoproterenol-induced oxidative stress on hemodynamic and ventricular functions in rats. Subcutaneous injections of isoproterenol (85 mg/kg for two consecutive days at 24 h interval) significantly decreased myocardial antioxidant enzymes; superoxide dismutase, catalase and glutathione peroxidase in heart. Isoproterenol-induced oxidative stress was also evidenced by significant depletion of reduced glutathione and increased formation of lipid peroxidation product, thiobarbituric acid reactive substances along with depletion of myocyte injury specific marker enzymes; creatine phosphokinase isoenzyme and lactate dehydrogenase. The deleterious outcome of oxidative stress on hemodynamic parameters and ventricular function were further evidenced by decreased systolic, diastolic and mean arterial blood pressure, heart rate, ventricular contractility; $[(+)\text{LVdP/dt}]$ and relaxation; $[(-)\text{LVdP/dt}]$, along with an increased left ventricular end diastolic pressure (LVEDP). Subsequent to changes in heart rate and arterial pressure, isoproterenol also decreased rate pressure product. Present study findings clearly demonstrate the detrimental outcome of isoproterenol induced-oxidative stress on cardiac function and tissue antioxidant defense and substantiate its suitability as an animal model for the evaluation of cardioprotective agents.

Keywords Arterial pressure · Heart rate · Cardiac function · Oxidative stress

Introduction

Catecholamines play an important role in the regulation of cardiac functions under physiological conditions. In low concentrations they exert positive inotropic action on the myocardium and, are beneficial in regulating cardiac function [1]. On the other hand, their higher concentrations produce deleterious effects on cardiac function and metabolism [1, 2]. Nevertheless, excessive release of catecholamines is responsible for the development of various cardiac dysfunctions, e.g. in cardiac remodeling following acute myocardial infarction, myocyte death in heart failure and myocardial lesions or infarction [1–5].

A synthetic catecholamine, isoproterenol is used to produce myocardial injury in animals that serves as an experimental model for the pharmacological evaluation of cardioprotective agents [6, 7]. The biochemical and histopathological changes occurring after administration of isoproterenol in rats have been well documented and demonstrated to be a deleterious outcome of isoproterenol-induced imbalance of oxidant and antioxidants in hearts [2–7]. Isoproterenol inflicted complex biochemical and structural changes leading to cell damage and necrosis show similarity with human myocardial infarction [1, 2]. However, few studies available to demonstrate the effect of isoproterenol-induced deteriorated biochemical defense on cardiac function [4–6].

Therefore, present study was undertaken to evaluate the effect of isoproterenol on myocardial antioxidant status, hemodynamics and ventricular function in the *in vivo* animal model. For a correlation between biochemical and

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functional changes, we measured arterial pressure; systolic, diastolic and mean arterial pressure (SAP, DAP and MAP), heart rate (HR), left ventricular peak positive pressure change {(rate of pressure development represents contractility; $(+)\text{LVdP/dt}$ }, left ventricular rate of peak negative pressure change {(rate of pressure decline represents relaxation; $(-)\text{LVdP/dt}$ } and left ventricular end-diastolic pressure (represents preload, LVEDP), and rate pressure product (indicates myocardial energy consumption) and the levels of endogenous antioxidants, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), lipid peroxidation product; thiobarbituric acid reactive substances (TBARS) and myocyte specific injury markers; creatine phosphokinase-MB isoenzyme (CK-MB) and lactate dehydrogenase (LDH) in heart.

Materials and Methods

Experimental Animals

Wistar male albino rats, weighing 150–200 g were used in the study. Study protocol was reviewed and approved by Institutional Animal Ethics Committee of All India Institute of Medical Sciences, New Delhi, India and conforms to Committee for the Purpose of Control and Supervision on Experiments on Animals. Rats were housed at standard laboratory conditions ($25 \pm 2^\circ\text{C}$, relative humidity $50 \pm 10\%$, 14 h light/10 h dark photoperiod) and fed pellet diet and tap water ad libitum.

Chemicals

All chemicals used in the study were of analytical grade and obtained from standard drug companies. Double distilled water was used for all biochemical estimations. Isoproterenol hemisulphate was obtained from Sigma Chemicals Co., St. Louis, USA. Creatine kinase-MB (CK-MB) isoenzyme detection kit was purchased from Randox Laboratories Ltd., USA. Isoproterenol powder was dissolved in normal saline and used within 10 min of its preparation.

Experimental Design

The animals were randomly divided into two groups, viz. control and experimental, having six rats in each. Animals of group I (control) were administered normal saline (1 ml/kg, s.c. at an interval of 24 h) on day 1 and 2. Rats of group II, (Isoproterenol-treated) were administered isoproterenol (85 mg/kg, s.c. at 24 h interval) on day 1 and 2. On day 3, 48 h after the first injection of saline or isoproterenol, hemodynamic and left ventricular function as well as tissue antioxidants were measured.

Measurement of Hemodynamic and Left Ventricular Dynamics

On day 3, animals of both experimental groups were anesthetized with pentobarbitone sodium (60 mg/kg; i.p.). Atropine (0.4 mg/kg) was administered along with anesthesia to maintain heart rate during surgery and to reduce trachea-bronchial secretion. Tracheostomy was performed and rat was ventilated with a positive pressure ventilator (Inco, Ambala, India) using compressed air at rate of 90-strokes/min and a tidal volume of 10 ml/kg. Ventilator setting and oxygen were adjusted to maintain arterial blood gas parameters within the physiological range. Left jugular vein was cannulated with polyethylene tube for continuous infusion of normal saline solution (0.9% NaCl). Right carotid artery was cannulated with a heparinized saline filled cannula. The cannula was connected with Cardiosys CO-101 (Experimentia, Hungary) using a pressure transducer and signals were amplified by an amplifier for measurement of arterial blood pressure; SAP, DAP and MAP as well as HR. Left thoracotomy was performed at the fourth-fifth intercostal space on left side and heart was exposed. After incising pericardium, heart was exteriorized by gentle pressure on ribs. A sterile metal cannula (1.5 mm bore) was introduced into the cavity of left ventricle from posterior apical region of heart for measuring left ventricular dynamics such as $(+)\text{LVdP/dt}$, $(-)\text{LVdP/dt}$ and LVEDP [6]. The cannula was connected to a pressure transducer (Gould Statham P23ID, USA) through a pressure-recording catheter on Polygraph (Grass 7D, USA). After the stabilization of 10 min, tracings were recorded on polygraph paper. Rate-pressure product, a marker of energy expenditure, was calculated by multiplying the systolic arterial pressure and heart rate and dividing the product by 100 [8].

Evaluation of Myocardial Enzymes

After recording hemodynamic and ventricular function, rats were sacrificed and the heart was excised for biochemical estimation. A 10% homogenate of heart was prepared in phosphate buffer saline (pH 7.4, 50 mM) and the aliquots of tissue homogenate were used for estimation of TBARS [9] and GSH [10]. Rest of the homogenate was cold centrifuged at 5,000 rpm for 20 min and supernatant was used for estimation of protein [11], SOD [12], CAT [13], GPx [14], CK-MB isoenzyme [15] and LDH [16].

Statistical Analysis

The data was analyzed using student-*t* test. The quantitative variables were presented as mean $\pm$ SD. *P* value of less than 0.05 was considered significant.

Table 1 Effect of isoproterenol on endogenous antioxidants

Groups	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	GSH ($\mu\text{mol/g}$ tissue)
Control	18.62 $\pm$ 2.14	25.40 $\pm$ 2.60	0.85 $\pm$ 0.18	4.85 $\pm$ 0.80
Isoproterenol	10.25 $\pm$ 1.70*	10.50 $\pm$ 2.12*	0.32 $\pm$ 0.11*	2.10 $\pm$ 0.65*
% Reduction	44.95	58.66	62.35	56.70

Values are expressed as mean $\pm$ SD

* $P < 0.05$

Results

Effect of Isoproterenol on Tissue Antioxidants and Marker Enzymes

Administration of isoproterenol significantly decreased the values of antioxidant enzymes; SOD, CAT and GPx in comparison to control group (Table 1). Concomitant to decreased GPx, a significant decrease in reduced form of glutathione; GSH was also observed (Table 1). In addition to producing depletion of antioxidant, isoproterenol also caused a significant rise in lipid peroxidation product; TBARS (Fig. 1). The myocyte injury marker enzymes, CK-MB and LDH were significantly reduced in myocardium in heart compared to control group (Table 2).

Effect of Isoproterenol on Hemodynamic and Ventricular Function

Isoproterenol caused a significant fall in arterial pressure; SAP, DAP and MAP as well as heart rate as compared to control group (Table 3). In addition to hemodynamic alteration, isoproterenol also significantly decreased (+)LVdP/dt, (–)LVdP/dt and rate pressure product as compared to control group (Table 4). Subsequent to fall in MAP, a significant increase in the LVEDP was also observed in animals administered isoproterenol in comparison with control group (Fig. 2).

Discussion

In myocardium, tissue defense system consists of antioxidant enzymes including SOD, CAT and GPx and reduced glutathione [17]. SOD plays a major role in controlling mitochondrial reactive oxygen species (ROS) generated during normal oxidative phosphorylation and protects cells against oxidative stress by catalytic removal of superoxide radicals and conversion to hydrogen peroxide [17]. After isoproterenol administration, a significant decrease in SOD indicate occurrence of oxidative stress and impaired mitochondrial energetic, required for normal cardiac function. CAT activity was also decreased which play a critical role in regulating ROS in myocardium by handling hydrogen peroxide, the product of SOD.

Activities of GSH-dependent antioxidant enzyme, GPx were also significantly reduced in myocardium after

Fig. 1 Levels of Lipid peroxidation product (TBARS) in heart. Values are expressed as mean $\pm$ SD of six rats. * $P < 0.05$

Table 2 Effect of isoproterenol on myocytes injury marker enzymes

Groups	CK-MB (IU/mg protein)	LDH (IU/mg protein)
Control	190.20 $\pm$ 4.72	228.41 $\pm$ 8.65
Isoproterenol	72.62 $\pm$ 3.20*	97.56 $\pm$ 6.48*
% Reduction	38.81	57.28

Values are expressed as mean $\pm$ SD

* $P < 0.05$ compared to control group

isoproterenol administration. GPx protects cellular and sub-cellular membranes from peroxidative damage by catalyzing removal of hydrogen peroxide via oxidation of GSH that is recycled from oxidized glutathione by the NADPH-dependent glutathione reductase [17]. The reduced activity of GPx in present study might be due to decreased availability of its substrate, GSH. GSH plays an important role in the regulation of cell functions and protect cells from ROS by replenishing GPx as well as scavenging ROS by reacting with superoxide radicals, peroxy

Table 3 Effect of isoproterenol on arterial pressure and heart rate

Groups	SAP (mmHg)	DAP (mmHg)	MAP (mmHg)	HR (beats/min)
Control	132 ± 9	104 ± 6	114 ± 7	390 ± 22
Isoproterenol	84 ± 12*	71 ± 8*	76 ± 8*	246 ± 31*
% Reduction	36.36	31.73	33.33	36.92

Values are expressed as mean ± SD

* $P < 0.05$

Table 4 Effect of isoproterenol on contractility and relaxation

Groups	(+)LVdP/dt (mmHg/s)	(-)LVdP/dt (mmHg/s)	Rate pressure product
Control	2550.75 ± 112.28	2675.55 ± 103.36	514.86 ± 34.40
Isoproterenol	1835.26 ± 130.25*	1755.65 ± 78.80*	207.45 ± 14.27*
% Reduction	28.5	34.38	59.70

Values are expressed as mean ± SD

* $P < 0.05$

Fig. 2 Left ventricular end diastolic pressure (LVEDP) in experimental groups. Values are expressed as mean ± SD of six rats. * $P < 0.05$

radicals and singlet oxygen following the formation of oxidized GSH and other disulfides [18]. Isoproterenol might have exceeded the ability of free radical scavenging enzymes to dismutate the ROS, resulting in myocyte injury and reduction of free radical scavengers [6].

Moreover, due to impaired antioxidant defense mechanism, it is quite likely that the free radicals are not effectively

neutralized. Thus, myocardium shows enhanced susceptibility to lipid peroxidation, a type of oxidative degeneration of polyunsaturated fatty acids which is recognized as one of the basic deteriorative reactions during myocardial ischemia [19]. Isoproterenol-induced rise in myocardial TBARS in present study has been suggested to be due to enhanced formation of free radicals and resultant oxidative stress.

Besides alteration in the endogenous antioxidants, changes in CK-MB isoenzyme and LDH have been considered as one of the important diagnostic markers of myocytes damage [20]. CK-MB isoenzyme is a marker for early detection of myocardial ischemic injury and LDH is a delayed marker of myocardial injury, begins to rise in 12–24 h following injury with peak levels in 2–3 days. Leakage of CK-MB and LDH from heart in present study after 48 h of isoproterenol administration precisely explains the ischemic injury of heart parallel to previous reports [6, 7]. When myocardial cells, containing CK-MB isoenzyme and LDH are damaged or destroyed due to deficient oxygen supply or glucose, the integrity of cell membrane gets disordered and it might become more porous and permeable or may rupture that result in the leakage of these enzymes.

Available literature dictates that ischemic tissues generate free radicals and other reactive species which bring about alteration of contractile function, arrhythmias and myocyte damage [4–6]. These changes are considered to be the consequence of imbalance between the formation of oxidants and the availability of endogenous antioxidants, which determines energy metabolism of heart [17]. Thus, perturbed oxidant and antioxidant balance may impair hemodynamic and contractile function of heart by deterioration of mitochondrial energetic and suppression of Ca^{2+} transport which causes intracellular Ca^{2+} overload and leads to cardiac dysfunction [21].

In present study, a significant reduction of arterial pressure, heart rate and ventricular contractility as well as relaxation along with a rise in LVEDP, might be ascribed to isoproterenol-induced oxidative stress. Reduction in arterial pressure values; SAP, DAP and MAP in the face of decreased HR indicates deranged sympathetic and parasympathetic input to the heart. It is possible that deteriorating myocardial contractility following isoproterenol-induced necrosis might be responsible for significant fall in MAP, a marker of afterload. In addition, absence of positive chronotropic effect indicated by (+) LVdP/dt in the face of a reduced MAP suggests impairment of conduction (AV block) of heart following ischemic injury. Normally, a fall in MAP due to ischemic injury is expected to reflexly increase HR and myocardial contractility by activating baroreceptors, which may subsequently result in reflex vasoconstriction thus worsening the imbalance, between myocardial oxygen demand and supply. However, none of these effects have been observed in present investigation due to ischemic injury to inotropic and chronotropic function of heart. Subsequently, reduced HR and MAP after isoproterenol administration may account for reduced rate-pressure-product, which is an approximation of myocardial oxygen consumption.

On the other hand, lusitropic effect indicated by (–)LVdP/dt was more markedly depressed suggest importunate diastolic dysfunction which might result in the persistence of elevated LVEDP, a surrogate marker of preload. In normal physiology, myocardium gets perfused during diastolic phase of cardiac cycle through coronary arteries. These arteries are poor in collaterals therefore, under ischemic condition; the subendocardial region of heart is most vulnerable to ischemic necrosis because of disproportionate reduction in blood flow to subendocardial region, which is subjected to greatest extra-vascular compression during systole. In addition, increased LVEDP exerts an outward force on ventricular wall that reduces blood flow to the subendocardial region.

Findings of present study clearly demonstrate that isoproterenol-induced oxidative stress contributes to hemodynamic impairment and ventricular dysfunction. As described earlier, isoproterenol-induced myocardial injury model is explicitly used for evaluating cardioprotective agents; hence this study may provide a hemodynamic insight of isoproterenol-induced myocardial necrosis.

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